

A microscopic image of a neuron, showing its cell body and branching processes. The neuron is highlighted with glowing red outlines, creating a striking contrast against the dark background. The text is overlaid on the upper portion of the image.

conversations WITH A neuron

Washington State University Vancouver | Photo by Levi Midnight

Volume 1 | Issue 1

EDITOR IN CHIEF
Cheyanne Lewis

SUPERVISING STAFF
Allison Coffin, PhD
Dale Fortin, PhD

TABLE *of* CONTENTS

PREFACE

[iv](#)

NEWS AND VIEWS

PART ONE: NEUROANATOMY

»» How does behavioral training influence sound frequency discrimination?	1
»» The unique properties between the cerebral cortex and sports-related concussive injuries	4
»» What makes us human? Newly discovered neurons may be the key	7
»» Hippocampal-prefrontal coherence linked to schizophrenia in mice could point to a potential psychosis treatment	10
»» Cutting out the middle man	13
»» TRPA1: A ghost ship in the membrane	16
»» Dopamine receptors aren't always dope	19
»» Hope for schizophrenia patients after discovery of brain cells associated with this mental chronic disorder	21
»» Eat your stress away!	23
»» Try not to forget this one	27
»» Disturbances of gamma-band activity and parvalbumin expression in schizophrenia	30
»» I took this new sleeping pill and nhjmjjj..... (it worked)	33
»» Forgot to brush your teeth? It might kill you	36
»» Taking shots to treat migraine pain: the effects of BOTOX injections on pediatric patients	39
»» Decision-making and reaction times: why you should be playing video games too	42
»» A possible cause for autism: abnormal VEN development	44
»» Musical enrichment for neurologically disordered children	46
»» The key to an effective anti-depressant regimen: brain-derived neurotropic factor	49
»» Functional prions are the central nervous system: how they influence memory!	53

PART TWO: NEUROPHYSIOLOGY

»» Cannabis helps your brain perform like new	57
»» Targeting chondroitin sulfate proteoglycans in order to reduce neural inflammation and promote oligodendrogenesis	61
»» The brain on cocaine: a closer look at the cellular changes to blame	64
»» Social anxiety isn't just for mammals anymore	67
»» Are you one of 70% of people that want to learn a foreign language? Have a nap and learn	69
»» Possible treatment to rehabilitate the brain from addiction	72
»» No Need for Surgery	75
»» Deep brain stimulation improves cognitive control in depressed patients	78
»» Women versus men, what do you want to eat? We might have an answer	81
»» Amyloid- β beta get outta here	84
»» Why it doesn't hurt to get enough sleep	87
»» Puzzled? Turn on the gamma waves	90
»» Don't go to bed angry! Good for your relationship and for your frontal cortex	93
»» Special K – Not just a cereal: ketamine as a novel treatment of depression	96
»» Salt on the brain: a possible explanation for the irresistibility of salty foods	99
»» Are pesticides safe to use with proper protections	102

SPOTLIGHT [104](#)

NEUROSCIENCE IN REVIEW

»» Bring more bugs into the lab! A review on the use of social invertebrate species as models in the study of learning and memory	105
»» Frequency discrimination in the primary auditory cortex and the influence of behavioral training	110
»» Aminoglycoside-induced hair cell death and potential therapies	114
»» A cold case: pediatric high grade glioma	120
»» Perineuronal nets influence on neuroplasticity and fear associations	124
»» Are vital proteins becoming a wrench in the gears?	128
»» Physical exercise and neuronal plasticity: review on the relationship between physical activity on enhanced learning and memory formation	132
»» Visual and auditory perceptual load applied to distracted driving	135
»» Cytokine storm signaling and potential therapies	139
»» Approaches to capturing nonlinear dynamics in auditory encoding	144

»» Perineuronal net modulation of parvalbumin interneurons and the excitatory/inhibitory balance	148
»» A review on one mechanism of aminoglycoside-induced ototoxicity and mitochondria specific treatments	152
»» Martial arts related neurological damage: a review	161
»» Racial and ethnic differences in postpartum depression and cortisol function: a literature review	164
»» The role of the gut microbiome on depression	169
»» Analyzing the cellular basis behind permanent central nervous system damage	173

preface

Welcome to the inaugural edition of *Conversations with a Neuron*! This issue, and the ones to follow, feature articles written by our Neuroscience majors at Washington State University Vancouver. The articles all started as class assignments for classes such as Neuroanatomy and the neuroscience Capstone course; senior-level courses that invite the students to dig into a topic in neuroscience that interests them, to think deeply, and to synthesize ideas. The end result, of course, is a graded assignment – we know our students work hard for their grades! In a traditional course, the grade would be the end – a paper written by the student, read and graded by the professor, and set aside.

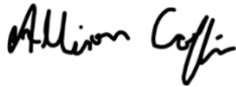
These papers are so much more than a chance to earn a grade! They show original thought and mastery of the subject matter, and they teach the professors something new every time. Hence, this journal. We wanted to showcase the great work of our students.

I particularly want to thank Cheyanne Lewis (B.S. Neuroscience 2019), a gifted writer and editor who volunteered to compile and edit this volume. Cheyanne did an amazing job editing every page, turning a disparate set of papers into a comprehensive body of work.

To learn more about our Neuroscience program, please visit our website at <https://cas.vancouver.wsu.edu/neuroscience>.

We hope you enjoy this issue... and learn something you are glad to know!

Sincerely,



Dr. Allison Coffin
Associate Professor of Neuroscience



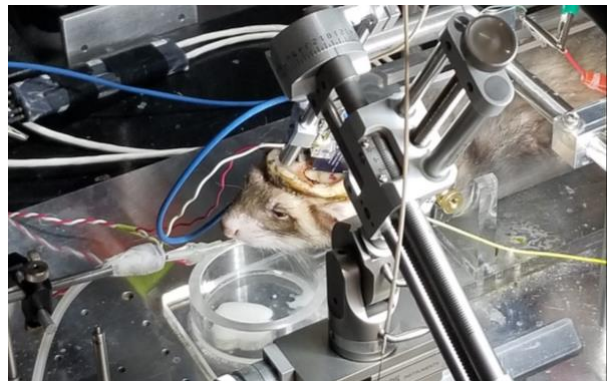


How does behavioral training influence sound frequency discrimination?

With hearing loss affecting 35 million Americans, ferrets have been used to study frequency discrimination in the auditory cortex in aims to improve current engineered sensory signaling systems.

Kawther Elolaimi

In a paper recently published in Nature Communications, Sophie Bagur et al., investigate the effects of behavioral training on the auditory cortex of ferrets.¹ Their goal was to understand how and where in the brain sensory representation transform into abstract percepts. Originally, primary sensory areas played a role in extracting and encoding high-level stimulus attributes, but recent studies in awake animals have challenged this perception and shown that information represented in primary areas depends on the behavioral state of the animals. This study supports the claims of previous studies challenging this perception while also focusing on the correlation between encoding from a sensory stimulus and behavioral driven response in the primary auditory cortex.



Exposed brain area where electrodes are inserted. *This figure shows the ferret in a soundproof booth with the sprout directly under his nose to sense the water to lick at the appropriate frequency. It also shows the implanted headpost on the ferret as well as electrode insertion into the exposed brain area.*

To gain a better understanding of the purpose of this research, let's understand the big picture a little better. Sensorineural hearing loss accounts for about 90% of reported hearing loss² affecting 35 million Americans.³ The root cause is from inner ear hair cell damage as well as from nerve damage which disrupts the transmission of sound from the inner ear to the brain.⁴ From the inner ear, sound is transmitted to the auditory cortex which is located in the temporal lobe. More specifically, the auditory cortex is located on the superior temporal gyrus in the temporal lobe⁵ and is essential for hearing and understanding speech.⁶ The auditory cortex is composed of two main regions, primary auditory cortex and the secondary auditory cortex. The primary auditory cortex (AI) is composed of neurons which function to decode the organized representation of a sound stimulus as well as differentiate frequencies into identifiable sound.^{7,8} The secondary auditory cortex (AII) is essential for sound detection and localization as well as auditory memory^{7,8} and language comprehension.⁵ Impairment of the auditory cortex to discriminate sounds is often an effect of hearing loss since frequency discrimination is important for speech and complex sound recognition. This study is using behavioral training on ferrets to further understand the importance of processing temporal sequences of sound in the auditory cortex.^{1,5,9} Without the

auditory cortex, ferrets lose the ability to discriminate between two complex sounds which have similar frequency components that differ in temporal sequence.⁵

During behavioral training, adult female ferrets were trained using aversive conditioning which is the use of something unpleasant to stop an unwanted behavior (via positive reinforcement) to discriminate between high tone and low tone sound frequencies. These ferrets were trained to lick water from a spout as shown in the figure below, during the presentation of a class of reference stimuli and to stop licking after the presentation of a different class of target stimuli to avoid a mild shock. During each session, the target sound frequency changed as to require the ferrets to continue to learn a new reward association. Once the animal learned the task, neuronal activity in A1 was recorded in response to the sound stimulus and the behavioral state of the ferrets as implied in the figure below. Prior to behavioral training, the ferrets were implanted with a headpost to expose the brain of the ferrets and allow the researchers to use electrodes to record neuronal activity as explicitly shown in the ferret in the figure below. The data was then analyzed and used to guide their objective which was to further understand the correlation between encoding from a sensory stimulus and behavioral driven response in the primary auditory cortex.

During analysis, they observed increased spontaneous activity in the A1 during task-engaged conditions but did not observe a significant difference between firing patterns between high vs. low sound frequencies. This research shows that the animals were actually able to learn the task and differentiate different tone frequencies which was shown in the lick rate. They learned to only lick the sprout at specific tone frequencies and avoid it during target sound frequencies. In addition, encoding of information strongly changed in between trials when the sound shifted from a random sound stimulus to the target sound frequency. This showed a comparison of the sound decoders in the brain between the duration that the sound was played, and the ferret was licking water vs. during the target sound stimulus when the ferret stopped licking to avoid getting shocked. The primary auditory cortex therefore appeared to strongly represent information about the stimulus class.

In comparison to other studies, this research group conducted their research on ferrets instead of rodents, cats, owl monkeys, etc., for instance, due to ferret's rapid ability to learn new tasks.^{10,11,12,13} Ferrets are considered smart and have a relatively similar hearing range frequency to humans, which in humans is 20 Hz to 20 kHz. With age, most people lose the ability to detect high frequency sounds while maintaining the ability to hear low frequency sounds for a longer time. A common solution has been hearing aids while those who are hearing impaired or deaf tend to use cochlear implants to detect sound. Hearing aids only amplify sounds while cochlear implants send sound signals to the brain.¹⁵ Cochlear implants are small complex electronic devices that provide people with a sense of sounds by replacing the function of the damaged parts of the inner ear.¹⁵ The implant consists of a small portion that is surgically placed in the inner ear and an external portion that sits behind the ear.¹⁵ Bilateral implantations have been introduced within the last decade with hopes of improving both speech perception in background noise and sound localization.¹⁴ There is evidence suggesting that binaural perception is possible with two implants, research results in humans are variable therefore ferrets have been used to explore potential contributing factors to these variable, inconsistent outcomes.¹⁴ The idea of using ferrets as an animal model has opened broad possibilities of using them to study potential protective effects of bilateral cochlear implantation on the developing central auditory pathway and develop novel neuroprosthetic therapies for use in humans.¹⁴ As shown not only in this study but additional studies as well, behavioral training enhances temporal processing parameters. This research aids in understanding frequency resolution in A1 and can be used to improve current engineered sensory systems for signaling processes.

REFERENCES

1. Bagur, Sophie, et al. "Go/No-Go task engagement enhances population representation of target stimuli in primary auditory cortex." *Nature communicators*, 28 June 2018, www.nature.com/naturecommunications
2. Clason, Debbie. "Common Causes of Sensorineural Hearing Loss." *Healthy Hearing*, 6 Feb. 2018, www.healthyhearing.com/report/50276-Common-causes-of-sensorineural-hearing-loss.
3. Kochkin, Sergei. "MarkeTrak VIII: 25-Year Trends in the Hearing Health Market." *MarkeTrak VIII: 25-Year Trends in the Hearing Health Market*, The Hearing Review, 1 Oct. 2009
4. "Sensorineural Hearing Loss." *American Speech-Language-Hearing Association*, ASHA, www.asha.org/public/hearing/Sensorineural-Hearing-Loss/.
5. Purves, Dale. "The Auditory Cortex." *Neuroscience. 2nd Edition.*, U.S. National Library of Medicine, 1 Jan. 1970, www.ncbi.nlm.nih.gov/books/NBK10900/.
6. SpinalCord.com. "Temporal Lobe." *SpinalCord.com*, www.spinalcord.com/temporal-lobe.
7. Gil-Loyzaga, Pablo. "Journey into the World of Hearing." *Cochlea*, 9 Dec. 2016, www.cochlea.eu/en/auditory-brain/thalamo-cortex/auditory-cortex-physiology.
8. "Auditory Cortex." *Brain Auditory Pathways - Auditory Cortex*, psych.athabascau.ca/html/Psych402/Biotutorials/26/cortex.shtml.
9. Schwartz, Zachary, and Stephen V David. "Focal Suppression of Distractor Sounds by Selective Attention in Auditory Cortex." *OUP Academic*, Oxford University Press, 9 Nov. 2017, academic.oup.com/cercor/article/28/1/323/4608048.
10. Populin, Luis C., and Tom C. T. Yin. "Behavioral Studies of Sound Localization in the Cat." *Journal of Neuroscience*, Society for Neuroscience, 15 Mar. 1998, www.jneurosci.org/content/18/6/2147.
11. Vollmer, Maike & E Beitel, Ralph. (2011). Behavioral training restores temporal processing in auditory cortex of long-deaf cats. *Journal of neurophysiology*. 106. 2423-36. 10.1152/jn.00565.2011.
12. Recanzone, G. H., and M. M. Merzenich. *Plasticity in the Frequency Representation of Primary Auditory Cortex Following Discrimination Training in Adult Owl Monkeys*. The Journal of Neuroscience, Jan. 1993.
13. Fritz, Jonathan, et al. "Rapid Task-Related Plasticity of Spectrotemporal Receptive Fields in Primary Auditory Cortex." *Nature News*, Nature Publishing Group, 28 Oct. 2003, www.nature.com/articles/nn1141.
14. Hartley, D. E., Vongpaisal, T., Xu, J., Shepherd, R. K., King, A. J., & Isaiah, A. (2010). Bilateral cochlear implantation in the ferret: a novel animal model for behavioral studies. *Journal of neuroscience methods*, 190(2), 214-28.
15. "Cochlear Implants." *National Institute of Deafness and Other Communication Disorders*, U.S. Department of Health and Human Services, 15 June 2018, www.nidcd.nih.gov/health/cochlear-implants.



The unique properties between the cerebral cortex and sports-related concussive injuries

Concussions are a large issue with a growing spotlight in sports; professionally, collegiately, and recreationally this is a problem commonly addressed. Maerlander et al's article "Concussion competencies: a training model for school-based concussion management"¹ chose to take a look at how concussive education can decrease risky behavior and overall cortical damage.

Jason Driver

Concussions are forms of mild traumatic brain injuries (mTBIs) from hits to the body that cause the brain to hit the inner skull and damage itself. The Centers for Disease Control and Prevention found that in 2014 2.87 million mTBI-related emergency department visits with 56,800 attributed deaths occurred in the United States every year².

Maerlander et al's study looks to combat the dangers of concussions through early education and teaching students the risks early on. Adolescents that receive concussions while being in school are put in one of the worst possible environments; an expectation of mental and physical exertion, bright lights, noises everywhere. All of these scenarios help to exacerbate the symptoms of a concussion^{2,3}.

When the skull is accelerated at a rate that results in the collision between the brain and cranium, there is shearing of brain cells and blunt trauma of the internal brain structures⁵. The trauma caused by these mTBIs leads to symptoms such as noise/light sensitivity, confusion, amnesia, ringing in the ears, slurred speech, failure to track with eyes, and temporary loss of consciousness^{6,7,8}. These symptoms arise from damage from unique cortical areas encoding for different senses. The article from Maerlander et al that is being focused on gives insight to how symptoms can be amplified in a school setting, further demonstrating the importance of educating students on concussion risk.

It's been observed that mild traumatic damage to the occipital lobe causes swelling and impairment in smooth pursuit and saccadic function⁹. Damage to the temporal lobe can cause problems with hearing; hypersensitivity, hyposensitivity, and some loss of function¹⁰. If damage is systemic enough, consciousness can be lost completely at the time of damage, and symptoms can

manifest themselves decades down the line¹¹. Figure 1 provides an example and explanation of area specific damage found in concussive injuries.

All of these neurological symptoms can be annoying in the short term, but concussions have been shown to have long term-debilitating effects and even shorten expected lifespan¹². Though the long-term systemic consequences of concussions are not fully known, and it is also not known how education of a young generation can lead to changes in this field of medicine.

Because of these risks, the focused research from Maerlander et al desired to educate adolescent student instructors about concussions in hopes to protect them. The study itself followed 102 participants staffed at schools primarily supporting grades 6-12 from 4 different schools. These staff members were given two trainings ranging between 2-3 hours each for a total of 4-5 hours. The hope was to provide school staff members with concussion management knowledge. This knowledge is given to help prevent concussion onset, diagnose possible concussions, and properly treat those with concussions. Participants were measured on two concussion competency tests produced by concussion experts; one test before and one test 5 months after training. Overall, there was a visible improvement on concussion competency scores among all tested groups, and 88% found the training to be satisfactory in the information given.

Maerlander et al's study was unique through not targeting coaches or students in sports, but other members of the school that these students will interact with. This concussion education allows for school staff to notice the signs of concussions and teach students about the science and dangers associated with concussions. Overall, only nine states have legislation requiring schools to have a protocol for managing concussed students as they return to academics; the hope is that through proving that useful information is retained more legislation may be put in place to help provide this education.

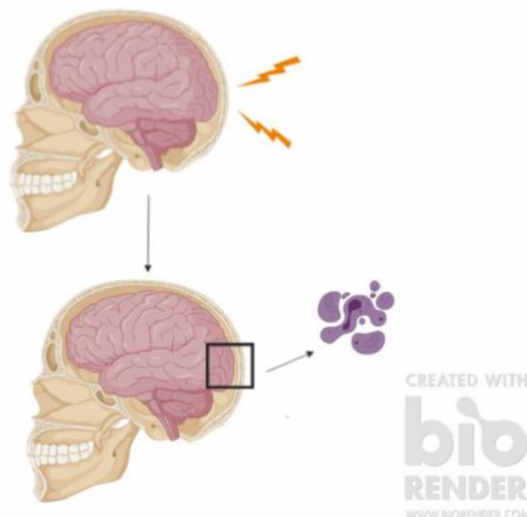


Figure 1. When a concussive blow occurs at the posterior portion of the skull, the occipital lobe will be most directly damaged. Possible hemorrhaging, inflammation, and cell death will occur in this area. This area specific damage is associated with symptoms such as temporary blindness, tunnel vision, and seeing stars after an impact occurs.

REFERENCES

1. Maerlander, A., Lichtenstein, J. D., Parent-Nichols, J., Higgins, K., & Reisher, P. (2019). Concussion competencies: a training model for school-based concussion management. *Concussion*, (0).
2. Centers for Disease Control and Prevention (2019). Surveillance Report of Traumatic Brain Injury-related Emergency Department Visits, Hospitalizations, and Deaths—United States, 2014. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services.
3. Halstead, M. E., McAvoy, K., & Brown, B. E. (2016). Expanding concussion laws not necessary for return to learning after concussion. *Pediatrics*, *138*(6), e20163194.
4. Ransom, D. M., Vaughan, C. G., Pratson, L., Sady, M. D., McGill, C. A., & Gioia, G. A. (2015). Academic effects of concussion in children and adolescents. *Pediatrics*, *135*(6), 1043-1050.
5. Nishimoto, T., & Murakami, S. (1998). Relation between diffuse axonal injury and internal head structures on blunt impact. *Journal of Biomechanical Engineering*, *120*(1), 140-147.
6. Wood, F., Novack, T. A., & Long, C. J. (1985). Post-concussion symptoms: Cognitive, emotional, and environmental aspects. *The International Journal of Psychiatry in Medicine*, *14*(4), 277-283.

7. Yamamoto, L. G., & Bart Jr, R. D. (1988). Transient blindness following mild head trauma: criteria for a benign outcome. *Clinical pediatrics*, 27(10), 479-483.
8. King, N. S., Crawford, S., Wenden, F. J., Moss, N. E. G., & Wade, D. T. (1995). The Rivermead Post Concussion Symptoms Questionnaire: a measure of symptoms commonly experienced after head injury and its reliability. *Journal of neurology*, 242(9), 587-592.
9. Cochrane, G. D., Christy, J. B., Almutairi, A., Busettini, C., Swanson, M. W., & Weise, K. K. (2019). Visuo-oculomotor Function and Reaction Times in Athletes with and without Concussion. *Optometry and Vision Science*, 96(4), 256-265.
10. Tremblay, S., De Beaumont, L., Henry, L. C., Boulanger, Y., Evans, A. C., Bourgouin, P., ... & Lassonde, M. (2012). Sports concussions and aging: a neuroimaging investigation. *Cerebral cortex*, 23(5), 1159-1166.
11. Kerr, Z. Y., Register-Mihalik, J. K., Kay, M. C., DeFreese, J. D., Marshall, S. W., & Guskiewicz, K. M. (2018). Concussion nondisclosure during professional career among a cohort of former National Football League athletes. *The American journal of sports medicine*, 46(1), 22-29.
12. Centers for Disease Control and Prevention. (2007). Nonfatal traumatic brain injuries from sports and recreation activities--United States, 2001-2005. *MMWR: Morbidity and mortality weekly report*, 56(29), 733-737.



What makes us human? Newly discovered neurons may be the key

Boldog et. al. have discovered a specialized GABAergic interneuron subtype within layer 1 cortex unique to the human brain. These rosehip interneurons have been shown to target layer 3 pyramidal neurons and work to inhibit back propagating, expressing great local control of distal dendrites.

Jessica Faraca

What is it that makes the human brain unique compared to other animals? This is a question many neuroscientists are interested in, especially when it comes to human's capacity for higher cognitive functioning. The work of Boldog et.al.¹ may be the clue needed to crack our understanding of the human brain. In their recently published article in Nature Neuroscience, Boldog et. al. share the discovery of a new neuron subtype unique to humans. These newly found cells, called rosehip neurons due to their shape, are found within layer 1 cerebral cortex. What makes these cells so interesting is their morphology and physiology, which is unlike anything found within rodent cortex. With the discovery of these interneurons insight can be gained as to how inhibition impacts cortical function of human cognition, and potentially how human cortex differs from other species.

Understanding the function and type of neurons within the cerebral cortex has been of interest since the time of Santiago Ramón y Cajal, and his findings supporting the neuron doctrine². Traditionally, when studying the mechanisms of our brains it is not uncommon to use animal models. Previously neuroscientist believed the variation in human and animal brains were only a result of their differing size and degree of elaboration³. The idea of variation in type of cells between species was not an idea widely held. However, as research techniques have advanced our ability to identify differences in neurons has increased drastically. One approach to researching cognition that has been garnering attention recently is a combination between genetics and cognitive neuroscience⁴. Through this new integrative research, it has been suggested that the synapse is an excellent starting point for global systems biology of the brains⁵. However, up until the findings of Boldog et.al. there was little evidence supporting differences between the types of neurons seen in humans compared to those of animals.

A typical approach for studies focusing on neurons of the neocortex is to complete electrophysiological recordings of brain slices. The electrophysiology of a cell provides information on the electrical behavior of neurons and the function of neural systems⁶. However, only completing an electrophysiology study does not consider other aspects of cortical interneurons. To gather a comprehensive understanding, the cells morphology was also investigated. Distinctions between various neurons in terms of their dendritic configuration, axonal structures, and shape/size of soma can indicate variations in function and type of neuron⁷. To detect genetic links between cortical interneurons the study utilized single nucleus RNA-sequencing technology. With RNA sequencing it is possible to identify known markers which can

then be used to classify neuronal subtypes^{8,9}. The objective of the project was to identify, and classify, neurons previously undiscovered. Boldog et.al. employed multiple methodologies, recording the cells electrophysiology, morphology, as well as sequencing single nucleus RNA allowing for thorough analysis of postmortem human cortex.

The team of researchers were able to identify GABAergic neurons of layer 1 via RNA-sequencing, which yielded cells aligning with known genetic markers. From these cells further, analysis and viewing was completed using a light microscope. In viewing the layer 1 GABAergic neurons an undescribed group of interneurons with large, rosehip-shaped axonal boutons forming compact arborizations were revealed. These rosehip cell boutons had an average measured volume approximately four times greater than that of neurogliaform cell boutons. After identifying these rosehip cells, mouse cortex was screened for similar genetic markers yielding no results, suggesting the type of neuron is specific to human cortex. Electrophysiology revealed rosehip cells had a lag that exceeded those of neurogliaform cells when responding to hyperpolarizing current pulses. It was found that rosehip interneurons received local excitatory inputs from layer 2/3 pyramidal cells, as visualized in Figure 1. the axons of layer 1 rosehip cells synapse with pyramidal cells in layer 3, providing inhibitory control via the release of GABA. These findings suggest that rosehip cells might specialize in the control of dendritic signal processing.

The findings of Boldog et. al. highlight how little is truly known about the human brain. Rather than prior propositions suggesting the relative size of neocortex is what differentiates human from animal, the discovery of rosehip interneurons implies there is more to the story. Instead of merely focusing on the gross anatomy, variations on the cellular level should be accounted for as well. Identifying these differences in cells and neural systems is a start to understanding the complex organ that is the brain. However, further research will need to be conducted analyzing what the implications of this inhibitory control means in respect to cognition.

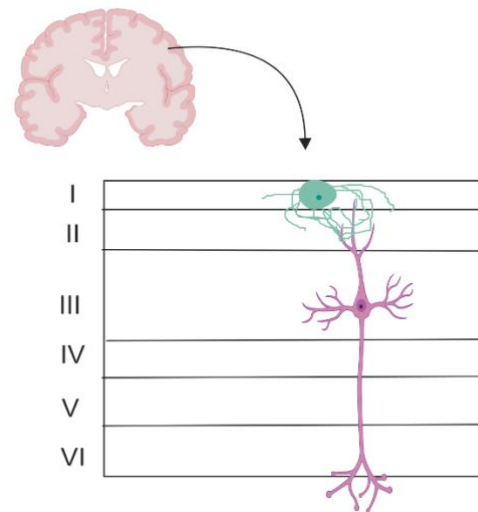


Figure 1. Depiction of newly discovered layer 1 rosehip interneurons (a) projecting to layer 3 pyramidal cells (b). Rosehip neurons synapse with dendrites of pyramidal cells expressing inhibitory control of cortical neurons.

REFERENCES

1. Boldog, Eszter, Trygve E. Bakken, Rebecca D. Hodge, Mark Novotny, Brian D. Aevermann, Judith Baka, Sándor Bordé, Jennie L. Close, Francisco Diez-Fuertes, Song-Lin Ding, Nóra Faragó, Ágnes K. Kocsis, Balázs Kovács, Zoe Maltzer, Jamison M. Mccorrison, Jeremy A. Miller, Gábor Molnár, Gáspár Oláh, Attila Ozsvár, Márton Rózsa, Soraya I. Shehata, Kimberly A. Smith, Susan M. Sunkin, Danny N. Tran, Pratap Venepally, Abby Wall, László G. Puskás, Pál Barzó, Frank J. Steemers, Nicholas J. Schork, Richard H. Scheuermann, Roger S. Lasken, Ed S. Lein, and Gábor Tamás. "Transcriptomic and Morphophysiological Evidence for a Specialized Human Cortical GABAergic Cell Type." *Nature Neuroscience* 21, no. 9 (August 27, 2018): 1185-195. doi:10.1038/s41593-018-0205-2.
2. "The Nobel Prize in Physiology or Medicine 1906." NobelPrize.org. Accessed April 18, 2019. <https://www.nobelprize.org/prizes/medicine/1906/cajal/biographical/>.
3. Preuss, Todd M. "Taking the Measure of Diversity: Comparative Alternatives to the Model-Animal Paradigm in Cortical Neuroscience." *Brain, Behavior and Evolution* 55, no. 6 (2000): 287-99. doi:10.1159/000066664.
4. De Geus, E. J. C., & Boomsma, D. I. (2001). A genetic neuroscience approach to human cognition. *European Psychologist*, 6(4), 241-253. <http://dx.doi.org/10.1027//1016-9040.6.4.241>

5. "Systems Biology in Neuroscience: Bridging Genes to Cognition." *Current Opinion in Neurobiology*. October 04, 2003. Accessed April 18, 2019. <https://www.sciencedirect.com/science/article/abs/pii/S0959438803001375>.
6. Suter, Kelly J., Bret N. Smith, and F. Edward Dudek. "Electrophysiological Recording from Brain Slices." *Methods* 18, no. 2 (1999): 86-90. doi:10.1006/meth.1999.0761.
7. "Petilla Terminology: Nomenclature of Features of GABAergic Interneurons of the Cerebral Cortex." *Nature Reviews Neuroscience* 9, no. 7 (2008): 557-68. doi:10.1038/nrn2402.
8. Krishnaswami, Suguna Rani, Rashel V. Grindberg, Mark Novotny, Pratap Venepally, Benjamin Lacar, Kunal Bhutani, Sara B. Linker, Son Pham, Jennifer A. Erwin, Jeremy A. Miller, Rebecca Hodge, James K. McCarthy, Martijn Kelder, Jamison McCarrison, Brian D. Aebermann, Francisco Diez Fuertes, Richard H. Scheuermann, Jun Lee, Ed S. Lein, Nicholas Schork, Michael J. McConnell, Fred H. Gage, and Roger S. Lasken. "Using Single Nuclei for RNA-seq to Capture the Transcriptome of Postmortem Neurons." *Nature News*. February 18, 2016. Accessed April 18, 2019. <https://www.nature.com/articles/nprot.2016.015>.
9. Lake, B. B., R. Ai, G. E. Kaeser, N. S. Salathia, Y. C. Yung, R. Liu, A. Wildberg, D. Gao, H.-L. Fung, S. Chen, R. Vijayaraghavan, J. Wong, A. Chen, X. Sheng, F. Kaper, R. Shen, M. Ronaghi, J.-B. Fan, W. Wang, J. Chun, and K. Zhang. "Neuronal Subtypes and Diversity Revealed by Single-nucleus RNA Sequencing of the Human Brain." *Science* 352, no. 6293 (2016): 1586-590. doi:10.1126/science.aaf1204.



Hippocampal-prefrontal coherence linked to schizophrenia in mice could point to a potential psychosis treatment

Abnormalities in hippocampal-prefrontal theta coherence played a role in the dysfunctional regulation of selective attention through short-term habituation leading to aberrant salience. Introducing the AMPA receptor subunit GLUA1 in the CA2/CA3 of the hippocampus in Gria1-deficient mice restored hippocampal-prefrontal coherence, linking GRIA1 to schizophrenia¹.

Maribel Garcia-Igueldo

The study conducted by Bygrave and colleagues supported a link between the lack of the gene GRIA1 in the hippocampus of mice brains and elevated levels of hippocampal-prefrontal theta coherence which underlined deficits in short-term habituation and could lead to aberrant salience, a player in schizophrenic patients exhibiting psychosis¹. Variations of the GRIA1 gene encode AMPA receptors that reside in the central nervous system and are important for transmitting excitatory signals between neurons². Previous research has concluded that dysfunctional AMPA receptors are associated with positive symptoms of schizophrenia such as hallucinations and delusions, thereby supporting the link between the schizophrenia and GRIA1². Oxford University has also shown that mice lacking AMPA receptors exhibited deficits in short-term habituation, defined as a decrease in response to a frequently repeated stimulus³. The inability to regulate short-term habituation might be a cause of aberrant salience, defined as attribution of significance to irrelevant stimuli that drives psychotic symptoms⁴. In a study investigating the “aberrant salience” model schizophrenic patients at high risk of psychosis were more likely to attribute salience to irrelevant stimulus, and their bias was positively correlated with their positive symptoms⁴. In addition, fMRI of neuronal responses to relevant and irrelevant stimulus indicated alterations in the hippocampus, as well as in the striatum and subcortical dopamine system⁴. Additional information relevant to the research led by Bygrave worth noting is the term local field potential (LFP) which refers to the electrical field recorded in the extracellular space of brain tissue referenced against a recording made either inside or outside the tissue using electrodes, which capture the signals being transmitted between the neurons around the electrode⁵. The data collected by Bygrave and colleagues pointed to the AMPA receptors in the CA2/CA3 of the hippocampus as a target for treating positive psychotic symptoms in schizophrenic patients¹. Further research must investigate possible road blocks to increasing GRIA1 protein levels in mice before it is considered as a treatment option for human subjects.

Simultaneous LFP recordings of the dorsal hippocampus and medial prefrontal cortex were collected while the control wildtype (WT) and the Gria1-deficient (Gria1^{-/-}) group performed hippocampus-dependent tasks assessing working memory and selective attention¹. In addition, a rescue (GLUA1^{CA2/3}) group was created by reintroducing the AMPA receptor subunit GLUA1 in the CA2/CA3 of the Gria1^{-/-} group¹. The groups performed the T-maze, Y-maze, and a novelty-induced locomotion test¹. The T-maze test measured exploratory behavior in rodents with central

nervous system disorders and is based on their willingness to explore novel environments⁶. Each trial consisted of a sample run in which mice were forced to leave the start arm and visit a goal arm, and a choice run in which mice could choose which arm to visit and were rewarded when they entered the previously unvisited arm. Each group was trained for seven days with 10 trials per day¹. The Y-maze test measured rodents' willingness to explore novel environments⁷. Each test was conducted once per rodent, in the sample phase the mice could explore the start arm and one goal arm and in the test phase the mice could explore all three arms freely¹. The novelty-induced locomotion test consisted of each mouse being able to freely explore plastic cages with saw dust for five minutes¹. The data was processed in Matlab using the open-source Chronux Tool-box and analyzed with the statistical methods ANOVA or Tukey-HSD post hoc tests¹.

The results of the data collected by Bygrave and colleagues concluded that hippocampal-prefrontal coherence mediated working memory and selective attention at certain frequency ranges¹. The T-maze test concluded that that the WT and GLUA1^{CA2/3} performed significantly better than the Gria1^{-/-} group which performed near chance levels¹. Hippocampal-prefrontal beta/gamma coherence was significantly greater for the WT and GLUA1^{CA2/3} group compared to the Gria1^{-/-} group¹. Hyperactivity of the Gria1^{-/-} group was observed compared to the WT and GLUA1^{CA2/3/4}. Constantly elevated levels of theta oscillations and hippocampal-prefrontal theta coherence in the Gria1^{-/-} group compared to the WT and GLUA1^{CA2/3} groups were recorded in novel environments¹. The Y-maze test concluded that the WT and GLUA1^{CA2/3} groups preferred the novel arm and the Gria1^{-/-} group performed at chance levels¹. Hippocampal-prefrontal theta coherence in the Gria1^{-/-} group remained elevated after the hippocampal-prefrontal coherence in the WT and GLUA1^{CA2/3} groups decreased in novel environments¹. In addition, running speeds were not dependent on neither familiar or novel environments and did not predict coherence¹.

The results of the study imply a link between schizophrenia and lack of AMPA receptors in the hippocampus. The T-maze test measured the spatial working memory of the three mice groups. The control group and Gria1-deficient mice reintroduced with the AMPA receptor subunit GLUA1 in the CA2/CA3 of the hippocampus learned to visit the previously unvisited arm of the maze to obtain a food reward. The Gria1-deficient mice did not exhibit spatial working memory and visited each arm by chance during the test phase of each trial. The GLUA1^{CA2/3} mice experienced significantly greater hippocampal-prefrontal beta/gamma coherence compared to the Gria1-deficient mice which indicated that spatial working memory was dependent on AMPA receptors in the CA2/CA3 of the hippocampus. The consistently elevated levels of theta oscillations in the dorsal hippocampus specific to the Gria1-deficient mice indicated a deficit in short-term habituation of attention which is common in patients with positive schizophrenic symptoms. The Spatial Novelty Preference (SPN) Y-maze test measured short-term habituation dependent on the hippocampus and the results indicated impaired preference between familiar and novel environments in the Gria1^{-/-} mice compared to the WT and GLUA1^{CA2/3} mice. In conclusion, the connection being regulated by the hippocampus was not controlled in the Gria1^{-/-} mice, but control was restored when the AMPA receptor subunit GLUA1 was introduced in the CA2/CA3 of the hippocampus. The inability of the Gria1-deficient mice to reduce their response to stimulus they have been previously exposed to could be the result of dysfunctional regulation of selective attention through short-term habituation which may lead to aberrant salience¹. As mentioned, aberrant salience is observed in schizophrenic patients⁸. It is important to study the mechanisms behind schizophrenia to develop treatments that can improve the quality of life for 3.2 million Americans and 1% of the population worldwide⁹. Schizophrenia is characterized by positive symptoms such as hallucinations and delusions, as well as negative symptoms such as lack of pleasure, trouble with speech, cognitive and thinking problems¹⁰. If the driving force of psychosis could potentially be treated by targeting AMPA receptors in the CA2/CA3 of the hippocampus then future research should build on the work of Bygrave and colleagues.

REFERENCES

1. Bygrave, A. M., Jahans-Price, T., Wolff, A. R., Sprengel, R., Kullmann, D. M., Bannerman, D. M., & Kätzel, D. (2019). Hippocampal–prefrontal coherence mediates working memory and selective attention at distinct frequency bands and provides a causal link between schizophrenia and its risk gene GRIA1. *Translational Psychiatry*, 9(1). doi:10.1038/s41398-019-0471-0
2. Kang, W. S., Park, J. K., Kim, S. K., Park, H. J., Lee, S. M., Song, J. Y., . . . Kim, J. W. (2012). Genetic variants of GRIA1 are associated with susceptibility to schizophrenia in Korean population. *Molecular Biology Reports*, 39(12), 10697-10703. doi:10.1007/s11033-012-1960-x
3. Sanderson, D. J., Lee, A., Sprengel, R., Seeburg, P. H., Harrison, P. J., & Bannerman, D. M. (2017). Altered balance of excitatory and inhibitory learning in a genetically modified mouse model of glutamatergic dysfunction relevant to schizophrenia. *Scientific Reports*, 7(1). doi:10.1038/s41598-017-01925-8
4. Roiser, J. P., Howes, O. D., Chaddock, C. A., Joyce, E. M., & Mcguire, P. (2012). Neural and Behavioral Correlates of Aberrant Salience in Individuals at Risk for Psychosis. *Schizophrenia Bulletin*, 39(6), 1328-1336. doi:10.1093/schbul/sbs147
5. Sharott, A. (2014). Local Field Potential, Methods of Recording. *Encyclopedia of Computational Neuroscience*, 1-3. doi:10.1007/978-1-4614-7320-6_723-1
6. Behavioral and Functional Neuroscience Laboratory. (n.d.). T Maze Spontaneous Alternation Test. Retrieved from <https://med.stanford.edu/sbfnl/services/bm/lm/t-maze.html>
7. Behavioral and Functional Neuroscience Laboratory. (n.d.). Y Maze Spontaneous Alternation Test. Retrieved from <https://med.stanford.edu/sbfnl/services/bm/lm/y-maze.html>
8. Barkus, C., Sanderson, D. J., Rawlins, J. N., Walton, M. E., Harrison, P. J., & Bannerman, D. M. (2014). What causes aberrant salience in schizophrenia? A role for impaired short-term habituation and the GRIA1 (GluA1) AMPA receptor subunit. *Molecular Psychiatry*, 19(10), 1060-1070. doi:10.1038/mp.2014.91
9. Schizophrenia Symptoms, Patterns And Statistics And Patterns. (n.d.). Retrieved from <https://www.mentalhelp.net/articles/schizophrenia-symptoms-patterns-and-statistics-and-patterns/>
10. Schizophrenia Symptoms: Positive and Negative Symptoms of Schizophrenia. (n.d.). Retrieved from <https://www.webmd.com/schizophrenia/schizophrenia-symptoms#1>



Cutting out the middle man

Using transdifferentiation as a means of regenerating specific cell types.

Ameen Baradar

Stem cell research has been the cornerstone of scientific endeavors in a decade of history. Recently, scientists may have discovered a way to genuinely reprogram cells to serve an uncanny regenerative purpose. The purpose of this research was to find an alternative method of reprogramming cells so that any and all inconveniences during future procedures can be avoided. In a study recently published in the Proceedings of the National Academy of Sciences of the United States of America, Tanabe and colleagues (2018) explore the future possibility of reprogramming specific cells to change from one cell type to another, all whilst avoiding the unnecessary steps in between¹. The regenerative applications that humans could harness go as far as possible treatments for several illnesses like liver disease or brain related diseases². But first, we must first take a look at the history of transdifferentiation to understand its progression throughout the years.

One of the earliest applications of transdifferentiation was an experiment testing the role of laminin (a protein), and basement membrane that separates tissue during differentiation of endothelial cells to produce capillary like structures³. In 2004, scientists explored tissue plasticity in mice to see how transdifferentiation can be used to reverse engineer epithelial cells into adipocytes in the mammary gland and vice versa⁴. After 2010, there was a significant growth in differentiation research in humans and animals. Recently, scientists have been able to experiment with tumor cells and explore future therapeutic remedies that involve transdifferentiation of the tumor cells⁵.

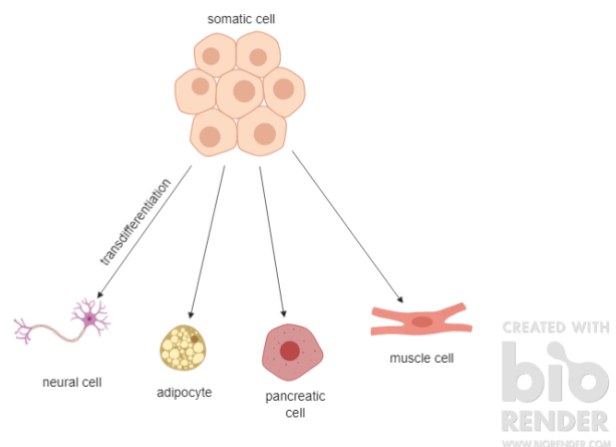


Figure 1. Potentials for transdifferentiation.

Tanabe and colleagues (2018) lab test the limits of transdifferentiation. Fibroblasts (a common cell type found in connective tissue) were obtained with a minimal invasive biopsy. One of the main tools used for inducing transdifferentiation to occur is CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). CRISPR has the ability to silence specific genes and prevent them from expressing them ever again⁶. Due to their scarce supply and the amount of time needed to regenerate, neuronal cells are a huge target for transdifferentiation. Tanabe, et al., were able to execute an experiment in which peripheral blood cells could potentially be transdifferentiated into brain cells. They were able to accomplish this through centrifugation of

adult blood and introduced four DNA transcription factors by electrically stimulating the blood cells. These DNA transcription factors were transfected (injected into a eukaryotic cell), in order for transdifferentiation to occur⁷.

The remarkable discovery that had been made in the Tanabe, et al., lab was that just from 1 mL of peripheral blood, greater than 50,000 brain cells were generated within one step. The brain cells that were generated from this experiment displayed great efficacy in firing action potentials and formation of fully-functional synapses. Other data that has been gathered was that these transdifferentiated brain cells derived from blood cells only obtain excitatory properties¹.

As a student of neuroscience, I find this type of research to be incredibly fascinating. The possibility to change the function of certain cells to do whatever we want seems like a dream. This type of research opens up so many new avenues in developing a treatment for terminal cases of cancer, and/or neurodegenerative diseases such as Alzheimer's, multiple sclerosis, and Parkinson's disease. There have been experiments that simply leave me dumbfounded such as using transdifferentiation to turn human dermal cells into viable skeletal muscle tissue⁸, or transdifferentiating specific cells to attain an immune response to harmful foreign bacteria⁹. Regardless of the infinite possibilities in the realm of transdifferentiation, I think it's important to remember that this type of research is still in its premature phase, and that the likelihood of these procedures being conducted on the public anytime soon is very little. I believe that though this type of research is very interesting, there may also be risks involved. Recently in an article by Cohen (2018)¹⁰, Dr. He Jiankui created the very first gene-manipulated babies (a set of twins) by using CRISPR to wipe out the genomic receptor for HIV in an attempt to make the fetuses resistant to the virus during their lifetime. This was countered with extreme backlash by the scientific community, and rightfully so in my opinion. There is still a lot we do not know about the future of transdifferentiation. There will have to be experiments that go throughout generations of offspring before we can even begin thinking about testing on humans.

If you took all of the knowledge that could possibly be collected about the human brain, and limited it so the length of one mile, it is in my humble opinion that we have achieved no further than 10 feet in that mile as scientists. I am very excited to learn more about this topic as it develops in the scientific community, and what treatments arise as a result of it.

REFERENCES

1. Tanabe, K., Ang, C. E., Chanda, S., Olmos, V. H., Haag, D., Levinson, D. F., ... Wernig, M. (2018). Transdifferentiation of human adult peripheral blood T cells into neurons. *Proceedings of the National Academy of Sciences of the United States of America*, 115(25), 6470–6475. doi:10.1073/pnas.1720273115
2. Grath, A., & Dai, G. (2019). Direct cell reprogramming for tissue engineering and regenerative medicine. *Journal of biological engineering*, 13, 14. doi:10.1186/s13036-019-0144-9
3. Kubota, Y., Kleinman, H. K., Martin, G. R., & Lawley, T. J. (1988). Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. *The Journal of cell biology*, 107(4), 1589–1598.
4. Morroni, M., Giordano, A., Zingaretti, M. C., Boiani, R., De Matteis, R., Kahn, B. B., ... Cinti, S. (2004). Reversible transdifferentiation of secretory epithelial cells into adipocytes in the mammary gland. *Proceedings of the National Academy of Sciences of the United States of America*, 101(48), 16801–16806. doi:10.1073/pnas.0407647101
5. Shekhani, M. T., Jayanthi, A. S., Maddodi, N., & Setaluri, V. (2013). Cancer stem cells and tumor transdifferentiation: implications for novel therapeutic strategies. *American journal of stem cells*, 2(1), 52–61.
6. Wang, C., Liu, W., Nie, Y., Qaher, M., Horton, H. E., Yue, F., Kuang, S. (2017). Loss of MyoD Promotes Fate Transdifferentiation of Myoblasts Into Brown Adipocytes. *EBioMedicine*, 16, 212–223. doi:10.1016/j.ebiom.2017.01.015
7. Eguchi, G., & Kodama, R. (1993). Transdifferentiation. *Current opinion in cell biology*, 5(6), 1023-1028.

8. Boularaoui, S. M., Abdel-Raouf, K. M., Alwahab, N. S. A., Kondash, M. E., Truskey, G. A., Teo, J. C. M., & Christoforou, N. (2018). Efficient transdifferentiation of human dermal fibroblasts into skeletal muscle. *Journal of tissue engineering and regenerative medicine*, *12*(2), e918-e936.
9. Sayed, N., Wong, W. T., Ospino, F., Meng, S., Lee, J., Jha, A., ... Cooke, J. P. (2014). Transdifferentiation of human fibroblasts to endothelial cells: role of innate immunity. *Circulation*, *131*(3), 300–309. doi:10.1161/CIRCULATIONAHA.113.007394
10. Cohen, J. (2018). 'I feel an obligation to be balanced.' Noted biologist comes to defense of gene editing babies. *Science*, *n.a*(n.a), n.a. 0.1126/science.aaw2120



TRPA1: A Ghost ship in the membrane

Transient Receptor Potential Ankyrin 1 is a calcium-permeable channel that plays a major role in pain perception, overactivity of these cation channels can induce hyperalgesia. A number of compounds belonging to various chemical families that were involved in animal/human studies have been demonstrated to act as antagonists to this TRP class of channel proteins and attenuate symptoms of hyperalgesia.

Micah Black

The old adage “nociception is pain perception, anti-nociception is the prevention of pain perception” has been a primary focus for researchers in the medical fields as well as the peripheral fields of chemical research. Nociceptive nervous tissue expresses myriad membrane-bound proteins, foremost of which are a specialized class of channel proteins referred to as transient receptor potential (TRP) proteins. They are expressed on primary afferent neurons¹, astrocytes², oligodendrocytes³ and Schwann cells⁴. When activated, TRP proteins electrically communicate information to the nociceptor regarding noxious or potentially harmful stimuli and can initiate flexion reflexes in the organism. Hyperalgesia (hypersensitivity to pain) has been attributed to a variety of genetic and environmental factors. Clinically, sleep deprivation has been attributed to hyperalgesia⁵ by generating chemicals that amplify pain signals through TRP pathways^{6,7}. Chemical imbalances due to other factors such as chemotherapy can also induce symptoms of hyperalgesia, initiating a cascade of activity on TRP pathways and effectively causing the organism to experience heightened levels of pain that is debilitating.

A study published by the Journal of Pharmaceuticals describes research involving derivatives of xanthine, oxime, and benzamide⁸ that have been demonstrated to prevent the TRP from activating (TRP antagonist). The oxime derivatives were not effective when taken orally by humans, but were clinically effective in rat models⁸. Conversely, a number of trichloro(sulfanyl)ethyl benzamide derivatives were demonstrated to exclusively effect human models as opposed to rat models⁹. TRP Ankyrin 1 (TRPA1) is implicated to participate in the model TRP nociception pathways. It is expressed in the above-listed cells and chemical mechanisms necessary for nociceptive signaling have been associated with TRPA1. In the case of sleep deprivation, NMDA-nitric oxide cascades as well as D-amino acid oxidase (DAAO) promote the activation of astrocytes in the dorsal horn of the spinal cord due to lack of sleep by generating metabolites that amplify nociceptive signals through TRPA1¹⁰ as illustrated in figures 1.1 and 1.2.

In clinical studies, animal test subjects were injected with the TRPA1 antagonists in the spinal cord around the dorsal horn. Spinal administration of these compounds resulted in attenuated hyperalgesia, supporting the hypothesis that TRPA1 is expressed in the spinal cord as well as the protein’s participation in the transduction of pain signals¹¹. Animal tests are useful for modeling conditions that result in pain hypersensitivity due to a variety of factors; ability to control their sleep patterns, availability of chemotherapy drugs implemented with symptoms of neuropathy/allodynia, and genetic modifications that make it possible to alter desired protein expression. Modulation of

TRPA1 expression in the nervous system of rat models has confirmed its presence in the viscera. Studying the TRP class of membrane-bound proteins provides a more complete picture of somatosensory and nociceptive pathways that are mediated by ligand-binding and metabolism of endogenous molecules induced by environmental stimuli as well as genetic factors.

Medical researchers strive to mitigate the side

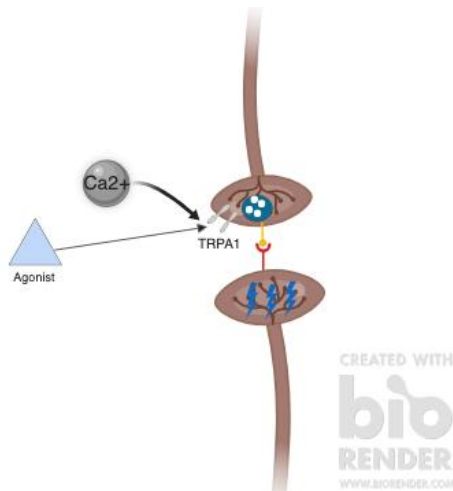


Figure 1.1. Action potential firing due to activation of TRPA1

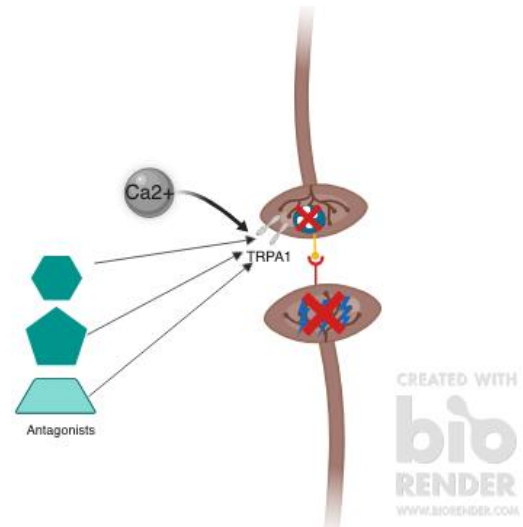


Figure 1.2. Action potential inhibited due to inhibition of TRPA1

effects of treatment for various health problems. Diabetic nerve pain, chemotherapy induced neuropathy/allodynia, sleep induced hyperalgesia, post-operative pain and noxious stimuli are examples of factors that interact with the transient receptor potential proteins and produce symptoms of acute or chronic pain. Lack of sensitivity to agonist molecules can cause the TRP proteins to not function properly, creating an alternative issue of the absence of nociceptive stimulation. Reviewing the existing literature regarding these topics will provide yet another component to a more complete understanding of the mammalian nervous system, effectively resolving a portion of the innate difficulties with regard to medical care and treatment.

REFERENCES

1. Story, G.M.; Peier, A.M.; Reeve, A.J.; Eid, S.R.; Mosbacher, J.; Hricik, T.R.; Earley, T.J.; Hergarden, A.C.; Andersson, D.A.; Hwang, S.W.; et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* 2003, *112*, 819–829.
2. Shigetomi, E.; Tong, X.; Kwan, K.Y.; Corey, D.P.; Khakh, B.S. TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. *Nat. Neurosci.* 2011, *15*, 70–80.
3. Hamilton, N.B.; Kolodziejczyk, K.; Kougioumtzidou, E.; Attwell, D. Proton-gated Ca²⁺-permeable TRP channels damage myelin in conditions mimicking ischaemia. *Nature* 2016, *529*, 523–527.
4. De Logu, F.; Nassini, R.; Materazzi, S.; Carvalho Gonçalves, M.; Nosi, D.; Rossi Degl'Innocenti, D.; Marone, I.M.; Ferreira, J.; Li Puma, S.; Benemei, S.; et al. Schwann cell TRPA1 mediates neuroinflammation that sustains macrophage-dependent neuropathic pain in mice. *Nat. Commun.* 2017, *8*, 1887.
5. Lautenbacher, S.; Kundermann, B.; Krieg, J.C. Sleep deprivation and pain perception. *Sleep Med. Rev.* 2006, *10*, 357–369.
6. Wei, H.; Koivisto, A.; Saarnilehto, M.; Chapman, H.; Kuokkanen, K.; Hao, B.; Huang, J.L.; Wang, Y.X.; Pertovaara, A. Spinal transient receptor potential ankyrin 1 channel contributes to central pain hypersensitivity in various pathological conditions in the rat. *Pain* 2011, *152*, 582–591.
7. Wiertelak, E.P.; Furness, L.E.; Horan, R.; Martinez, J.; Maier, S.F.; Watkins, L.R. Subcutaneous formalin produces centrifugal hyperalgesia at a non-injected site via the NMDA-nitric oxide cascade. *Brain Res.* 1994, *649*, 19–26.

8. McNamara, C.R.; MandelBrehm, J.; Bautista, D.M.; Siemens, J.; Deranian, K.L.; Zhao, M.; Hayward, N.J.; Chong, J.A.; Julius, D.; Moran, M.M.; et al. TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. USA* 2007, *104*, 13525–13530.
9. Klionsky, L.; Tamir, R.; Gao, B.; Wang, W.; Immke, D.C.; Nishimura, N.; Gavva, N.R. Species-specific pharmacology of Trichloro(sulfanyl)ethyl benzamides as transient receptor potential ankyrin 1 (TRPA1) antagonists. *Mol. Pain* 2007, *3*, 39.
10. Zhang, G.H.; Lv, M.M.; Wang, S.; Chen, L.; Qian, N.S.; Tang, Y.; Zhang, X.D.; Ren, P.C.; Gao, C.J.; Sun, X.D.; et al. Spinal astrocytic activation is involved in a virally-Induced rat model of neuropathic pain. *PLoS ONE* 2011, *6*, e23059.
11. Chen, J.; Joshi, S.K.; DiDomenico, S.; Perner, R.J.; Mikusa, J.P.; Gauvin, D.M.; Segreti, J.A.; Han, P.; Zhang, X.F.; Noforatos, W. et al. Selective Blockade of TRPA1 Channel Attenuates Pathophysiological Pain Without Altering Noxious Cold Sensation or Body Temperature Regulation. *Pain* 2011, *152*, 1165-1172.



Dopamine receptors aren't always dope

The presence of specific allelic variants of the polymorous dopamine receptor D₄ (DRD₄) is shown to be predictors of intensified neurodegeneration in frontotemporal dementia-spectrum patients.

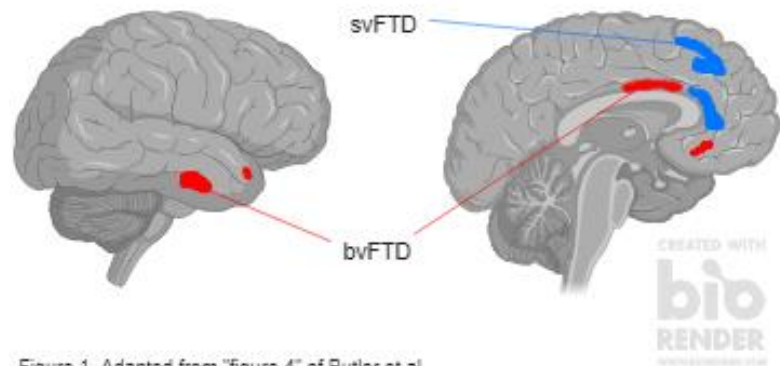
Shelby Brock

Frontotemporal dementia (FTD) and other neurodegenerative diseases have devastating effects on loved ones and their families. With 50 million global sufferers of dementia¹, the understanding of this and similar neurodegenerative diseases is vital to innovating patient care. However, not much is known about how genotypic to phenotypic variables link in these patients. In a paper published in *NeuroImage: Clinical*, Butler et al. found two allelic variants of the dopamine receptor D₄ (DRD₄) associated with increased atrophy in FTD patients². This clinically tested link between genotype and atrophy patterns is significant to further the method of FTD diagnosis and potentially develop drugs to target this receptor's pathways.

Researchers under the grant of the National Institute of Aging (NIA) found reason to hypothesize the known functional polymorphic DRD₄ in relation to brain decay based on previously-published studies by Gennatas et al., which cites the polymorphic Val₁₅₈Met allele variants within the COMT gene, the inhibition of which can alter working memory and decision making^{3,4}. This study also resembles a 2018 study on the polymorphic effect of DISC1 on dopamine systems as indicators of risk of psychosis in schizophrenia patients³. The researchers in this study hypothesized that patients with non-wild type variations of the DRD₄ receptors would exhibit atrophy in regions where the receptor was most prevalent, which was in frontotemporal regions. DRD₄ is a G-coupled protein which, by inhibition of adenylyl cyclase⁵ affects the second-messenger system of dopamine, a known deficit which is present in FTD patients.

Three groups composed of 337 total participants (healthy controls, Alzheimer's (AD) patients, and various FTD patients) underwent genomic testing, structural MRI scans, and cognitive testing. The groups were compared using linear modeling of the gray matter maps of healthy controls vs. AD, controls vs. FTD, and AD vs. FTD groups. Genomic testing identified a wild type allelic variant and two variants that dampened the effect of D₄, which were hypothesized to exacerbate frontal atrophy. The MRI scans used voxel-based morphometry (VBM) to analyze the intensity of gray matter in brains. Cognitive tests included the CDR sum-of-boxes test, NPI tests, and MMSEs to measure the impairment of function in individuals. Analysis between behavior variant FTD (bvFTD) and semantic variant primary progressive aphasia (svPPA) FTD patients was done using voxel-based morphometry (VBM), which statistically measures and illustrates gray matter structures. All participants were age-matched, had relatively even sex ratios, and education levels, which prevents the interaction of external factors.

After analysis of collected data from participants, the results were not significant for AD patients, but were within FTD patients, with controls and regular symptom-specific atrophy patterns used as comparison. Fifty patients with bvFTD and twenty-six with svPPA and non-wild type variants showed increased gray matter atrophy within the right anterior-inferior insula, dorsal and ventral anterior cingulate cortex, ventromedial prefrontal cortex, and orbitofrontal cortex. The results between the FTD-spectrum patients are illustrated in Figure 1 with a general mapping of the (DRD4) regions where atrophy patterns were amplified beyond syndrome-specific patterns. The hypothesis of DRD4 variant involvement in neurodegeneration was therefore proven correct, but further investigation with more FTD patients was requested by researchers to expand on this correlate.



This study demonstrated the ability to reconcile the link between genetics and behavior as well as addition to scientific literature on the involvement of DRD4 polymorphism on neurodegenerative disease. The identification of DRD4 variants that affect is one step forward in the advancement of patient treatment by emphasizing the need for genomic testing to better perform individual-specific care. The confirmation of receptor involvement in FTD-spectrum atrophy also secondarily aids in the confirmation of FTD (versus a common misdiagnosis of AD). This goes beyond treating an illness; it's about treating a *person*. With these findings, more specific drugs can potentially be created to target DRD4 variants known to aggravate atrophy as well as improve current preventative therapy methods.

REFERENCES

1. "Dementia." (12 Dec 2017) *World Health Organization*, World Health Organization, www.who.int/en/news-room/fact-sheets/detail/dementia
2. Butler, P., Chiong, W., Perry, D., Miller, Z., Gennatas, E., Brown, J., Seeley, W. (2019). Dopamine receptor D4 (DRD4) polymorphisms with reduced functional potency intensify atrophy in syndrome-specific sites of frontotemporal dementia. *NeuroImage: Clinical*, 23, 101822. doi: 10.1016/j.nicl.2019.101822
3. Farrell, S. M., Tunbridge, E. M., Braeutigam, S., & Harrison, P. J. (2012). COMT Val(158)Met genotype determines the direction of cognitive effects produced by catechol-O-methyltransferase inhibition. *Biological psychiatry*, 71(6), 538–544. doi:10.1016/j.biopsych.2011.12.023
4. Gennatas, E. D., Cholfin, J. A., Zhou, J., Crawford, R. K., Sasaki, D. A., Karydas, A., . . . Seeley, W. W. (2012). COMT Val158Met genotype influences neurodegeneration within dopamine-innervated brain structures. *Neurology*, 78(21), 1663-1669. doi: 10.1212/wnl.0b013e3182574fa1
5. DRD4 dopamine receptor D4 [Homo sapiens (human)] - Gene - NCBI. (2019, April 21). Retrieved from <https://www.ncbi.nlm.nih.gov/gene/1815>



Hope for schizophrenia patients after discovery of brain cells associated with this mental chronic disorder

There are limited studies underlying the basis of schizophrenia that can be used to model experiments to expand the genomic basis of schizophrenia and help alleviate its symptoms more effectively than the treatments available today. That is exactly what a recent study has discovered. This study incorporated knowledge of the brain from single-cell RNA sequencing and analyzed whether specific loci associated with schizophrenia were mapped on specific cells in the brain.

Petru Buracioc

Schizophrenia is one of diverse mental disorders affecting millions of people with minimal scientific and genetic basis which impedes experimental modeling that can successfully alleviate its symptoms with minimal side effects. Finding the genetic basis of this neurological disorder is important in understanding more about schizophrenia and other similar neurological disorders. In a paper recently published in *Nature Genetics*, Nathan Skene and other neuroscientists investigate the brain cell types that are involved in schizophrenia. The primary finding of this article is the specific cell types in the brain associated with schizophrenia and how each cell correlates with this psychiatric disease using information about the brain from single-cell RNA sequencing.

Schizophrenia is a psychotic illness described by delusions, hallucinations, and bizarre behavior. Schizophrenia symptoms could be negative and positive. Negative symptoms involve the reduced expression of emotions, memory impairment, difficulty, and difficulty initiating goal-directed behavior. Positive symptoms include delusion, hallucinations, and disorganized behavior¹. One of the neuromodulatory systems in the brain that is responsible for symptoms in schizophrenia is dopamine. Dopaminergic pathway especially the one described in figure 1 is found to be abnormal as well as the prefrontal cortex where the principal cells are pyramidal cells among schizophrenic patients².

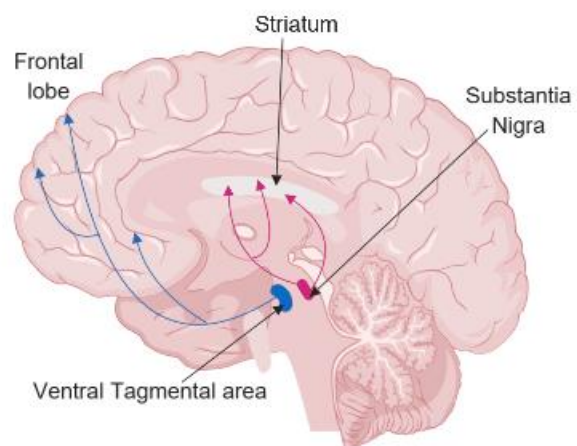


Figure 1: A sagittal section of the brain shows the dopamine modulatory system in the brain. The dopaminergic system arising from the ventral tegmental area and projecting to the neocortex (which contains pyramidal cells) was determined to cause schizophrenia. The second dopaminergic system originates from the substantia nigra and points to the striatum where the MSNs release GABAergic neurotransmitters.

The data used in this study was the human-mouse homolog list. The cell-type specificity or the genes correlating to certain cells was calculated using a metric system. MAGNA, a program used for gene set analysis was used to analyze the association for gene-level schizophrenia statistics to the cell-type-specific expression. Whether the brain cell types were associated with schizophrenia, CLOZUK which is a genome-wide association finding was used. Cell-type specificity of gene expression was determined from the scRNA-seq data from Karolinska Institutet. The brain regions identified from the genome associations with schizophrenia in this study were determined to be neocortex, striatum, hippocampus, hypothalamus, and midbrain³.

This study found clear connections between human genomic findings for schizophrenia in multiple studies and specific brain cell types using single-cell RNA sequencing. Four cells out of 24 main brain cell types were found to have clear connections to schizophrenia. These cells are MSNs, pyramidal cells in hippocampal CA1, pyramidal cells in the somatosensory cortex and cortical interneurons which were determined to play significant roles in schizophrenia³.

This study is significant because it connects the genome findings for schizophrenia and specific cell types, opening the door for a deeper understanding of the cells associated with this disease. With the results of this study, in vivo and in vitro experiments could be done to study the genes that produce the symptoms of Schizophrenia. Since Schizophrenia is associated with memory dysfunction and decreased cognitive function, therefore, parts of the brain such as the neocortex, hippocampus, and the hypothalamus are involved in schizophrenia as concluded with the cells identified in this study. Additionally, taking into consideration the dopamine hypothesis associated with schizophrenia that increased dopamine pathways increase symptoms of this disorder could lead to a better understanding of the basis of schizophrenia and how what can be done to help alleviate the symptoms of schizophrenia.

REFERENCES

1. Bear, M. F., Connors, B. W., Paradiso, M. A. (2016). Neuroscience: *Exploring the Brain*, Fourth Edition Waddington, J. L. (1993). Schizophrenia: developmental neuroscience and pathobiology. *The Lancet*, 341(8844), 531-532.
2. Meyer-Lindenberg A., *Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia*. Nature Neuroscience volume 5, pages267–271. <https://doi.org/10.1038/nn804>
3. Skene N.G., *Genetic identification of brain cell types underlying schizophrenia*. Nature Genetics volume 50, pages825–833 (2018), <https://doi.org/10.1038/s41588-018-0129-5>
4. Ranganath, C., Minzenberg, M. J., & Ragland, J. D. (2008). The cognitive neuroscience of memory function and dysfunction in schizophrenia. *Biological psychiatry*, 64(1), 18-25.
5. Paz, R. D., Tardito, S., Atzori, M., & Tseng, K. Y. (2008). Glutamatergic dysfunction in schizophrenia: from basic neuroscience to clinical psychopharmacology. *European neuropsychopharmacology*, 18(11), 773-786.
6. Seeman, P. (1987). Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse*, 1(2), 133-152.
7. Jentsch, J. D., & Roth, R. H. (1999). *The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia*. *Neuropsychopharmacology*, 20(3), 201-225.



Eat your stress away!

Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice.

The Microbiota-Gut-Brain Axis is a mysterious wonderland full of Nobel prize scientific discoveries to be made. Everything your body builds is dependent on what you ingest, from your energy supplies to the levels of hormones your brain has available to communicate with your body. Eating seems to have a greater undisclosed impact on our mood, motivation and accomplishments that we are just beginning to understand.

Vicente Chavez

The gut-brain axis is the term used for communication that occurs between our gut and brain. The microbiota-gut-brain axis specifically refers to the complex environment of microorganisms that inhabit our stomachs and its ability to communicate with our brain via the gut signaling pathways. The gut microbiota composition typically referred to as the gut microbiome (bacterial environment) has been implicated in a variety of central nervous system disorders, cancer, inflammation and obesity^{1,2,3}. A beneficial microbiome includes great diversity of species to effectively balance each other's many inflammatory and regulatory signaling chemicals⁴. The number of bacterial cells inside a "standard person's" gut is upwards of 3.8×10^{13} ^(5,6). This number is unfathomable for many and is a large reason the gut chemistry is so complex. A variety of substances can influence the microbiota including probiotics, prebiotics and antibiotics⁷. Probiotics are live beneficial bacteria that naturally occur in all-time favorite foods such as yogurt, miso soup and kimchi. Prebiotics are plant fibers found in the skins of fruit, vegetable roots and beans. Prebiotics are typically indigestible facilitating growth of our good anti-inflammatory bacteria^{8,9}. Antibiotic administration has proven to be a difficult task because it indiscriminately effects all bacteria¹⁰. The microbiome involves and impacts neural, humoral, immune and metabolic pathways¹¹. Modulating the microflora offers potentially new avenues to develop novel therapeutics for complex stress-related Central Nervous System (CNS) disorders where there is huge medical need present¹².

A recent study conducted over the course of ten weeks by Burokas et al., set out to investigate the impacts of prebiotics such as Fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) (both soluble fibers) as microbiome manipulators. These prebiotics are found in common food items, FOSs are in red onions, bananas and nutrition bars. GOSs can be found in cashews, hummus dip and soy milk^{13,14}. The study's aim was to understand their potential roles as anxiolytics (anxiety-reducer) and as antidepressants¹⁵. Mice were given FOS, GOS or a combination of both for three weeks, with the goals of nurturing a beneficial microbiome. Anxiety, depression, cognition, stress response and social behavior were measurements of this study. Tests included marble burying, elevated plus maze, resident-intruder test and novel object recognition test^{16,17,18}. Many of the tests identified baseline anxiety, depression and stress levels by recording levels of certain chemicals such as corticosterone, IL-6 and TNF-Alpha implicated in the immune system and environmental stress¹⁹. Results indicated that chronic prebiotic FOS+GOS treatment exhibited both antidepressant and anxiolytic effects in response to stress.

FOS+GOS treatment reduced stress-induced corticosterone release and effected hippocampus and hypothalamus genetic expression indicating long-term brain changes (Figure 1,2 & 3).

These findings support our growing understanding of the impacts of what we choose to eat on our mental health and most notably physical health. It is evident that our societies fundamental lifestyle and diet changes occurred too quickly for our body to effectively adapt. Our bodies inability to adequately evolve have led to a rise in health problems coined “diseases of civilization”²⁰. The convenience of fast food and a high-sugar/sodium Western Diet has been implicated in a wide range of mental, physical and community-based health problems such as depression, obesity and lack of medical resources^{21,22,23}. There is a huge call to action to pay greater attention to one’s diet. There is no perfect diet for any particular individual, but more focus on vegetables, fruits and nuts is recommended as our body has evolved around these food sources²⁴. More research is needed to positively influence the direction of policy surrounding food, medicine and most importantly people’s decision making.

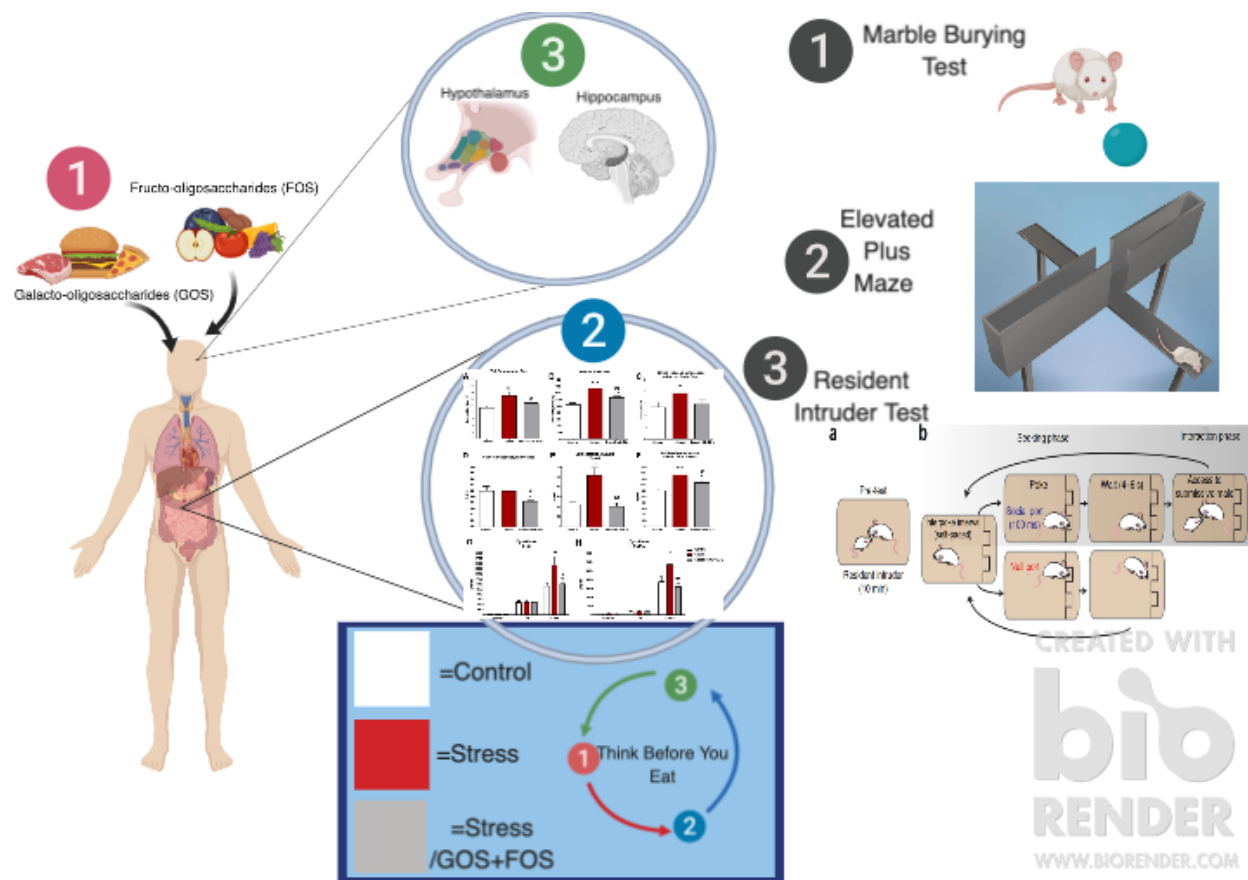


Figure 1. Representative outline of the study and tests employed to measure behavioral responses. Red 1 indicates the two compounds investigated in this study. Blue 2 represents the study results seen more clearly in Figure 2 below. Green 3 represents the long-term changes that occur in the brain in response to the types of food ingested. The take home message is Think Before You Eat. Black/White 1,2 and 3 are examples of the many behavioral tests included in this study (1,2 and 3; Marble Burying Test, Elevated Plus Maze and Resident Intruder Test respectively).

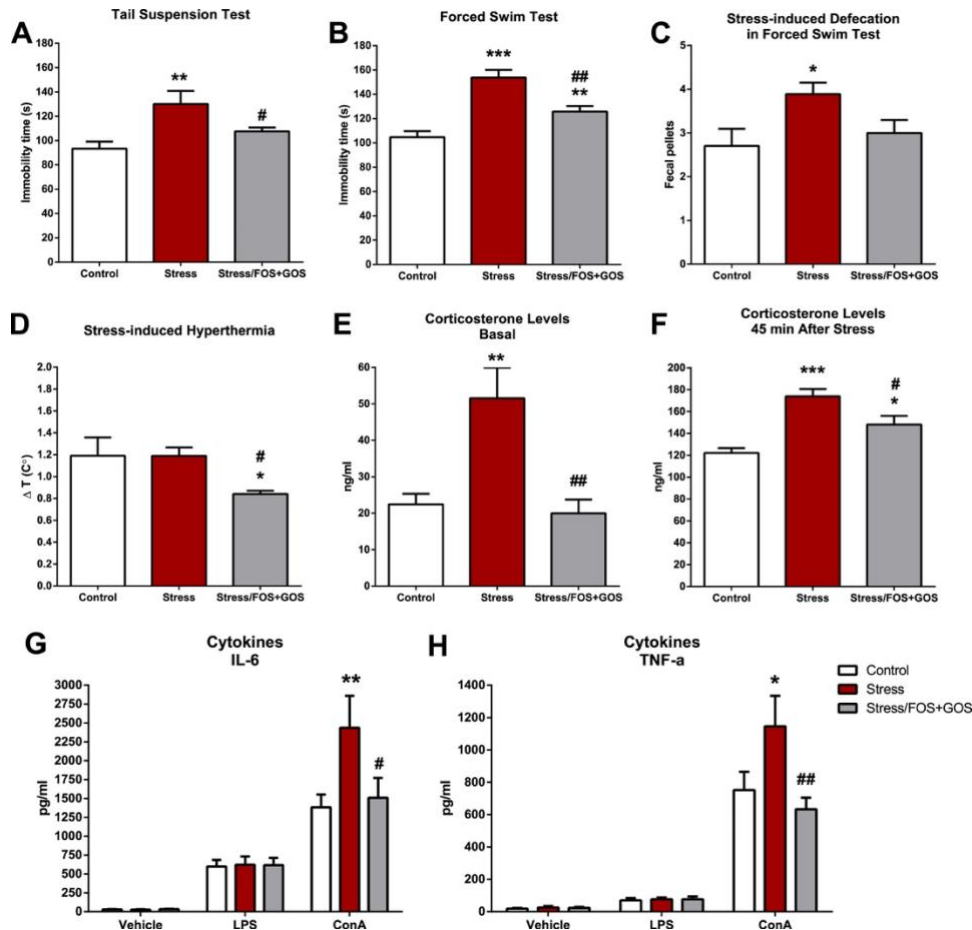


Figure 2. Closer look of the figure used in Figure 1. Test suspension test, forced swim test, stress-induced defecation in forced swim test, stress-induced hyperthermia, corticosterone levels basal, corticosterone levels 45 min after stress, cytokines IL-6, and cytokines TNF-alpha results included for a closer interpretation.

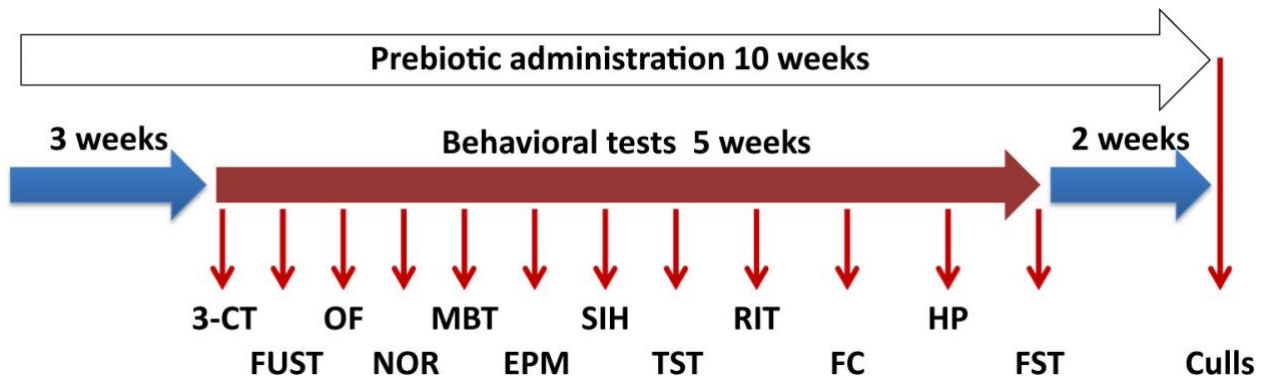


Figure 3. Supplemental methods. Total of 10 weeks prebiotic administration. Behavioral testing occurred on a gradient from least stressful to most. Stress-induced hyperthermia occurred 30 minutes before testing that occurred between 8 am and 4 pm (between 8 am and 12 noon for the forced swim test). “Week 4: 3-CT, three-chamber test; FUST, female urine sniffing test; OF, open field; NOR, novel object recognition test. Week 5: MBT, marble burying test; EPM, elevated plus maze; SIH, stress-induced hyperthermia. Week 6: TST, tail suspension test; RIT, resident-intruder test. Week 7: FC, fear conditioning. Week 8: HP, hot plate; FST, forced swim test and blood collection. Week 10: animals are culled and tissue is collected.”

REFERENCES

1. Wang, Y., & Kasper, L. H. (2014). The role of microbiome in central nervous system disorders. *Brain, behavior, and immunity*, 38, 1-12.
2. Honda, K., & Littman, D. R. (2012). The microbiome in infectious disease and inflammation. *Annual review of immunology*, 30, 759-795.
3. Tilg, H., & Kaser, A. (2011). Gut microbiome, obesity, and metabolic dysfunction. *The Journal of clinical investigation*, 121(6), 2126-2132.
4. Bercik, P., Collins, S. M., & Verdu, E. F. (2012). Microbes and the gut-brain axis. *Neurogastroenterology & Motility*, 24(5), 405-413.
5. Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS biology*, 14(8), e1002533. doi: 10.1371/journal.pbio.1002533
6. Holzapfel, W. H., Haberler, P., Snel, J., Schillinger, U., & in't Veld, J. H. H. (1998). Overview of gut flora and probiotics. *International journal of food microbiology*, 41(2), 85-101.
7. Preidis, G. A., & Versalovic, J. (2009). Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. *Gastroenterology*, 136(6), 2015-2031.
8. Looijer-Van Langen, M. A., & Dieleman, L. A. (2008). Prebiotics in chronic intestinal inflammation. *Inflammatory bowel diseases*, 15(3), 454-462.
9. Goubeyre, P., Denery, S., & Bodinier, M. (2011). Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. *Journal of leukocyte biology*, 89(5), 685-695.
10. Lindenbaum, J., Rund, D. G., Butler Jr, V. P., Tse-Eng, D., & Saha, J. R. (1981). Inactivation of digoxin by the gut flora: reversal by antibiotic therapy. *New England Journal of Medicine*, 305(14), 789-794.
11. Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: stress, health and disease. *Mammalian Genome*, 25(1-2), 49-74.
12. Rea, K., Dinan, T. G., & Cryan, J. F. (2016). The microbiome: a key regulator of stress and neuroinflammation. *Neurobiology of stress*, 4, 23-33.
13. Niittynen, L., Kajander, K., & Korpela, R. (2007). Galacto-oligosaccharides and bowel function. *Scandinavian Journal of Food and Nutrition*, 51(2), 62-66.
14. Yun, J. W. (1996). Fructooligosaccharides—occurrence, preparation, and application. *Enzyme and microbial technology*, 19(2), 107-117.
15. Burokas, A., Arbolea, S., Moloney, R. D., Peterson, V. L., Murphy, K., Clarke, G., ... & Cryan, J. F. (2017). Targeting the microbiota-gut-brain axis: prebiotics have anxiolytic and antidepressant-like effects and reverse the impact of chronic stress in mice. *Biological psychiatry*, 82(7), 472-487.
16. Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*, 204(2), 361-373.
17. De Boer, S. F., Lesourd, M., Mocaer, E., & Koolhaas, J. M. (1999). Selective antiaggressive effects of alnespirone in resident-intruder test are mediated via 5-hydroxytryptamine_{1A} receptors: A comparative pharmacological study with 8-hydroxy-2-dipropylaminotetralin, ipsapirone, buspirone, eltoprazine, and WAY-100635. *Journal of Pharmacology and Experimental Therapeutics*, 288(3), 1125-1133.
18. Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of neuroscience methods*, 14(3), 149-167.
19. Groenink, L., Van Der Gugten, J., Zethof, T., Van Der Heyden, J., & Olivier, B. (1994). Stress-induced hyperthermia in mice: hormonal correlates. *Physiology & behavior*, 56(4), 747-749.
20. Trowell, H. C., & Burkitt, D. P. (Eds.). (1981). *Western diseases, their emergence and prevention*. Harvard University Press.
21. Adlercreutz, H. (1990). Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. *Scandinavian Journal of Clinical and Laboratory Investigation*, 50(sup201), 3-23.
22. Carrera-Bastos, P., Fontes-Villalba, M., O'Keefe, J. H., Lindeberg, S., & Cordain, L. (2011). The western diet and lifestyle and diseases of civilization. *Res Rep Clin Cardiol*, 2(1), 15-35.
23. Manzel, A., Muller, D. N., Hafler, D. A., Erdman, S. E., Linker, R. A., & Kleinewietfeld, M. (2014). Role of "Western diet" in inflammatory autoimmune diseases. *Current allergy and asthma reports*, 14(1), 404.
24. Cordain, L. (2010). *The Paleo Diet revised: lose weight and get healthy by eating the foods you were designed to eat*. Houghton Mifflin Harcourt.

Try not to forget this one

This study uses visual experiences and an instruction to disregard them in order to activate memory processing.

Elisei Cosovan

This experiment was done in order to test and observe what regions of the brain are activated or inhibited in intentional forgetting and how the brain would respond to the instruction of either remembering or forgetting a visual presentation. An enhancement of memory processing in the sensory cortex is necessary in order to intentionally forget recent visual occurrences. In a recent study, Wang, Placek, and Lewis-Peacock pursued an experiment to test the activation of the ventral temporal cortex when subjects were instructed to deliberately forget a visual representation and whether or not the instruction would cause memories to weaken and be forgotten. An enhancement of memory processing in the sensory cortex is observed when participants are told to forget a visual experience rather than when they are instructed to remember it. The results of this experiment display an increase in memory processing after a TBF (to be forgotten) item compared to a TBR (to be remembered) item.

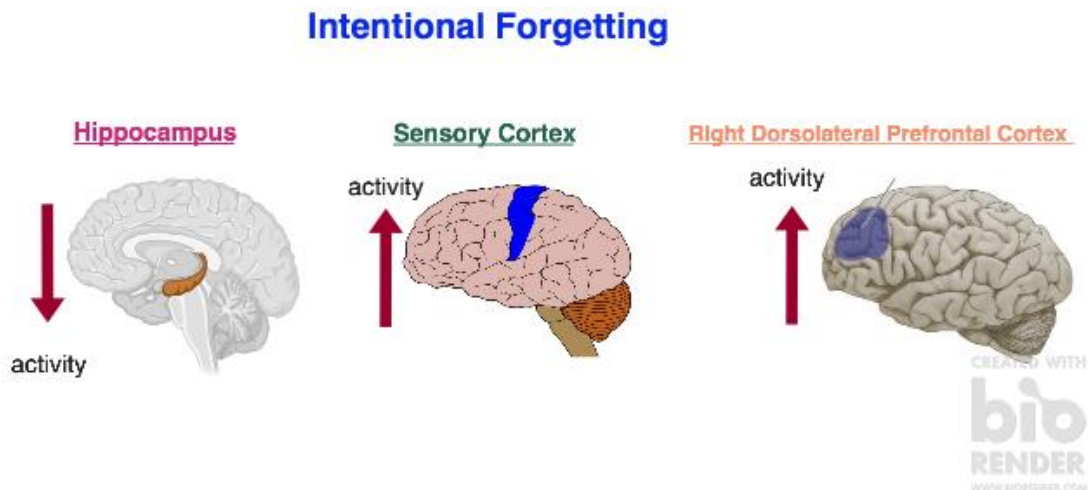


Figure 1. To intentionally forget a visual experience, memory processing in the sensory cortex must be enhanced, along with an increase in the activity of the right dorsolateral prefrontal cortex and a decrease in activity in the hippocampus.

Forgetting is a feature of the mind in which information that is not relevant, or that is no longer needed is removed – allowing one to retain necessary and important information². The brain is capable of automatically erasing unnecessary information, but people can also do this voluntarily³. Inhibitory processes in the frontal region of the brain allow for suppression of such memories. In

other terms, in order to successfully forget new memories, an increase in activity in the right dorsolateral prefrontal cortex and a decrease in the activity of the hippocampus is needed (Figure 1).

Functional magnetic resonance imaging (fMRI) was used to collect data from an experiment in which twenty-four male and female participants looked at various images (faces, and scenes) and were told to either remember (TBR) or to forget (TBF) the given image. Pattern classifiers were used to decode the human fMRI. Three phases were conducted for each of the participants in order to complete the experiment. The first two, which were done in the MRI scanner were the localizer phase, and the encoding phase. The third phase – recognition – was performed away from the scanner. In the first phase, subjects were presented with various images and were instructed to respond whether each image had previously been shown or not. In the encoding phase, subjects were presented with images and instructed to either remember or to forget them. A total of 252 items were presented. In the third phase, and additional 252 images were added to the list and subjects were given a recognition memory task in which they had to give confident judgements of ‘definitely old, probably old, probably new and definitely new’ to the images presented. A ‘definitely old, probably old’ response meant that the image was previously seen by the given subject¹.

When accounting for the results of the given experiment, the focus remained on the activity in the ventral temporal cortex – an input to convergence zones responsible for long-term memory storage. Higher levels of memory processing were observed in the ventral temporal cortex when participants were told to forget the visual experience. Identification of images followed by a TBF instruction was high for faces, and for scenes (97.7% and 98.0%, respectively). An increase in the activity of the dorsolateral prefrontal cortex, posterior cingulate and precuneus was observed when images were successfully forgotten. These results were consistent with previous studies done by Wyile and Rizio and in conjunction with the notion that intentional forgetting involves a certain amount of memory activation which helps in forgetting. The data collected also revealed that forgetting was most successful when moderate levels of memory activation were heightened. When mental representation of a visual experience is boosted, the result is memory weakening through local inhibitory processes in neural activity and its homeostatic regulation.

This study shows that memory was retained more after a TBF instruction rather than a TBR instruction, which indicates that increased memory processing was involved in TBF items. Since forgetting requires more memory processing, the ‘forgotten’ items became more distinct when shown again. These results have been predicted by other research – such as the NMPH – but also present intentional forgetting as an effortful cognitive process¹. Altogether, this study showed that in order to forget an item, or to weaken an unwanted memory, its level of activation should be raised rather than lowered. The results of this study are somewhat significant. Even though the results of this experiment have already been shown in previous studies – such as the one done by the NMPH – they help to concrete findings of how memory is processed and retained. One weakness seen in this study is that the TBR items might not have been remembered as well due to the large amount of TBR items that were displayed to the participants – one after another¹. Disregarding such weaknesses, what stood out in this experiment is how memory was retained based on given instructions and how much more memory activation is needed in the TBF instruction.

REFERENCES

1. Tracy H. Wang, Katerina Placek, Jarrod A. Lewis-Peacock (2019) More is less: increased processing of unwanted memories facilitates forgetting. *Journal of Neuroscience* 11 March 2019, 2033-18; DOI: 10.1523/JNEUROSCI.2033-18.2019

2. Kim, G., Norman, K. A., & Turk-Browne, N. B. (2017). Neural differentiation of incorrectly predicted memories. *Journal of Neuroscience*, 3272–16.
3. Bjork EL, Bjork RA, Anderson MC. (1998) Varieties of goal-directed forgetting. In J. M. Golding and C. MacLeod (Eds.), *Intentional forgetting: Interdisciplinary approaches*. (pp.103-137). Hillsdale, NJ: Erlbaum.
4. Basden, B. H., Basden, D. R., & Gargano, G. J. (1993). Directed forgetting in implicit and explicit memory tests: A comparison of methods. *Journal of Experimental Psychology: Learning, Memory, & Cognition*, 19, 603-616.
5. Benoit RG, Davies DJ, Anderson MC (2017). Reducing future fears by suppressing the brain mechanisms underlying episodic simulation. *PNAS*, 113, 52: E8492-E8501. PMID: 27965391
6. Fawcett, J. M., Taylor, T. L., & Nadel, L. (2013). Intentional forgetting diminishes memory for continuous events. *Memory*, 21:6, 675-694. PMID: 23301998.
7. Fawcett, Jonathan M., and Tracy L. Taylor. "Forgetting Is Effortful: Evidence from Reaction Time Probes in an Item-Method Directed Forgetting Task." *Mem Cognit* 36, no. 6 (2008): 1168–81. doi:10.3758/MC.36.6.1168. PMID 18927035
8. Gelfand, H., & Bjork, R. A. (1985, November). On the locus of retrieval inhibition in directed forgetting. Paper presented at the meeting of the Psychonomic Society, Boston, MA.
9. Newman, EL and Norman KA. (2010). Moderate excitation leads to weakening of perceptual representations. *Cereb. Cortex*. 20, 2760 -2770. PMC2951848.
10. Norman, K. A., Newman, E. L. & Detre, G. A neural network model of retrieval-induced forgetting. *Psychol. Rev.* 114, 887–953 (2007).
11. Pastötter B, Bäuml KH. (2007) The crucial role of postcue encoding in directed forgetting and context-dependent forgetting. *J Exp Psychol Learn Mem Cogn.* 33(5):977-82. PMID:17723074.
12. Zacks, Rose T., Gabriel Radvansky, and Lynn Hasher. "Studies of Directed Forgetting in Older Adults." *Journal of Experimental Psychology: Learning, Memory, and Cognition* 22, no. 1 (1996): 143.



Disturbances of gamma-band activity and parvalbumin expression in schizophrenia

In schizophrenia, increases in NMDA receptor (NMDAR) inhibition and glutathione (GSH) depletion coexist, but are separate processes. This has led to new insights behind gamma band oscillations and parvalbumin (PV) expression disturbances in schizophrenia and other disorders.

Abigail Gligor

Schizophrenic pathomechanisms have been thought to involve oxidative stress from decreases of excitatory receptors (NMDA receptors) and an antioxidant, glutathione (GSH), that lead to dysfunctions of specific interneurons that express parvalbumin (PV) and synchronous brain waves called gamma oscillations. However, it was unclear what NMDAR hypofunction and decreased presence of GSH individually contributed to changes in the parvalbumin containing interneurons and gamma oscillations. Until researchers Hasam-Henderson et al. (2018) conducted a study that explored the contributions of NMDARs and GSH each have in the mechanism underlying schizophrenia. They assessed different time points of cultured hippocampal slices during development to see the effects of NMDAR inhibition on oxidative stress and glutathione levels as well as changes in gamma oscillations and PV expression. As a result, inhibition of NMDARs and GSH synthesis both produced oxidative stress. On the contrary, NMDAR inhibition reduced gamma oscillation frequency and the loss of PV was delayed. Depletion of GSH caused immediate decreases in PV and increased power of gamma oscillations. These findings indicate that the different mechanisms coexist, but are separate in schizophrenia.¹

Gamma oscillations are synchronous neural activity from information processing and exchange in the brain, which is altered in schizophrenia.^{2,3} PV, another aspect behind schizophrenia, surrounds GABAergic interneurons and contributes to synchrony by mediating pyramidal cell inhibition.⁵ Individuals affected by schizophrenia show decreased expression of PV and GABA synthesizing enzyme glutamic acid decarboxylase-67 (GAD67).^{6,7} Also, it has been reported that genetic deletion of NMDAR disturbs cortical gamma oscillations and decreases PV and GAD67 expressions.^{8,9} NMDAR hypofunction and oxidative stress have been linked together.¹⁰ Oxidative stress contributions in schizophrenia are further supported by decreased levels of the antioxidant, GSH.^{6,11} Mutations in the synthesis of GSH have been shown to result in decreases of PV expression and altered gamma oscillations.¹² It is unclear if network alterations in schizophrenia result directly from NMDAR hypofunction or if they are from the oxidative stress produced by NMDAR hypofunction.

The authors, Hasam-Henderson et al, conducted their study by culturing 750 hippocampal slices from 150 rats for several days. NMDARs and GSH inhibition were pharmacologically done in vitro from day 1 until the assessed time points, which were day 3, 10, or 15. The tissue was assessed for PV using immunohistochemistry and for GSH using a Bradford protein assay. Then, the tissue was imaged using a confocal microscope for visualizing PV. The oxidation of proteins was measured by an oxyblot assay. Electrophysiology was done to observe gamma oscillations.¹ All

of these assessments were done on day 3, 10, or 15 of being cultured. A summary of the methods is described in Figure 1.

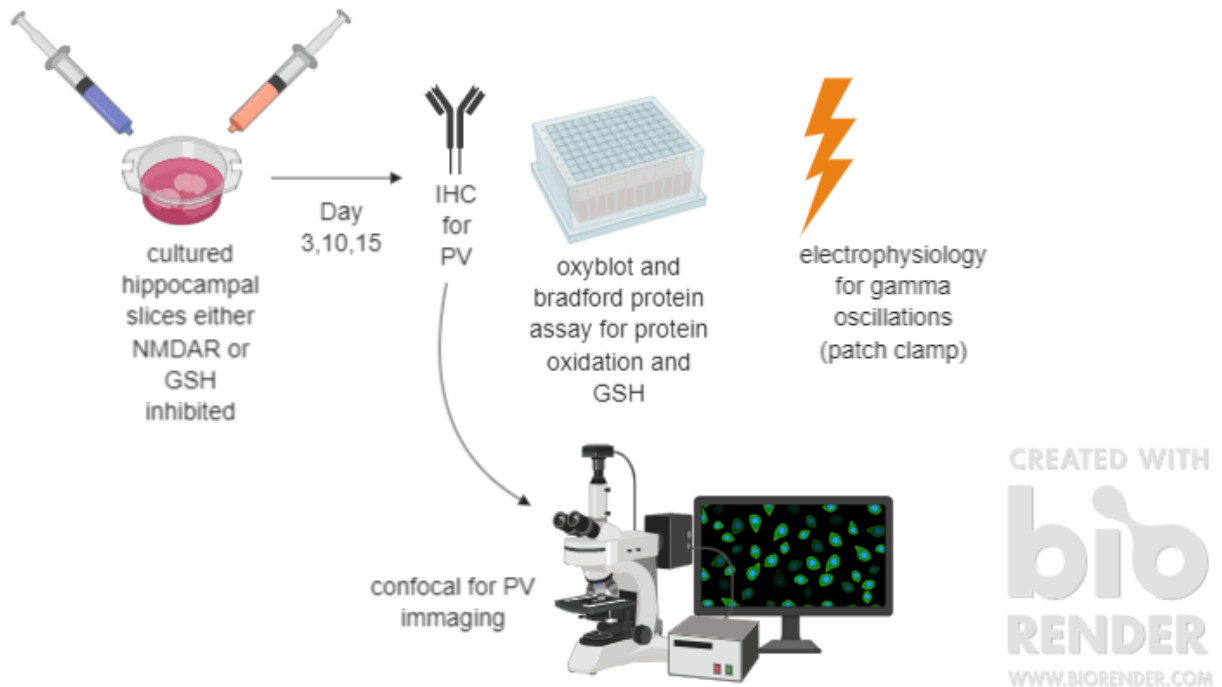


Figure 1. Summary of methods. Hippocampus slices from neonatal rats were cultured and NMDAR or GSH were inhibited. On day 3, 10, or 15 PV, oxidative stress, gamma oscillations, and GSH were evaluated using immunohistochemistry, protein assays, patch clamping, and confocal microscope.

The results of this study indicated several important aspects behind the roles of NMDARs and GSH in oxidative stress, PV expression, and gamma oscillation patterns. First, it was shown that when NMDARs are inhibited, there is an increase of protein oxidation without affecting the levels of GSH. To add on, NMDAR inhibition decreases the frequency of gamma oscillations before affecting PV expression. In another portion of the experiment, oxidative stress alone was shown to disturb PV expression while also increasing the oscillation powers of gamma waves. Another portion showed that a general increase in neuronal activity improved the survival of interneurons even though there was high oxidative stress. Overall, the main findings of this paper was that NMDAR inhibition led to decreased gamma oscillation frequency and a delayed loss of PV, whereas, GSH depletion let to an immediate reduction in PV, but an increase of gamma oscillation power.¹

Hasam-Henderson et al provide crucial links to the mechanism of which NMDAR and GSH provide for the oxidative stress, PV expression, and gamma wave oscillations behind the development of schizophrenic brains. Beforehand, it was unclear if NMDAR hypofunction and GSH depletion were part of the same mechanism. Instead, it was found that even though they coexist with each other in schizophrenia, that they are separate processes. This study's exploration of gamma oscillations can be used for other areas of research such as substance abuse disorder. Information processing and exchange in the brain produce brain oscillations or gamma waves and in substance abuse disorder there is a change in this process. The authors in

this paper speculated that the reason PV expression reduced PV, but increased gamma oscillation power could be attributed to the oxidative protective effects of perineuronal nets.¹³ The nets most likely were degraded in the presence of oxidative stress, which increased neuroplasticity that enhanced gamma oscillations.¹ In terms of substance abuse disorder, this paper's findings open new avenues to researching the mechanism behind addiction using gamma oscillations. Overall, the study conducted provided more information on PV expression, oxidative stress, and gamma wave oscillations as a result from manipulating NMDARs and GSH.

REFERENCES

1. Hasam-Henderson, L.A. et al. NMDA-receptor inhibition and oxidative stress during hippocampal maturation differentially alter parvalbumin expression and gamma-band activity. *Nature*, (2018).
2. Unhlaas, P.J. & Singer, W. Abnormal neural oscillations and synchrony in schizophrenia. *Nat. Rev. Neurosci.* 11, 100-113, (2013).
3. Gonzalez-Burgos, G et al. GABA Neuron Alterations, Cortical Circuit Dysfunction and Cognitive Deficits in Schizophrenia. *Neural Plasticity*, 1-24, (2011).
4. Traub, R.D. et al. Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. *J. Physiol.* 493, 471-484 (1996).
5. Do, K.Q. et al. Redox dysregulation, neurodevelopment, and schizophrenia. *Curr. Opin. Neurobiol.* 19, 220-230, (2009).
6. Powell, S.B. et al. Behavioral and neurochemical consequences of cortical oxidative stress on parvalbumin-interneuron maturation in rodent models of schizophrenia. *Neuropharmacology* 62, 132201331, (2012).
7. Belforte, J.E. et al. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat. Neurosci.* 13, 76-83, (2010).
8. Korotkova, T. et al. NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. *Neuron* 68, 557-569, (2010).
9. Cabungcal, J.H. et al. Juvenile antioxidant treatment prevents adult deficits in a developmental model of schizophrenia. *Neuron* 83, 1073-1084, (2014).
10. Gysin, R. et al. Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence. *Proc. Natl. Acad. Sci. USA* 104, 16621-16626, (2007).
11. Steullet, P. et al. Redox dysregulation affects the ventral but not dorsal hippocampus: impairment of parvalbumin neurons, gamma oscillations, and related behaviors. *J. Neurosci.* 30, 2547-2558, (2010).
12. Volman, V. et al. Downregulation of parvalbumin at cortical GABA synapses reduces network gamma oscillatory activity. *J. Neurosci.* 31, 18137-18148, (2011).
13. Cabungcal, J.H. et al. Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proc. Natl. Acad. Sci. USA* 110, 9130-9135, (2013).



I took this new sleeping pill and nhjmjjj..... (it worked)

An Oxford research study, conducted by Kempf, Song, Talbot and Miesenböck, found evidence that the ratio of different states of NADPH had a direct effect on Shaker potassium channels which cause sleeping. This research helps shed light on the mechanisms of sleeping and could help those suffering from insomnia as well as develop better sleeping pills.

Gavin Harvey

Sleep is still a process that is shrouded in mystery. Currently little is known as to why we need sleep just that when we do not get it a series of processes occur that effect metabolism, cellular repair, and cognitive function in a detrimental manner^{1,2}. It is known that certain neurons in the Suprachiasmatic nucleus regulate the circadian rhythm in the mammalian hypothalamus³ as well as others that fire when one is asleep but remain inhibited while awake. It has been proposed that we sleep to repair damage and relieve oxidative stress accumulated while awake⁴ and this is a major area of study. In this study there was a relationship found between A-type currents through Shaker's $K_v\beta$ subunit (the channel that is on the neurons that seem to trigger a sleeping state) and oxidative bonding to NADPH. If we could better understand the mechanisms that influence how and why we sleep, we could determine why some individuals experience sleep related illnesses like insomnia. We could also develop medication to aid in better rest and recovery without deleterious side effects.

In this study the purpose was to find a connection between metabolism, oxidation and sleep as these processes have been linked to the processes of aging and disease^{5,6}. The animal used in this study was the fruit fly *Drosophila* because it has a small, identifiable sleep-inducing neurons^{7,8} which project to the dorsal Fan-shaped Body (dFB). These induce sleep when they are activated, which means they emit a constant flow of action potential called an Alpha current (I_A). The mechanism is somewhat complex but, simply put, it involves two types of ion channel enzymes. Essentially during waking periods, the potassium ion channel called Sandman causes the activation of these sleep switch neurons to be irregular and harder to achieve. Then some unidentified mechanisms cause the ion channels in the cell membrane to be transported to a storage area within the cytoplasm (the inside of the cell). During this process the previously inhibited Shaker ion channels then become active (these are active during sleep). This then results in the neurons becoming active and expressing an I_A that appears to activate the state of sleep in the subject.

In this experiment several different experiments were performed on the Shaker channel protein. The first focused on mutations of the subunit of Shaker called the Hyperkinetic. In this experiment it was determined that flies that did not have mutated Hyperkinetic, one that resulted in its improper oxidoreductase activity, found that flies with the mutation showed insomniac behavior while the wild-type flies without it did not. Then levels of redox histories were compared between sleep-deprived flies and a control group of rested flies. To do this the researchers labelled mitochondria of the dFB with a florescent protein that would react when oxidized by reactive

oxygen species (ROS). They determined that the level of sleep deprivation correlated to an almost equal augmentation in the light detected which correlated with previous research⁹.

Now the primary test in this experiment was performed by introducing a flavoprotein into dFB neurons of the *Drosophila*. These proteins would activate when exposed to a specific blue light. This activated protein would react causing superoxide product which would then cause NADPH to reduce to NADP+. The researchers would expose the flies to the light for 9 minutes during a 30-minute observation. The flies with the protein were quiescent while the control flies without the protein showed no change in their sleeping patterns. This sleep time was measured by periods of inactivity at least 5 minutes in duration, but a notable effect was that the protein-containing flies showed quiescent periods of over an hour. Also, flies with a removed Hyperkinetic showed resistance to induced sleep.

The implications here are that when the ratio of NADP+ was increased an increase in Shafer activation lead to quiescence. A simplistic rendering of this idea is illustrated here in Figure 1. This induced sleep was found not to occur if the Hyperkinetic group was removed, though. This would support that these groups act like sensors for the ratio of redox reactions and the response in kind begins yet unidentified mechanisms. The same mechanisms that remove the Sandman proteins from the membrane, promote activation of Shaker channels, and result in a stable I_A of the dFB neurons.

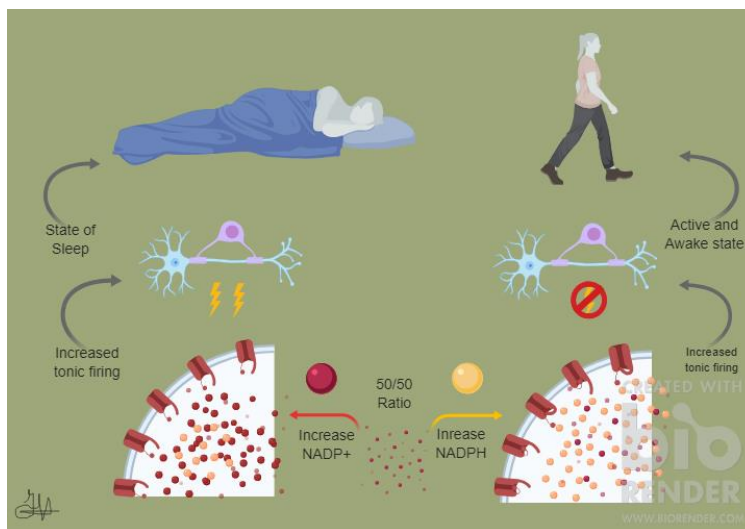


Figure 1

This has implications in making appropriate judgments in sleep disorders. These can occur in anyone and currently impacts around a third of US inhabitants¹⁰. These issues can arise from elevated stress levels as well as physiological conditions like mutated Hyperkinetic subcomponents in Shakers channels. This could improve sleep inducing medication by improving the effectivity of the medication and reducing the side effects. Currently there are various options for sleep inducing drugs. The three main sedative hypnotics are: benzodiazepines, barbiturates, and hypnotics¹⁰. These have issues such as problematic long-term diminishing effects, addictive characteristics, they can lead to fatal overdoses and a host of idiosyncratic effects. There are melatonin targeting medications that have much less severe side effects, but the strength of their effect is less than the other sleep aids¹¹. The research could indeed develop effective medication to help even insomniacs as well as gain more insight to the mechanisms that control sleeping.

REFERENCES

1. Rechtschaffen, A., Gilliland, M. A., Bergmann, B. M., & Winter, J. B. (1983). Physiological correlates of prolonged sleep deprivation in rats. *Science*, 221(4606), 182-184.
2. Ross, J. J. (1965). Neurological findings after prolonged sleep deprivation. *Archives of neurology*, 12(4), 399-403.
3. Sherin, J. E., Shiromani, P. J., McCarley, R. W. & Saper, C. B. (1996). Activation of ventrolateral preoptic neurons during sleep. *Science* 271, 216–219.
4. Hill, V. M. et al. (2018). A bidirectional relationship between sleep and oxidative stress in *Drosophila*. *PLoS Biol.* 16, e2005206.
5. Balaban, R. S., Nemoto, S. & Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell* 120, 483–495.

6. Orr, W. C. & Sohal, R. S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 263, 1128–1130.
7. Donlea, J. M., Thimgan, M. S., Suzuki, Y., Gottschalk, L. & Shaw, P. J. (2011). Inducing sleep by remote control facilitates memory consolidation in *Drosophila*. *Science* 332, 1571–1576.
8. Donlea, J. M., Pimentel, D. & Miesenböck, G. (2014). Neuronal machinery of sleep homeostasis in *Drosophila*. *Neuron* 81, 860–872.
9. Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochem. J.* 417, 1–13.
10. Side Effects of Sleeping Pills: Common and Potentially Harmful Side Effects. (n.d.). Retrieved 2018, from <https://www.webmd.com/sleep-disorders/guide/understanding-the-side-effects-of-sleeping-pills#1>
11. Melatonin: Uses, Side Effects, Interactions, Dosage, and Warning. (2018). Retrieved from <https://www.webmd.com/vitamins/ai/ingredientmono-940/melatonin>



Forgot to brush your teeth? It might kill you

A new study found that long-term exposure to a periodontal bacterium called Porphyromonas gingivalis is dangerous in ways beyond gum disease, including being a causative agent of Alzheimer's disease.

Gavin Lockard

The mechanisms behind the development of Alzheimer's disease are yet to be fully understood. It is known that certain bacterial and viral infections can lead to increases incidences of Alzheimer's disease^{1,2}. A paper was recently published by Iliovski et al. in the journal Public Library of Science One in 2018 titled "Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice". A bacterium called *Porphyromonas gingivalis* is a known causative agent of gum disease, but has now been additionally linked to brain inflammation and neurodegeneration, indicators of Alzheimer's disease³. The higher severity of gum disease is linked to higher amounts of bacteria present in the bloodstream^{4,5}. Periodontal bacteria and its potentially toxic products can then travel through the bloodstream to distant targets, including the brain^{6,7,8}. Indeed, *Porphyromonas gingivalis* has been identified in the human brain of post-mortem patients suffering from Alzheimer's disease⁹. This suggests that oral health is important for the prevention of the neurodegenerative effects that lead to Alzheimer's disease.

Alzheimer's disease is the most common form of mental failure observed in elderly people, amounting to 60-80% of dementia instances¹⁰. The associated neuropathology includes a buildup of excess protein called amyloid-beta which forms "plaques", as well as a different protein called tau which forms "tangles" after it is overactivated via hyperphosphorylation¹¹. These amyloid-beta plaques and tau tangles degenerate neurons and are ultimately deadly¹¹. Seemingly unrelated, periodontitis, or gum disease, results in the destruction of gum tissue and surrounding bone by an exaggerated host immune response to periodontal bacteria. Periodontitis is common, with an incidence of 50% in adults¹². As mentioned earlier, periodontal bacteria and its toxins can enter the bloodstream, and target the brain and other organs. What's new to the subject field is that the periodontal bacteria in the blood may lead to neurodegeneration and inflammation³.

This primary study³ was conducted using a mouse model. Mice were divided equally into two groups, a control and an experimental. Mice received oral applications of either solvent alone in the control condition, or solvent containing *Porphyromonas gingivalis* in the experimental condition. This was done twice a day on Mondays, Wednesdays, and Fridays every week for 22 weeks. Mice were euthanized, and their brains were removed and fixed. Tissue staining and fluorescence microscopy was performed to detect levels of amyloid-beta, intact neurons, degenerating neurons, *Porphyromonas gingivalis*, and inflammatory factors, such as microglia and several cytokines³.

Alzheimer's disease targets the hippocampus, a brain region involved in memory^{13,14}. *Porphyromonas gingivalis* and its toxins were found in the hippocampi of the mice in the

experimental condition. Inflammation was also observed in the hippocampi of experimental mice, which was seen with an increased number of proinflammatory cytokines, microglia, and astrocytes. Intact neuronal cells were fewer in experimental mice than control mice. Consequently, the number of degenerating neurons was greater in the experimental condition than the control condition. Gene expression for the APP (amyloid precursor protein) gene was increased in the experimental condition, suggesting an increase in amyloid-beta protein aggregation. Additionally, amyloid-beta was detected in the hippocampus and frontal cortex of experimental mice, but not in control mice. This amyloid-beta could continue on to form plaques. Hyperphosphorylated (overactivated) tau protein was also observed in the experimental mice³. Figure 1 shows all of the aforementioned experimental findings.

The Ilievski et al. study was the first to show that chronic periodontal pathogen exposure can lead to neuropathophysiology that mimics what is seen in Alzheimer's. Seemingly unrelated, periodontitis and Alzheimer's are often comorbid. This suggests that oral health needs to be taken seriously. However, this study was performed in mice, and though they are mammals and were exposed to the same periodontal bacteria, there is not a direct translation. It is not ethical to induce permanent periodontitis in humans, so mice will have to do, but the gap between rodent and human must be considered. Future work should develop drugs that can interfere with *Porphyromonas gingivalis* or its toxins, and prevent its debilitating effects that can lead to Alzheimer's. Beyond periodontal bacteria, other strange or previously unconsidered etiologies for Alzheimer's should continue to be investigated.

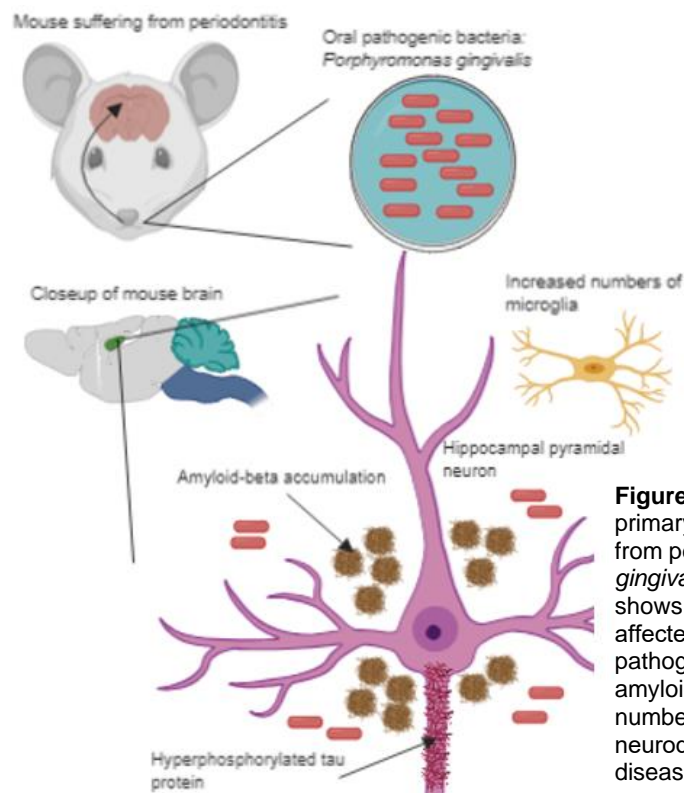


Figure 3. This figure demonstrates the findings from the primary study conducted by Ilievski et al. A mouse suffering from periodontitis has an increased amount of *Porphyromonas gingivalis* present in their mouth microbiota. This figure also shows that the neurons present in the hippocampus are affected by Alzheimer's-like symptoms due to the oral pathogenic bacterial toxins. This includes an accumulation of amyloid-beta, hyperphosphorylated tau protein, and increased numbers of microglia, astrocytes, and cytokines. This indicates neurodegeneration and inflammation, hallmarks of Alzheimer's disease.

REFERENCES

1. Itzhaki RF, Lathe R, Balin BJ, Ball MJ, Bearer EL, Braak H, et al. (2016). Microbes and Alzheimer's Disease. *J Alzheimers Dis.* 51(4):979–84.
2. Miklossy J. Alzheimer's disease—a neurospirochetosis. Analysis of the evidence following Koch's and Hill's criteria. *J Neuroinflammation.*
3. Ilievski, V., Zuchowska, P. K., Green, S. J., Toth, P. T., Ragozzino, M. E., Le, K., ... Watanabe, K. (2018). Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice. *PLOS ONE.* 13(10).
4. Hujuel PP, White BA, García RI, Listgarten MA. (2001). The dentogingival epithelial surface area revisited. *Periodontal Res.* 36:48-55.
5. Zelkha SA, Freilich RW, Amar S. (2010). Periodontal innate immune mechanisms relevant to atherosclerosis and obesity. *Periodontol 2000.* 54: 207-221.
6. Iwai T. (2009). Periodontal bacteremia and various vascular diseases. *J Periodontal Res.* 44:689–694.
7. Forner L, Larsen T, Kilian M, Holmstrup P. (2006). Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *J Clin Periodontol.* 33:401–407.
8. Ilievski V, Bhat UG, Suleiman-Ata S, Bauer BA, Toth PT, Olson ST, et al. (2017). Oral application of a periodontal pathogen impacts SerpinE1 expression and pancreatic islet architecture in prediabetes. *J Periodontal Res.* 52(6):1032–1041.
9. Poole S, Singhrao SK, Kesavalu L, Cutis MA, Crean S. (2013). Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. *J Alzheimers Dis.* 36(4):655–77.
10. Thies, W., Bleiler, L. (2013). Alzheimer's disease facts and figures. *Alzheimer's Dement.*, 9: 208-245.
11. Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological Alterations in Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine.* 1(1).
12. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. (2015). Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. *J Periodontol.* 86(5):611–22.
13. Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, et al. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurol.* 12(4):357–67.
14. Moodley KK, Chan D. (2014). The hippocampus in neurodegenerative disease. *Front Neurol Neurosci.* 34:95–108.



Taking shots to treat migraine pain: the effects of BOTOX injections on pediatric patients

Caleb Man

Migraine is a complex and a painful disorder. In fact, it is the third most common disorder that people have worldwide¹, and it is subcategorized into two main types: migraine with aura and migraine without aura². Put simply, migraine is a type of headache that can cause severe dysfunction in a person's daily life³. Not all migraine experiences are the same, however. This is especially evident when comparing them between men and women. To give a simple example, more men tend to transition from episodic migraines (less than 15 headache days per month) to chronic migraines (15 or more headache days per month) than women⁴. Regardless of sex, migraine pain, in general, can range from moderate to severe. The pain is often characterized by pulsating-throbbing pain on one side of the head, with the pain worsening following any physical exertion⁵. The pulsating-throbbing pain can also be accompanied by nausea, and vomiting, and an increased sensitivity to light and sound⁶. It is common for migraine patients to suffer from neck pain. What is strange, however, is that neck stiffness and neck pain are considered to be a symptom of migraine, rather than a cause⁷.

It appears that the symptoms of migraine are better known than the actual cause. Therefore, while it is not entirely clear what causes migraine or the aura accompanying it, one thing is clear – a migraine is able to impair neurological and even gastrointestinal functioning³. Of these two, neurological problems have received more attention. One hypothesis that is out there is that migraine aura results from cortical spreading depolarization and then hyperpolarization⁸. As a result, symptoms of aura can include visual changes, tingling, numbness, or dyspraxia⁸; dyspraxia, in short, is the inability to execute planned or skilled movements⁹. Problems with language and memory are also common⁸. Another interesting symptom of aura, although more rare than other symptoms, is olfactory hallucinations¹⁰. Finally, in addition to the pain, hypoperfusion associated with migraines is also associated with a reduction to the blood supply to the retina microcirculation and the death of ganglion cells¹¹. This could be one explanation as to why migraine patients experience visual problems.

Migraines are a serious and painful disorder that many people experience, but there are treatments available for them. Currently, migraine pain is treated by using nonsteroidal antiinflammatory drugs (NSAIDs), acetaminophine, triptans, and several other classes of drugs¹². A fairly new treatment is onabotulinumtoxin A, or as it is more commonly known as, BOTOX. Botox has been used to treat migraines in adult patients for several years now, but not much is known about its effects on pediatric patients. Therefore, the purpose of this paper is to dive deeper into how BOTOX can be used to treat pediatric patients.

In a paper recently published by Shah, Calderon, Wu, Grant, and Rinehart (2018), the researchers investigated the effects of BOTOX on 11 pediatric patients over a span of 5 years. This study is

important because over 9 % of the pediatric population is estimated to suffer from migraines, and it is a leading cause for visits to the emergency department and for school absences¹².

The researchers selected participants that had refractory migraine. Refractory migraine was defined as having 15 or more migraine days per month (for at least three months) with the added criteria that neuropathic medication or other abortive or preventive medication did not help resolve the migraine symptoms. Once they were deemed candidates for the treatment, parental consent was obtained and the participants were administered the BOTOX injections throughout the head, neck, and shoulders¹². See Figure 1 for common locations where a patient is likely to receive their BOTOX injections.

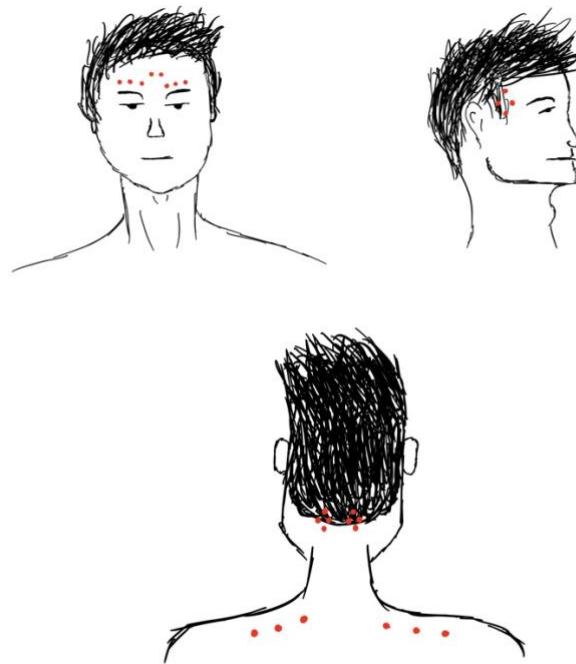


Figure 1. When BOTOX injections are given, they are given in the general locations where the red dots are. The qualified specialist administering the BOTOX can change the locations of the injection sites slightly to better treat each individual's migraines.

Essentially, BOTOX's method of action involves targeting neuromuscular junctions and by inhibiting the release of migraine related neuropeptides (like substance P) and glutamate¹². Therefore, it serves as a preventative treatment more so than an abortive one. When the researchers administered the BOTOX to the patients, they found a statistically significant improvement, specifically in the frequency, intensity, and duration of the migraines¹². The researchers were concerned about a potential placebo effect, but the results showed a great enough improvement that they were clinically significant as well. When compared to standard treatments, the participants who received the BOTOX treatment showed a 30% decrease in median head pain scores. An important consideration with any treatment, especially new ones, is the side effects that they entail. Standard medications currently used to treat migraines, like triptans, may cause undesirable side effects like sedation, rebound migraines, or even withdrawal.

BOTOX does not have any of these side effects and so far, there are no known serious side effects¹².

Considering that this study is one of the first to test the effects of BOTOX on pediatric patients, more studies with larger samples need to be conducted to really get a true assessment of how effective BOTOX is in these patients. The study was conducted well, and it provided a solid starting point for what could potentially be a widely used treatment for migraines. Future research into the effects that BOTOX may have on migraines with aura may also be beneficial; as aura brings with it a whole host of symptoms that many migraine sufferers are familiar with. This study did not state if the patients were diagnosed with migraine with aura or migraine without aura, and it is possible that patients that have migraine with aura may respond differently than those without it.

REFERENCES

1. Viana, M., Sances, G., Linde, M., Ghiotto, N., Guaschino, E., Allena, M., Goadsby, P. J., & Tassorelli, C. (2017). Clinical features of migraine aura: Results from a prospective diary-aided study. *International Headache Society, 37*, 979-989. doi:10.1177/0333102416657147
2. Cadiot, D., Longuet, R., Bruneau, B., Treguir, C., Carsin-Vu, A., Corouge, I., Gomes, C., & Proisy, M. (2018). *International Headache Society, 38*, 949-958. doi:10.1177/0333102417723570
3. Ekinci, M., Ceylan, E., Çağatay, H. H., Keleş S., Hüseyinoğlu, N., Tanyıldız, B., Çakıcı, Ö., & Kartal, B. (2014). Retinal nerve fibre layer, ganglion cell layer and choroid thinning in migraine with aura. *BMC Ophthalmology, 14*, 1-6
4. Scher, A., Wang, S., Katsarava, Z., Buse, D. C., Fanning, K. M., Adams, A. M., & Lipton, R. B. (2018). Epidemiology of migraine in men: Results from the Chronic Migraine Epidemiology and Outcomes (CaMEO) Study. *International Headache Society, 39*, 296-305. doi:10.1177/0333102418786266
5. Diener, H., Holle-Lee, D., Nägel, S., Dresler, T., Gaul, C., Göbel, H., Heinze-Kuhn, K., Jürgens, T., Kropp, P., Meyer, B., May, A., Schulte, L., Solbach, K., Straube, A., Kamm, L., Förderreuther, S., Gantenbein, A., Petersen, J., Sandor, P., & Lampl, C. (2019). Treatment of migraine attacks and prevention of migraine: Guidelines by the German Migraine and Headache Society and German Society of Neurology. *Clinical and Translational Neuroscience, 1*-40. doi:10.1177/2514183X18823377
6. Lin, Y., Liang, C., Lee, J., Lee, M., Chu, H., Tsai, C., Lin, T., & Yang, F. (2019). Association of suicide risk with headache frequency among migraine patients with and without aura. *Frontiers in Neurology, 10*, 1-8. doi:10.3389/fneur.2019.00228
7. Luedtke, K., Starke, W., & May, A. (2018). Musculoskeletal dysfunction in migraine. *International Headache Society, 38*, 865-875. doi:10.1177/0333102417716934
8. Petrusic, I., Dakovic, M., Kacar, K., & Zidverc-Trajkovic, J. (2017). Migraine with aura: Surface-based analysis of the cerebral cortex with magnetic resonance imaging. *Korean Journal of Radiology 19*, 767-776. doi:https://doi.org/10.3348/kjr.2018.19.4.767
9. Miller, M., Chukoski, L., Zinni, M., Townsend, J., & Trauner, D. (2014). Dyspraxi, motor function and visual-motor integration in autism. *Behavior Brain Research 269*, 95-102. doi: http://dx.doi.org/10.1016/j.bbr.2014.04.011
10. Mainardi, F., Rapoport, A., Zanchin, G., & Maggioni, F. (2017). Scent of aura? Clinical olfactory hallucinations during a migraine attack (OHM). *International Headache Society, 37*, 154-160. doi: 10.1177/0333102416630580
11. Iyigundogdu, I., Derle, E., Asena, L., Kural, F., Kibarogul, S., Ocal, R., Akkoyun, I., & Can, U. (2018). Relationship between white matter hyperintensities and retinal nerve fiber layer, choroid, and ganglion cell layer thickness in migraine patients. *International Headache Society, 38*, 332-339. doi: 10.1177/0333102417694882
12. Shah, S., Calderon, M., Wu, W. D., Grant, J., & Rinehart, J. (2018). Onabotulinumtoxin A (BOTOX®) for prophylactic treatment of pediatric migraine: A retrospective longitudinal analysis. *Journal of Child Neurology, 33*, 580-586. doi:10.1177/0883073818776142



Decision-Making and Reaction Times: Why you should be playing video games too

When comparing people who play action video games (AVGPs) to those who play non-action video games (NAVGP), there was an increase reaction time in AVGPs as well as an enhanced ability to make decisions and prioritize.

Briana Mason

Cognition tends to increase in people who play action video games because they have practice in diverting attention and reacting to changing stimuli. The study looked at the difference between action and non-action video game players to see if there was a difference in ability to process information. It was found that those who played more action video games such as *Call of Duty* were better equipped to prioritize stimuli and process information more effectively than to someone who plays *Candy Crush* in their free time. Increasing cognition by playing video games may be able to help people who are struggling with mental processing, and for individuals to increase their reactivity for professions that require quick reflexes such as sports, medicine and law enforcement^{1,2,3}.

Some theories on video games describe the gain to be only pertinent to that in which the game itself is played. Other theories suggest there may be other real world applications, and that women may see even more benefit than men^{4,5}. Action video games accelerate stimuli and processing that is not seen in most ordinary life and pushes the mind⁶. Action games exercise navigating complicated settings with abrupt events that requires gamers to track multiple objects, allocate their resources and manage their perceptual abilities in aiming with fast reaction times⁷. In being able to experience something that is not really happening to the gamer, the gamer must use their anterior N1 component, measured in this experiment, in what is called a “modality shift”⁸.

The study took four people who play action video games and three people who do not play action video games but play other video games instead. To qualify for playing action video games, the participants had to play an average of nine hours a week for two years. They had participants look at a screen with a left and right visual field and played an arrow on either half for 1500 msec. The participant’s eyes were monitored for horizontal eye movements while probes were placed on their scalp, which was hooked up to an EEG that monitored their brain waves.

The study found that most individuals either split their attention as to be able to switch between looking between both sides of the visual field or that participants chose to look at one side really well and hardly respond to the opposite side of the visual field. An interesting finding was that there was no differences in the striate, a brain region that processes position. This is unusual because typically when processing arrows, the orientation is important but both gamers and non-action gamers had the same capabilities within this study. Along with a lack of differences in the striatum, there was no difference in the visual cortices or their ability to discriminate between objects. There was a correlation between N1, the area that helps gamers shift into the game as

well as dividing attention, and the use of the opposite side of the brain. P300 (P3) was also tested and is important for how someone reacts to a stimulus, whether it is there or not, and in the study the larger the P3 response was, the more accurate the gamer was at detecting the arrows. Posterior P1 was also monitored, an area that helps in attention, and there was a significant difference between those who were divided, unattended and focused but that did not correlate with gamers and non-gamers⁹.

Significant differences were found in the action gamer's abilities were found in their heightened perception and ability to hold attention. They were also better at dividing their attention to be able to process more information and not miss queues on the opposite side of the visual field. In addition to this, the action gamers were also better at allocating importance to certain stimuli, to be able to tell when a certain stimulus is noteworthy and valuable to process. The participants also had an increased contralateral processing, which means that when the information gets processed, the action gamers had a higher ability to process what they were seeing. The effects lasted after game play but were not reported for how long. The study could have been obscured, however, if the non-gamers found the task too difficult while the gamers found it too easy.

Playing action video games for an average of 9 hours a week over a course of two years may have the ability to significantly increase attention flexibility. People who play action video games can better divide their attention and prioritize in high stress situations. They also have an increase in perceptual abilities due to navigating a screen in which they must perceive a three-dimensional world in a two-dimensional capacity, which has applications in the future of surgery where using cameras are becoming more integrated to lessen the chances of infection.

REFERENCES

1. Green, C.S., Bavelier, D. (2012). Learning, attentional control, and action video games. *Current Biology*. 22(6): R197-R206.
2. Boot, W.R., Champion, M., Blakely, D.P., Wright, T., Souders, D.J., Charness, N. (2013). Video games as a means to reduce age-related cognitive decline: attitudes, compliance and effectiveness. *Front. Psychol.*
3. Green, C.S., Bavelier, D. (2015). Action video game training for cognitive enhancement. *Current Opinion in Behavioral Sciences*. 4: 103-108.
4. Boot, W.R., Blackely, D.P., Simons, D.J. (2011). Do action video games improve perception and cognition. *Frontiers in Psychology*.
5. Feng, J., Spence, I., Pratt, J. (2007). Playing an action video game reduces gender differences in spatial cognition. *Psychological Science*.
6. Dye, M.W.G., Green, S., Bavelier, D. (2009). Increasing speed of processing with action video games. *Current Directions in Psychological Science*.
7. Spence, I., Feng, J. Video games and spatial cognition. (2010). *Review of General Psychology*.
8. Tollner, T., Gramann, K., Muller, H.J., Eimer, M. (2009). The anterior N1 component as an index of modality shifting. *J Cog Neurosci*. 21(9):1653-69.
9. Zhang, W., Li, H., Chen, J., Chen, N., Liu, X., Wang, D., Shen, J. (2014). Posterior P1 and early frontal negativity reflect developmental changes in attentional distraction during adolescence. *Brain Cogn*.
10. Colzato, L.S., van den Wildenberg, W.P.M., Zmigrod, S., Hommel, B. Action video gaming and cognitive control: playing first person shooter games is associated with improvement in working memory but not action inhibition. *Psychological research*. 77(2) 234-239.
11. Focker, J. Mortazavi, M., Khoe, W., Hillyard, S.A. (2018). Neural correlates of enhanced visual attentional control in action video game players: an event-related potential study. *Journal of Cognitive Neuroscience*. 31(3): 1-15.



A Possible Cause for Autism: Abnormal VEN Development

Decreased number of von Economo neurons in individuals with ASD, could be inhibiting intuition, social bonding and social reward due to lack of dopamine, serotonin and vasopressin receptors associated with this type of neurons.

Janee Meengs

Autism spectrum disorder (ASD) is a developmental disorder that affects social behavior and communication. The symptoms are generally identified during the first two years of life¹. The Mayo Clinic² describes common signs of ASD as the resistance of cuddling, performing activities that lead to self-harm and difficulty in social interactions. As of yet, there is no identified cause of ASD. However, a specialized type of neuron, called von economo neurons (VENs) could hold the answers to an ASD individuals' lack of social intuition, response to rewards and social bonding, partially explaining a neurobiological cause for the social behaviors of individuals with ASD. An article published in Trends in Cognitive Science, identifies three receptor types on the VENs whose chemical signal, when activated, could lead to feelings of intuition, social bonding and social reward. The authors dive further to link the lack of the VENs in the brain area related to cognitive and emotional processing, called the anterior cingulate cortex (ACC) to ASD^{3,4}.

The three receptors types on the VENs are vasopressin 1a (V1a), dopamine 3 (D3) and serotonin 2b (5-HT2b). Genetic variations of the gene that codes for the V1a receptor has been linked to activation of the emotional center of the brain specifically during emotional processing of facial expressions⁵. D3 receptors have been previously linked to cognition. Cognition is responsible for many mental processes, such as attention, memory and comprehension, which make it vital for understanding, adaptation and applying knowledge to given situations⁶. The D3 receptor has also been thought to signal when rewards are to be expected in uncertain circumstances³. The 5-HT2b receptors are highly expressed on the VENs but rarely in other areas of the brain³. However, the VENs are found in relatively large proportions in the human stomach and intestines³. Other research has implicated 5-HT2b receptors as modulators of dopamine neurons possibly signaling punishment. Further, mice lacking the 5-HT2b receptors behave similarly to mice addicted to cocaine, where cocaine addiction increases the levels of dopamine in several brain areas^{3,7}. Therefore, the D3 receptors signal reward and the 5-HT2b receptors could oppose those signals and create balance between reward and punishment³. Together,

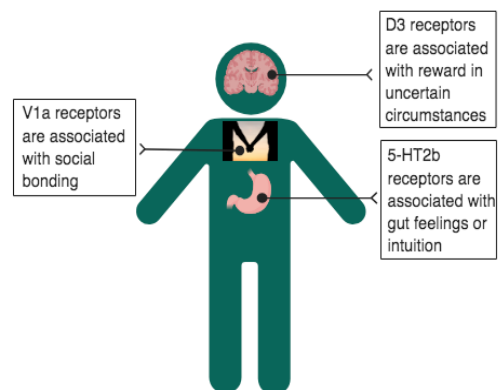


Figure 4. Associations of the three receptors found on von economo neurons of the anterior cingulate cortex.

these three receptors and their signaling allow us to read others' emotions and connect, expect rewards and have gut feelings when something is wrong (Figure 1).

This study first compared the total VEN number in apes and newborn, child and adult humans. Immunocytochemistry is a technique used to identify proteins; used here to visualize the V1a, 5-HT2b and D3 receptors on the VENs in the ACC of humans⁸. They found that VENs are concentrated in the right hemisphere and only 15% of total VENs are present at birth and increase until 4 years old. To link the VENs to ASD, the axon fibers that carry information were observed and showed the neuronal connections are disorganized in ASD individuals, which could be due to an abnormal growth pattern during development. Individuals with ASD also showed a relatively lower number of the VENs in the ACC.

The authors concluded that the VENs in those with ASD fail to develop properly which is partially responsible for the lack of intuition, social bonding and response to rewards due to the lack of receptors associated with these complex functions. Further research to manipulate number and connections to find a causal relationship between VENs and their associated receptors to the social behaviors would be more convincing in linking these things directly to ASD to truly be considered a partial cause.

REFERENCES

1. NIMH, National Institute of Mental Health. (2018). Autism Spectrum Disorder. Retrieved from <https://www.nimh.nih.gov/health/topics/autism-spectrum-disorders-asd/index.shtml>
2. Mayo Clinic. (2018). Autism Spectrum Disorder. Retrieved from <https://www.mayoclinic.org/diseases-conditions/autism-spectrum-disorder/symptoms-causes/syc-20352928>
3. Allman, J.M., Watson, K.K., Tetreault, N.A. and Hakeem, A.Y. (2005). Intuition and Autism: A Possible Role for Von Economo Neurons. *Trends in Cognitive Sciences* 9: 367-373
4. Bush, G., Luu, P. and Posner, M.I. (2000). Cognitive and Emotional Influences in Anterior Cingulate Cortex. *Trends in Cognitive Sciences* 4: 215-222
5. Umbricht, D., del Valle Rubido, M., Hollander, E., McCracken, J.T., Shic, F., Scahill, L., Noeideke, J., Boak, L., Khwaja, O., Squassante, L., Grundschober, C., Kletzl, H. and Fontoura, P. (2016). A Single Dose, Randomized, Controlled Proof-of-Mechanism Study of a Novel Vasopressin 1a Receptor Antagonist (RG7713) in High-Functioning Adults with Autism Spectrum Disorder. *Neuropsychopharmacology* 42: 1914-1923
6. Nakajima, S., Gerretsen, P., Takeuchi, H., Caravaggio, F., Chow, T., Le Foll, B., Mulsant, B., Pollock, B. and Graff-Guerrero, A. (2013). The Potential Role of Dopamine D3 Receptor Neurotransmission on Cognition. *European Neuropsychopharmacology* 23: 799-813
7. Doly, S., Quentin, E., Eddine, R., Tolu, S., Fernandez, S.P., Bertran-Gonzales, J., Valjent, E., Belmer, A., Vinals, X., Callebert, J., Faure, P., Meye, F.J., Herve, D., Robledo, P., Mameli, M., Launay, J., Maldonado, R. and Maroteaux, L. (2017). Serotonin 2B Receptors in Mesoaccumbens Dopamine Pathway Regulate Cocaine Responses. *Journal of Neuroscience* 37: 10372-10388
8. Burry, R. (2011). Controls of Immunocytochemistry. *Journal of Histochemistry and Cytochemistry* 59: 6-12



Musical enrichment for neurologically disordered children

A new study finds music therapy an effective addition to treatment for children with severe neurological disorders, enhancing the children's attentional and emotional capacities.

Trent Pratt

This study used a two-armed parallel group design to test the effectiveness of a music therapy treatment for children with severe neurological disorders. The control group received only a standard neurorestoration program, while the experimental received an additional music therapy prior in addition to standard occupational and speech therapy. The purpose was to improve the social interactions by promoting changes in attention and emotional responses and to explore whether music therapy could be more broadly utilized for a range of disorders. The results showed improved attention and communication and changes in brain plasticity in the children, supporting the hypothesis that music therapy is an effective addition to the treatment regimen for the rehabilitation of patients across a wide range of disorders.

It is without question that music plays a major role in human life, an aspect of humanity that transcends borders and generations. Previous research indicates that people consider music an important aspect of their lives and listening to music is an activity commonly engaged in¹. Similar research also found that when people are asked why music is a part of their lives, the answer is because music induces and regulates emotion². The social-emotional nature of music is credited with the sustained prominence music has throughout human culture^{3,4}. Regarding music and the brain, there is plenty of research that confirms differences in brain structures between musicians and non-musician. For example, music training has been shown to affect the development of the planum temporale, a structure of the secondary auditory cortex, which is involved in the conciliation of verbal memory⁵. In addition, the processing of music has been associated with the limbic and paralimbic regions of the brain, including regions involved in reward pathways (Zald, 2011). The concept of music therapy is defined as the use of music to modify brain processes by engaging the attention and interest of the subject and by confirmation of this engagement effect and its consequences⁶. There appears to be few studies that have evaluated the effectiveness of music therapy in treating a broad range of neurological disorders using appropriate methods to gauge behavioral and physiological outcomes. Because of the extensive research supporting various effects of music on brain structures involving cognitive, sensorimotor, and emotional processing⁷, this study was geared toward exploring the benefits of music therapy in addition to standard neurorestoration therapy for children with severe neurological disorders.

A two-arm parallel group design in which an experimental music therapy group was compared to a control group, where both groups received a standard neurorestoration program but the experimental group received additional music therapy. All children who participated had significant problems in motor, cognitive, and in particular communication abilities. There were no differences in the neuropsychological impairments between both groups. The neurorestoration program involved seven hours of motor, language, occupational, physical stimulation and

neuropsychology therapies, with each lasting an hour. The music therapy protocol involved children listening to different musical excerpts and focusing their attention on specific aspects of the music, such as shifting melody dynamics and rhythmic patterns. Two basic procedures were designed to stimulate either sustained or selective attention. In the sustained attention procedure, the child was required to throw a ball to another child in synchrony with changes in musical cues, and in the selective attention procedure, the child was required to focus on one instrument and ignore the others. The protocol was designed to increase the levels of sustained and selective attention and verbal and nonverbal communication between children with various neurological disorders. The music therapy protocol was administered in ten-minute sessions directly before the standard speech and occupational therapies. In all cases a final ERP Mismatch Response (event-related potential) evaluation was carried out at the end of the therapy period in order to measure brain plasticity. ERP Mismatch Responses have been used in children to determine maturation of the brain and to study specific childhood neurological disorders⁸. Additionally, a questionnaire was administered that used a number of well-established procedures from previous works in order to examine a wide range of behavioral results on a 5-point scale, with 1 being the lowest possible and 5 as the highest possible score. The questionnaire, administered by the therapists, incorporated aspects of standard and validated behavioral questionnaires in order to gauge improvements in motor, social, emotional, and cognitive areas. The effectiveness of music therapy was measured using the ERP Mismatch Response evaluation and the behavioral questionnaire to assess the results from multiple perspectives.

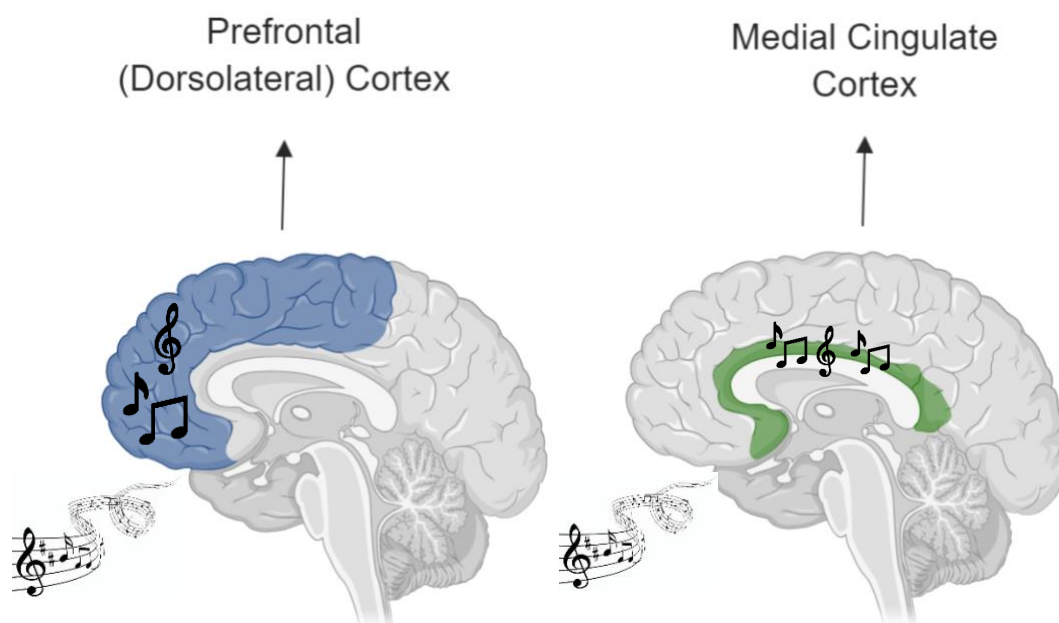


Figure 1

Overall, the results support the target questions: first, the effectiveness of MT in addition to standard neurorestoration therapy in real world situations, and second, the music therapy-specific changes in brain plasticity (Figure 1). The purpose of this protocol was to use the music therapy as a platform to train the children in other nonmusical areas, such as attention and communication. As opposed to previous works studying music therapy in relation to specific disorders, this study demonstrated music therapy's success across a mixed patient sample. Additionally, the program was also effective in enhancing the children's motivation and social behavior, which could account for the boosts in communication, cooperation and social awareness. An important note about the methodology is that the measurements were difficult to relate to the brain regions associated with

responses to the addition of music therapy. The median cingulate cortex and the prefrontal and dorsolateral cortex were identified with potential music therapy-induced effects. The ERP-LORETA analysis identified the medial cingulate cortex to be involved in the attentional network activated by cognitively demanding tasks, the processing and perception of pain, emotion, stimulus salience, action-reward associations, and premotor functions^{9,10,11}. The same analysis also identified the prefrontal cortical areas, which are largely associated with cognitive functions, and the dorsolateral prefrontal cortex, which is activated primarily during tasks requiring executive function¹².

REFERENCES

1. Rentfrow, P. J., and Gosling, S. D. (2003). The do re mi's of everyday life: the structure and personality correlates of music preferences. *J. Pers. Soc. Psychol.* 84, 1236–1256. doi: 10.1037/0022-3514.84.6.1236
2. Dubé, L., and Le Bel, J. (2003). The content and structure of Laypeople's concept of pleasure. *Cogn. Emot.* 17, 263–295. doi: 10.1080/02699930302295
3. Huron, D. (2006). *Sweet Anticipation: Music and the Psychology of Expectation*. Cambridge, MA: MIT Press.
4. Fitch, W. T. (2005). The evolution of music in comparative perspective. *Ann. N. Y. Acad. Sci.* 1060, 29–49. doi: 10.1196/annals.1360.004
5. Frisk, V., and Milner, B. (1990). The roll of the left hippocampal region in the acquisition and retention of story content. *Neuropsychologia* 28, 349–359.
6. Thaut, M. H. (2005). *Rhythm, Music and the Brain: Scientific Foundations and Clinical Applications*. New York, NY: Routledge.
7. Koelsch, S. (2009). A neuroscientific perspective on music therapy. *Ann. N.Y. Acad. Sci.* 1169, 374–384. doi: 10.1111/j.1749-6632.2009.04592.x
8. Liu, H. M., Chen, Y., and Tsao, F. M. (2014). Developmental changes in mismatch responses to Mandarin consonants and lexical tones from early to middle childhood. *PLoS ONE* 9:e95587. doi: 10.1371/journal.pone.0095587
9. Bush, G. (2009). "Dorsal anterior midcingulate cortex: roles in normal cognition and disruption in attention-deficit/hyperactivity disorder," in *Cingulate Neurobiology and Disease*, ed B. A. Vogt (New York, NY: Oxford University Press), 245–274.
10. Shackman, A. J., Salomons, T. V., Slagter, H. A., Fox, A. S., Winter, J. J., and Davidson, R. J. (2011). The integration of negative affect, pain and cognitive control in the cingulate cortex. *Nat. Rev. Neurosci.* 12, 154–167. doi: 10.1038/nrn2994
11. Rushworth, M. F., Behrens, T. E., Rudebeck, P. H., and Walton, M. E. (2007). Contrasting roles for cingulate and orbitofrontal cortex in decisions and social behaviour. *Trends Cogn. Sci.* 11, 168–176. doi: 10.1016/j.tics.2007.01.004
12. Kane, M. J., and Engle, R. W. (2003). Working-memory capacity and the control of attention: the contributions of goal neglect, response competition, and task set to Stroop interference. *J. Exp. Psychol. Gen.* 132, 47–70. doi: 10.1037/0096-3445.132.1.47
13. Bringas, M. L., Zaldivar, M., Rojas, P. A., Martinez-Montes, K., Chongo, D. M., Ortega, M. A., . . . Valdes-Sosa, P. A. (2015). Effectiveness of music therapy as an aid to neurorestoration of children with severe neurological disorders. *Frontiers in Neuroscience*, 9. doi:10.3389/fnins.2015.00427



The key to an effective anti-depressant regiment: brain-derived neurotrophic factor

Jonah Stickney

Major depressive disorder is hyper-prevalent in today's society. The National Institute of Mental Health estimates that 17.3 million adults in the United States have had at least one major depressive episode, 7.1% of all adults (NIMH, 2019). There are significant symptoms of MDD that may impede the lives of those living with it; reduced motivation, severe changes in diet and sleep, and in some, suicidal ideation. Part of the tenacity of MDD is the ability for these symptoms to further exacerbate the negative affect caused by depression in a positive feedback loop. If we better understood how to effectively treat these symptoms, it may improve the treatment outcomes of millions. One of these symptoms that is not fully understood is memory disruption. In recent study published in 2019 and performed by German scientists, brain derived neurotrophic factor (BDNF) was correlated with the memory restoration in MDD patients being treated with antidepressants¹. Understanding this correlation, BDNF levels could be used as a measure of antidepressant efficacy in patients who have impaired memory. Exogenous BDNF could also prove to be a novel treatment to treat memory impairment.

BDNF serum, blood and hair levels have recently been used in many research projects as a measure for the severity of various multiple disorders (especially depression, chronic stress and psychosis spectrum disorders). Measured thusly, BDNF seems to be an antagonist to cortisol when it comes to mental health prognosis². Why is there so much focus on measuring this specific neurotrophin as opposed to nerve growth factor or neurotrophins 3 and 4? In healthy individuals, BDNF has widespread expression both within and outside of the central nervous system. On a microscopic level, BDNF is a potent regulator for synaptic plasticity via LTP, especially within the hippocampus. BDNF acts on the tyrosine kinase B receptor (TrkB) so serum BDNF is often measured alongside level of TrkB expression (Figure 1)³. BDNF levels alter neurotransmitter release, glutamate receptor density, protein synthesis and structural plasticity at dendritic spines which sum to affect on learning and memory³. If this is the local effect of BDNF, what is its global effect? This is the topic of interest for the research project of discussion.

The study of interest was an investigation into a large clinical trial (n=173) aiming to improve depression treatment: is there a significant difference in outcome between groups who receive early medication change (EMC) and those who receive treatment as usual (TAU). Anti-depressants have a slow clinical onset and therefore treatment as usual consists of waiting three weeks before determining whether changing antidepressant dosage or prescription is appropriate. Early medication change as a treatment is defined by changing antidepressant (escitalopram to venlafaxine) in non-responding patients after only 2 weeks. A battery of memory tests known collectively as the Verbal Learning and Memory Test (VLMT) were performed three times during the experiment; at baseline, day 28 and day 56. Blood was drawn to measure plasma BDNF levels at baseline, day 14 and day 56. Depression level was monitored throughout using the Hamilton Depression Rating Scale (HAMD). ANCOVAs were performed between HAMD scores and learning and delayed recall, corrected for age, IQ and sex. Memory performance and plasma

BDNF (pBDNF) was compared using repeated measures ANOVAs. Significance level was set at an alpha of 0.05.

Learning performance improved for the group regardless of treatment but this may be due to increased familiarity to the memory test. Most comparisons made statistically with BDNF in this project were found to be non-significant. Depression severity was not correlated with memory or learning performance. Similarly, plasma BDNF and memory performance were not correlated. In fact, in a different study BDNF serum levels did not differ between those with and without depression (Dols *et al.*, 2015). There was one group in this experiment that did show significance. In the sample, 36% of patients showed memory deficits at baseline, 24% had impaired learning and 32% had delayed recall of word lists. Approximately 40% of these patients had memory normalization during the course of the experiment. In this subgroup of patients (those who initially had memory impairments and recovered), pBDNF and normalization of memory were significant when compared to those with persistent impairments. It is important to note that this significance is not an increase of BDNF during the experiment but rather a higher BDNF at baseline that was significant in predicting whether a patient would have normalization of memory (Average pBDNF was 1218.5 ug/ml for the normalization group and 769.1 ug/ml for the persistent impairment group). This implies that BDNF possibly predisposes anti-depressants to normalize impaired memory.

There is a study similar to this one in which BDNF was measured during an Electro-convulsive therapy of treatment resistant depression. BDNF was measured before and after ECT treatment and was compared to depression score also measured by the Hamilton Depression Rating Scale. In addition, the intensity of the induced seizure was measured by the Seizure Quality Index (SQI). Interestingly, as with the main study of interest, BDNF did not correlate significantly with many factors, including initial and final HAMD scores, age, or rate of remission. Similar to the previous study however there was significance between BDNF and an indirect measure of remission rather than the actual outcome itself; BDNF and SQI score were significantly correlated similar to the correlation between BDNF and memory normalization⁴.

The relationship between depression and memory functioning is highly elusive. Some studies have shown that memory problems often persist after remission of depression, while others show improvement of memory from a successful antidepressant treatment⁵ (Heinz *et al.*, 2012). The previous studies support this. Perhaps this is due to depression's etiology not being understood mechanistically, and in fact depression may be an umbrella term for different syndromes with entirely different etiologies. This is not a divisive statement, especially within clinical psychology. For example, Cushing's syndrome is sometimes misdiagnosed as treatment resistant depression⁶. It is possible that in some cases, memory disruption is an exacerbating cause to the depression while in others, depression is co-morbid with memory disruption unrelated to the depression etiology. This theory would explain why BDNF would not correlate with remission of depression across different treatments, but would only correlate with those suffering from a specific form of depression. In this view, BDNF allows for antidepressant treatment to be more effective (as measured by SQI and memory normalization) but in some cases this is not enough to alleviate depression.

Even though BDNF is not the best measure for depression outcomes, it is still an important factor to consider in the mechanism of depression and its treatment. A team in China recently introduced a traditional Chinese anti-depressant named XingPiJieYu into their lab for study. This extract from the herb of the same name greatly increased serum BDNF in rodents exposed to chronic unpredictable stress⁷. This is exciting for the future of antidepressant treatment in which XPJY may be co-prescribed with another anti-depressant to increase its efficacy. This is a promising

view but it may be too reductive to view the complex hormonal system would function this way. The UK government funded a large clinical trial (n=165) to see if co-prescribing anti-glucocorticoids with antidepressants could better treat depression because chronically elevated cortisol is a common symptom of depression. Unfortunately the study found this co-prescription had no effect on outcome⁸. Will BDNF treatment follow the same pattern or will XPIJY cause a revolution in depression treatment?

While we wait for clinical trials, what can we do to increase our BDNF? Exercise. It has been well established that aerobic exercise increases systemic BDNF levels, with one study finding that even a single bout of exercise significantly increases BDNF in rodents⁹. Two separate studies in particular have gone one step further and have implicated this interaction to be able to predict memory performance, one in rats and the other in young adult humans^{10,11}. Understanding hormonal systems is vital to develop better clinical therapies.

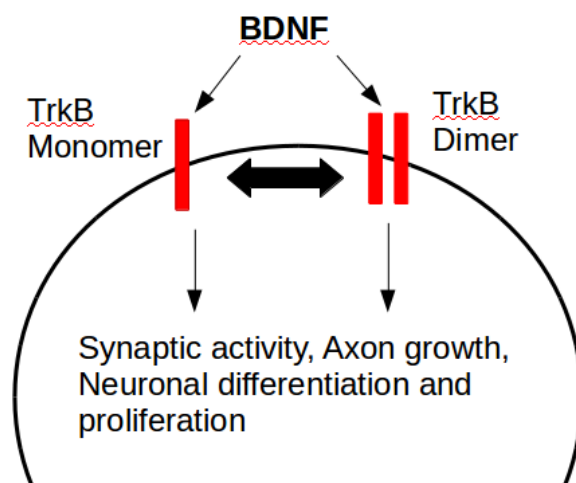


Figure 1. A basic overview of the BDNF-TrkB system. It has been theorized that a large part of the functioning of BDNF is based on the membrane ratio between TrkB monomerization and dimerization. BDNF has a large impact of DNA expression and its effects go well beyond memory and learning, including formation of the CNS by influencing proliferation and differentiation of neurons¹².

REFERENCES

- Engelmann J, Wagner S, Wollschlager D, Kaaden S, Schlicht KF, Dreimuller N, Braus DF, Muller MB, Tuscher O, Frieling H, Tadic A, Lieb K. (2019). Higher BDNF plasma levels are associated with a normalization of memory dysfunctions during an antidepressant treatment. *European Archives of Psychiatry and Clinical Neuroscience*. Mar 30 [Epub ahead of print]
- Linz R, Puhmann LMC, Apostolakou F, Mantzou E, Papassortiriou I, Chrousos GP, Engert V, Singer T. (2019) Acute psychosocial stress increases serum BDNF levels: an antagonistic relation to cortisol but no group differences after mental training. *Neuropsychopharmacology*. Apr 16 [Epub ahead of print]
- Leal G, Bramham CR, Duarte CB. (2017) BDNF and Hippocampal Synaptic Plasticity. *Vitamins and Hormones*. 104:153-195.
- Kranaster L, Hellweg R, Sartorius A. (2019) Association between the novel seizure quality index for the outcome prediction in electroconvulsive therapy and brain-derived neurotrophic factor serum levels. *Neuroscience Letters*. Apr 9;704:164-168.
- Hinkelmann K, Moritz S, Botzenhardt J, Muhtz C, Wiedemann K, Kellner M, Otte C. (2012) Changes in cortisol secretion during antidepressive treatment and cognitive improvement in patients with major depression: A longitudinal study. *Psychoneuroendocrinology*. May;37(5):685-692.
- Anil Kumar BN, Grover S. (2016) Cushing's Syndrome Masquerading as Treatment Resistant Depression. *Indian Journal of Psychological Medicine*. May-Jun;38(3):246-8.
- Wang C, Guo J, Guo R. (2017) Effect of XingPiJieYu decoction on spatial learning and memory and cAMP-PKA-CREB-BDNF pathway in rat model of depression through chronic unpredictable stress. *BMC Complementary & Alternative Medicine*. Jan;17:73.
- Ferrier IN, Anderson IM, Barnes J, Gallagher P, Grunze HCR, Haddad PM, House AO, Hughes T, Lloyd AJ, Mamasoula C, McColl E, Pearce S, Siddiqi N, Sinha B, Speed C, steen N, Wainwright J, watson S, Winter FH, McAllister-Williams RH; the ADD Study Team. (2015) Randomised controlled trial of Antiglucocorticoid

- augmentation (metyrapone) of antiDepressants in Depression (ADD Study). *Efficacy and Mechanism Evaluation*. Jun.
9. Venezia AC, Quinlan E, Roth SM. (2017) A single bout of exercise increases hippocampal Bdnf: influence of chronic exercise and noradrenaline. *Genes, Brain and Behavior*. May 26;16(8)
 10. Loprinzi PD. (2019) Does brain-derived neurotrophic factor mediate the effects of exercise on memory? *The Physician and Sportsmedicine*. Apr 19 [Epub ahead of print]
 11. Whiteman AS, Young DE He X, Chen TC, Wagenaar RC, Stern CE, Schon K. (2014) Interaction between serum BDNF and aerobic fitness predicts recognition memory in healthy young adults. *Behavioral Brain Research*. Feb 1;259:302-12.
 12. Gupta V, You Y, Gupta Veer, Klistorner, Graham S. (2013) TrkB Receptor Signalling: Implications in Neurodegenerative, Psychiatric and Proliferative Disorders. *International Journal of Molecular Sciences*. May 13;14(5):10122-10142.
 13. Sorri A, Jarventausta K, Kampman O, Lehtimaki K, Bjorkqvist M, Tuohimaa K, Hamalainen M, Moilanen E, Leinonen E. (2018) Effect of electroconvulsive therapy on brain-derived neurotrophic factor levels in patients with major depressive disorder. *Brain and Behavior*. Nov;8(11):ed01101



Functional prions are in the central nervous system; how they influence memory!

Prions are an incredible class of proteins which offer many beneficiary functions to the central nervous system, such as cytoplasmic polyadenylation element-binding protein (CPEB), its role in long-term memory and synaptic plasticity. Prions also serve as a harmful misfolded protein within the nervous system, this caused all prions to classically be identified as the forefront for neurodegenerative illnesses. TIA-1 is also a crucial functioning prion and it's known for its role during stress in the brain and programmed cell death. It is now known to be a key member of normal cell biology.

Victor Cosovan

Prions are a new class of proteins; they are a proteinaceous infectious particle which lacks nucleic acids but alter protein structure upon contact with specific neurological structures. Originally prions were found to be etiological agents of neurodegenerative diseases such as, Creutzfeldt–Jakob disease, kuru, and transmissible encephalopathies. The study of specific functional prions is important in understanding how synapses are formed and how the mechanisms function. By understanding the role of CPEB in synaptic function, many medicines for neurodegenerative diseases and other synaptic affiliated disorders can be restored or vindicated for the patients. TIA-1 is a wonderful example of a prion structure in which it facilitates normal cellular function in the duration of the cell's life span, but TIA-1 also has situations when it activates mechanisms which may lead to pathological and physiological irregularities leading to many forms of neurological disease.

A great representation of a prion is most formally known as its acronym form, CPEB (cytoplasmic polyadenylation element-binding protein). Recently, a hypothesis has emerged that prion like, self-templating mechanisms also underlie a variety of neurodegenerative disorders such as, amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease. New found evidence suggests that a variety of neurodegenerative conditions that are not transmissible between individuals as prions traditionally may nevertheless be caused by self-templating, prion-like mechanisms such as CPEB and TIA-1 or other prion like

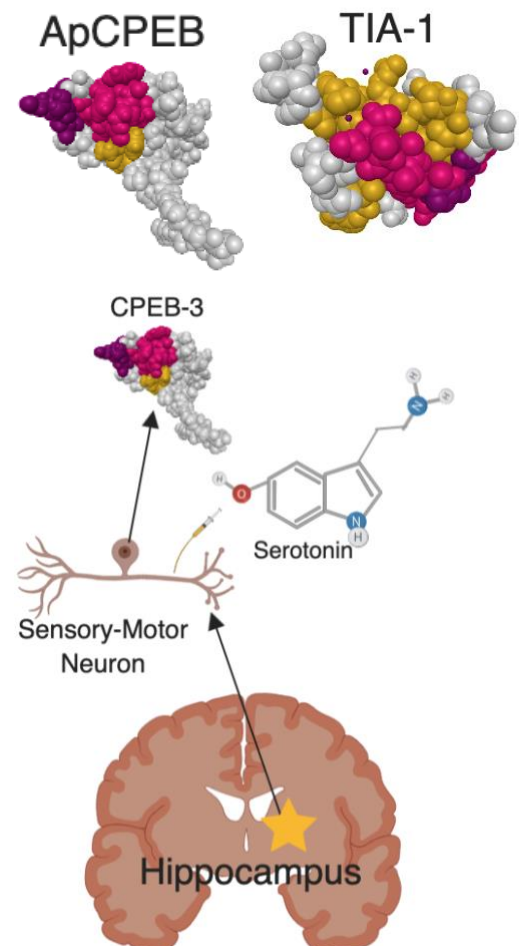


Figure 5. The persistence of synaptic plasticity and memory storage requires CPEB-3-mediated protein synthesis in the hippocampus. Serotonin pulse injections for synaptic memory-consolidation.

proteins. In this experiment a marine sea snail was stimulated for a siphon reflex and under vivo and vitro, the learning of the 5-HT marked synapses were observed for 72 hours for a change in gene expression in the synapse and for presence of CPEB and its role in the two types of memory construction. It is still needed to be researched about how that prion-like proteins can contribute to normal physiological processes in the nervous system, for only a few cases have been identified and studied. Prions pose a crucial role in neurophysiology and are needed for synaptic regulation.

THE ROLE OF CPEB IN APLYSIA

This prion like structure is known and named for its characteristic of binding multiple adenosine bases which causes a change in the protein shape, thus function too. For this study, the CPEB is tested within the cytoplasm of a sensory-motor neuron in a marine sea snail, *Aplysia californica* due to the simplicity of its nervous system. CPEB was examined for how conversion from a soluble to a prion-like conformation regulates protein synthesis at the synapse, thus modulating both the maintenance of synaptic plasticity and long-term memory. Aplysia is the subject because it has a number of simple reflexes that can be modified by different forms of learning. One of the tested examples includes the gill-withdrawal reflex when the siphon of the snail is rapidly withdrawn due to stimuli. The neural circuit of the snail's reflex has an important monosynaptic connection in where sensory neurons that innervate the siphon make direct connections with the motor neurons, thus moving the gill in the reflex mechanism. As behavioral memory works, there are two forms of memory, short-term (a few minutes) to long-term memory (days or weeks) with in the synaptic plasticity of the sensory and motor neurons. The short-term memory form depends on modifications of preexisting proteins mediated in part by protein kinase A which will strengthen the preexisting connections. Also, for the long-term form of memory, it is required that both new protein synthesis and CREB-mediated gene expression, which drives the remodeling of current existing synapses and to form new synaptic connections. In the role of synaptic plasticity, gene products are sent to all synapses for the mechanism of long-term memory synapse in each neuron because of the release of 5-HT (serotonin) is a marker of the synapses which will receive these gene products. The pathway for synapse specific long-term memory is a bidirectional process between the synapse and the nucleus. The synaptic marker for stabilization in the neuron was found to be activation of transitionally dominant mRNAs. CPEB was found to be the activator of dominant mRNAs in the process of elongation of the poly-A tail. Polyadenylation-dependent translational control requires a cis-acting cytoplasmic polyadenylation element (CPE) in the 3' UTR of the mRNA where CPEB can bind and recruit a variety of partner proteins to further an action. It was found that ApCPEB can be activated at the terminal by just a single pulse of 5-HT (Figure 1). The aggregated conformation that is the active form of CPEB promotes translation of target mRNAs, it is not harmful to the cell, usually prions in their aggregated state can bare harm. Using green florescent protein, they were able to tag CPEB and follow it through its aggregated state and use the florescent dye to image cells upon stimulation. The results show that the two forms of CPEB work in sequence and play a role in synaptic consolidation.

Another study focused on detecting aggregated ApCPEB by adding a florescent marker on them. After 5-HT pulses on the sensory-motor neuron on Aplysia, it was found that more aggregated ApCPEB were found in the synapses. It was predicted that blocking aggregated ApCPEB would then destabilize the maintenance of long-term facilitation of the synapses, but actually after injecting the blocking anti-body, synaptic facilitation did not persist beyond 48 hours after Ab464-injected cells. Thus, it is supported that the multimeric form of ApCPEB is involved in long-term stabilization of synaptic activity. Learning-induced activation of ApCPEB initiates a cellular positive feedback loop within the synapses, whereas ApCPEB promotes the activation of more ApCPEB

through specific conformational changes within the synapse. Thus, this is potentially a powerful mechanism for generating a self-perpetuating local signaling at the synapse.

A study in the CPEB in the *Drosophila* brain suggests that the expresser epigenetic prion like protein, Orb2/ CPEB are acting to stabilize activity-dependent changes within the synapse. Prion like conversion of Orb2 is necessary for long-term memory because failure to form Orb2 amyloid leads to the loss of facilitation in sensory-motor neuron synapses on inhibition of the ApCPEB oligomers. The break in the mechanism for ApCPEB is a leading factor in failure to sustain or form consolidated memories due to the lack of synaptic regulation. Flies with Orb2 deletion fail to show characteristics of long-term memory tasks and functions.

CPEB IN THE MAMMALIAN BRAIN

In recent studies, they searched for CPEB orthologs in the mouse brain and identified four isoforms of CPEB, CPEB-1 to CPEB-4. Of these four isoforms, CPEB-3 resembles ApCPEB because it is neuronally specific translational regulator with an unstructured Q/N-rich domain at its N-terminus and RNA-binding domains at its C-terminus. This resembles the make-up of prion like structures. The studies in hippocampal neurons provide evidence that mCPEB-3, like its invertebrate orthologs, ApCPEB and Orb2, displays the basic features of prion proteins within the nervous system (Figure 1). It was found that CPEB-3 binds to and also represses the translation of target mRNAs as a regulatory in long-term facilitation. When CPEB-3 is activated by specific post-translational modifications during active learning, CPEB-3 was able to transition from a soluble protein into an aggregated form, which thus promotes the translation of the AMPA receptor. This process leads to maintenance of synaptic activity in the synapse. In order to determine the role of CPEB-3 in synaptic plasticity and memory, a conditional knockout strain of CPEB-3 was generated and tested. It was revealed that CPEB-3-mediated protein synthesis is crucial for the maintenance of protein synthesis dependent long-term potentiation (LTP), but as found, not for short-term LTP or memory acquisition due to a different mechanism of synaptic regulation. Furthermore, CPEB-3 has shown that loss of the N-terminus, which is necessary for its aggregated form, loses its ability to maintain long-term synaptic plasticity and memory. In this process it can be concluded that AMPA receptor subunits, GluA1 and GluA2 provide the first evidence for a prion-like mechanism to sustain memory in the mouse brain, during both the processes of consolidation and maintenance.

PRIONS LEAD TO PATHOLOGICAL ILLNESS AND MAJOR NON-TRANSMITTABLE DISEASE

Unlike pathological prions, aggregated prions within the nervous system are regulated physiologically and pose no current threat as traditionally thought to be. CPEB expression is regulated by microRNAs (miRNAs). Therefore, a neuron-specific miRNA, like miRNA-22 inhibits ApCPEB mRNA through many 3' UTR binding sites and it is downregulated by serotonin by increasing and even enhancing long-term memories. Whereas, over expression will inhibit long-term facilitation of the synapse and block the formation or consolidation of new memories. The prion, ApCPEB has coiled-coil α -helices that can mediate prion-like oligomerization, unlike β -sheets, coiled-coils are responsive to cellular and environmental signals, therefore the prion can be regulated. This is what sets functional prions apart from pathologically harmful prions.

The pathogenic proteins associated with Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis are each non-transmittable disorder, for example amyloid β , in Alzheimer's disease and huntingtin, in Huntington's disease follow a spontaneous switch from a

regular conformation to a self-propagating, aggregated structure that is cytotoxic. In greater detail, yeast prions are characterized by their ability to pass on phenotypes from mother to daughter cell in a stable, non-Mendelian manner and also without involvement of nucleic acid-based mechanisms within the cells.

ADDITIONAL FUNCTIONAL PRIONS IN THE BRAIN

There are not just the basic prions in regulating synaptic function, there are other remarks about prions in the central nervous system. TIA-1 for example, was identified as a functional prion-like protein in the mouse hippocampus. TIA-1 can facilitate the assembly of cytoplasmic aggregates known as stress granules in response to extreme environmental condition, or when the cell is under 'stress.' This functionality is what allows the prion to be a key member in programmed cell death. Like the previous prions discussed, TIA-1 aggregates are amyloid-rich and show heritability in a yeast assay. TIA-1 is important in regulatory function which prevents neurodegenerative changes in the brain.

REFERENCES

1. Rayman, J. B., & Kandel, E. R. (2017). Functional prions in the brain. *Cold Spring Harbor perspectives in biology*, 9(1), a023671. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5204323/>
2. Si, K., & Kandel, E. R. (2016). The role of functional prion-like proteins in the persistence of memory. *Cold Spring Harbor perspectives in biology*, 8(4), a021774.
3. Casadio, A., Martin, K. C., Giustetto, M., Zhu, H., Chen, M., Bartsch, D., ... & Kandel, E. R. (1999). A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell*, 99(2), 221-237.
4. Fioriti, L., Myers, C., Huang, Y. Y., Li, X., Stephan, J. S., Trifilieff, P., ... & Kandel, E. R. (2015). The persistence of hippocampal-based memory requires protein synthesis mediated by the prion-like protein CPEB3. *Neuron*, 86(6), 1433-1448.
5. Fiumara, F., Fioriti, L., Kandel, E. R., & Hendrickson, W. A. (2010). Essential role of coiled coils for aggregation and activity of Q/N-rich prions and PolyQ proteins. *Cell*, 143(7), 1121-1135.
6. Fiumara, F., Rajasethupathy, P., Antonov, I., Kosmidis, S., Sossin, W. S., & Kandel, E. R. (2015). MicroRNA-22 gates long-term heterosynaptic plasticity in *Aplysia* through presynaptic regulation of CPEB and downstream targets. *Cell reports*, 11(12), 1866-1875.
7. Frey, U., & Morris, R. G. (1997). Synaptic tagging and long-term potentiation. *Nature*, 385(6616), 533-536.
8. Gebauer, F., & Richter, J. D. (1996). Mouse cytoplasmic polyadenylation element binding protein: an evolutionarily conserved protein that interacts with the cytoplasmic polyadenylation elements of *c-mos* mRNA. *Proceedings of the National Academy of Sciences*, 93(25), 14602-14607.
9. Gilks, N., Kedersha, N., Ayodele, M., Shen, L., Stoecklin, G., Dember, L. M., & Anderson, P. (2004). Stress granule assembly is mediated by prion-like aggregation of TIA-1. *Molecular biology of the cell*, 15(12), 5383-5398.
10. Hake, L. E., & Richter, J. D. (1994). CPEB is a specificity factor that mediates cytoplasmic polyadenylation during *Xenopus* oocyte maturation. *Cell*, 79(4), 617-627.
11. Hou, F., Sun, L., Zheng, H., Skaug, B., Jiang, Q. X., & Chen, Z. J. (2011). MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell*, 146(3), 448-461.
12. Huang, Y. S., Kan, M. C., Lin, C. L., & Richter, J. D. (2006). CPEB3 and CPEB4 in neurons: analysis of RNA-binding specificity and translational control of AMPA receptor GluR2 mRNA. *The EMBO journal*, 25(20), 4865-4876.
13. Keleman, K., Krüttner, S., Alenius, M., & Dickson, B. J. (2007). Function of the *Drosophila* CPEB protein Orb2 in long-term courtship memory. *Nature neuroscience*, 10(12), 1587.
14. Krüttner, S., Stepien, B., Noordermeer, J. N., Mommaas, M. A., Mechtler, K., Dickson, B. J., & Keleman, K. (2012). *Drosophila* CPEB Orb2A mediates memory independent of its RNA-binding domain. *Neuron*, 76(2), 383-395.
15. Martin, K. C., Casadio, A., Zhu, H., Yaping, E., Rose, J. C., Chen, M., ... & Kandel, E. R. (1997). Synapse-specific, long-term facilitation of *aplysia* sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell*, 91(7), 927-938.
16. Morgan, M., laconig, A., & Muro, A. F. (2010). CPEB2, CPEB3 and CPEB4 are coordinately regulated by miRNAs recognizing conserved binding sites in paralog positions of their 3'-UTRs. *Nucleic acids research*, 38(21), 7698-7710.

Cannabis helps your brain perform like new

Chronic low doses of THC can restore your brain power and help you perform as if you were half your age.

Vincente Chavez

The brain is complex with a network of many different neuron clusters with different functions. Everything from your genetics to your environment influences the way your neurons communicate. Drugs can influence and alter brain function and communication pathways with a wide range of mechanisms. Recently a lot of pharmacotherapy research focus has gravitated towards the endocannabinoid system (ECS) because of the vast reach it has in the body¹. Initially banned for sale or use with the Marijuana Tax Act of 1937, later replaced with The Controlled Substance Act of 1970, research was stalled and non-existent for a long time. The isolation of endocannabinoid chemicals in the 1960s paved the way for the discovery of cannabinoid receptors such as CB1^{2,3,4}. Since these initial findings, medical potential and money have driven research into this system with an ever-increasing number of physiological functions⁵.

New research implicating the ECS in a variety of homeostatic tasks, such as energy balance and hormone regulatory functions, sheds light to the critical role the ECS plays in our body⁶. CB1 receptors are predominately found in the brain as neuronal plasma membrane receptors⁷. They are also responsible for binding delta-9-Tetrahydrocannabinol (THC), the main psychoactive compound of cannabis⁸. When activated, CB1 receptors produce a cascade effect that inhibits neurotransmitter release impacting communication among neurons. Interestingly, deletion of CB1 receptors in mice has been found to produce age-related cognitive impairment, highlighting the potential role that the ECS plays in the molecular mechanisms of aging^{9,10}. Brain aging is highly associated with cognitive decline and the role and impact of the ECS as many other internal systems on the aging process remains unclear. Aging mice have been shown to have decreasing ECS activity comprised of CB1 receptor expression, as well as a decrease in 2-arachidonyl glycerol (2-AG) and anandamide¹¹. 2-AG and anandamide are both molecules produced within the body that may bind to CB1 receptor¹². Gaining great attention is new research finding improved cognitive function in old mice receiving chronic low doses of THC¹³. As any drug addiction is important to consider.

Research has revealed potential increase in dependence and increased risk taking in adolescences^{14,15}. Fortunately, this new research uncovers room for older adults to reap the benefits. This study was conducted with two main groups of mice, THC-rich and THC-poor; three sub-groups young, mature and old (2, 12 and 18 months old respectively). THC was delivered through minipumps that were implanted, releasing 3 mg/kg bodyweight for a month. Tests including Morris Water Maze (MWM), training and reversal training phase, novel object recognition test and social recognition test were accomplished (Figure 1). These tests were conducted to test a variety of cognitive function such as spatial memory and learning, memory impairments and long-term memory^{16,17,18}. The goal of the tests was to determine if prolonged THC exposure in low dosage had lasting effects on learning and memory. Effects of age and

treatment on spatial learning and memory were assessed with the MWM. The mature and old mice treated with THC performed at the level of THC-free 2-month-old mice indicating improved spatial learning and memory. Secondly effects of learning flexibility were assessed with the reversal phase testing which entails training of an animal to respond differentially to two stimuli such as approach or avoidance, rewarding and punishing conditions when responding inappropriately. The reversal phase would include the reversal of reward and punishment rewarding what was previously punished and vice versa. “Training an animal two tricks, punishing them when they respond incorrectly. After a while, the rewards and punishment get switched, testing how long it takes for the animal to change their behavior” (Piercy from neuroanatomy). The THC mature and old mice once again showed improved cognition and improved learning flexibility. Long-term spatial memory was tested with Novel Object Location Recognition and Social Recognition Test. Once again, the cognition improved in mature and old mice that had been treated with THC (Figure 1).

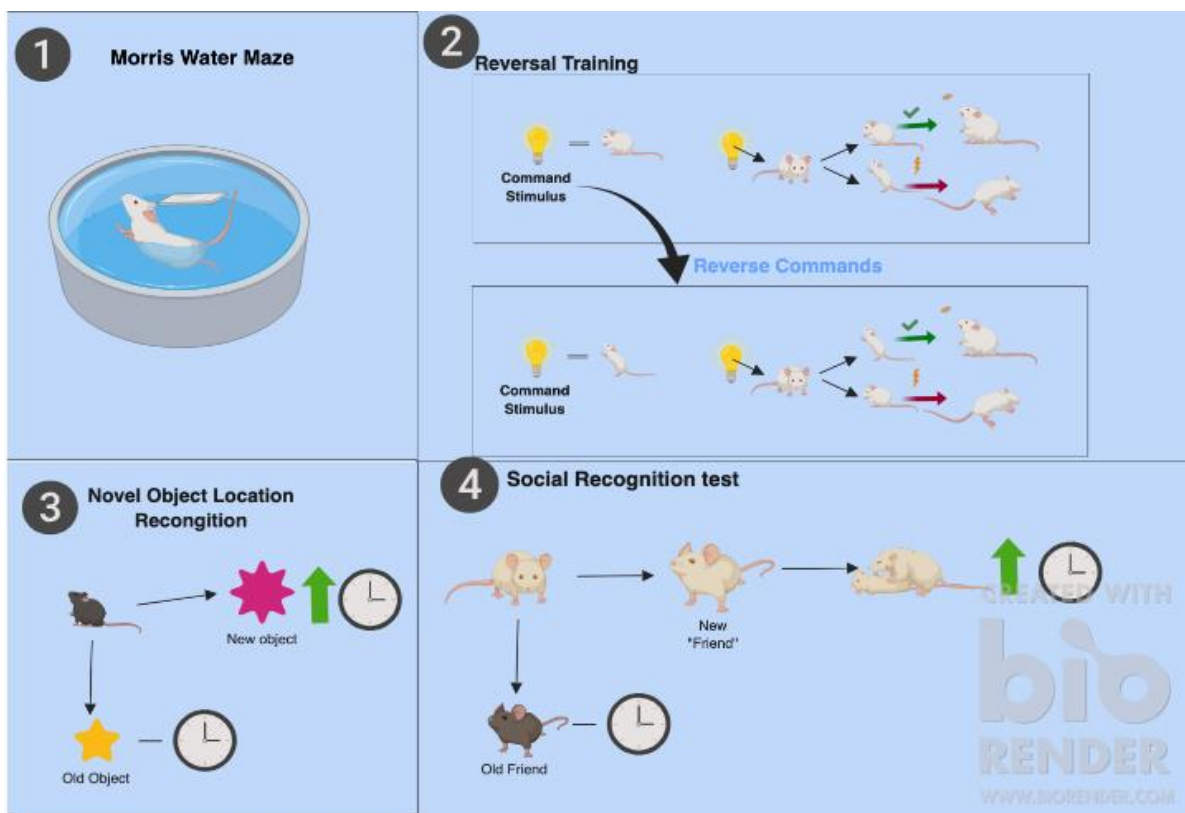


Figure 6. 1) Morris Water Maze to test spatial learning and memory 2) Reversal Training to test for learning flexibility 3) Novel Object Location Recognition and 4) Social Recognition test to test long-term memory. (Bilkei-Gorzo et al. 2017)

Each test showed decline of THC-free cognition in mature and old mice when compared to young mice. Concurrently THC-rich cognition improved in mature and old mice and resembled patterns of THC-free young mice (Figure 2). This evidence points towards internal mechanisms that alleviate stress on the brain helping mature and old mice perform with greater accuracy and more efficiency. This research is promising for older adults that seek to gain brain performance abilities as age induces decline in brain function. The stunted research of THC has limited our knowledge, but with ever growing legalization and societal acceptance more research is needed into the impacts on the youth and the chronic impacts on adult health.

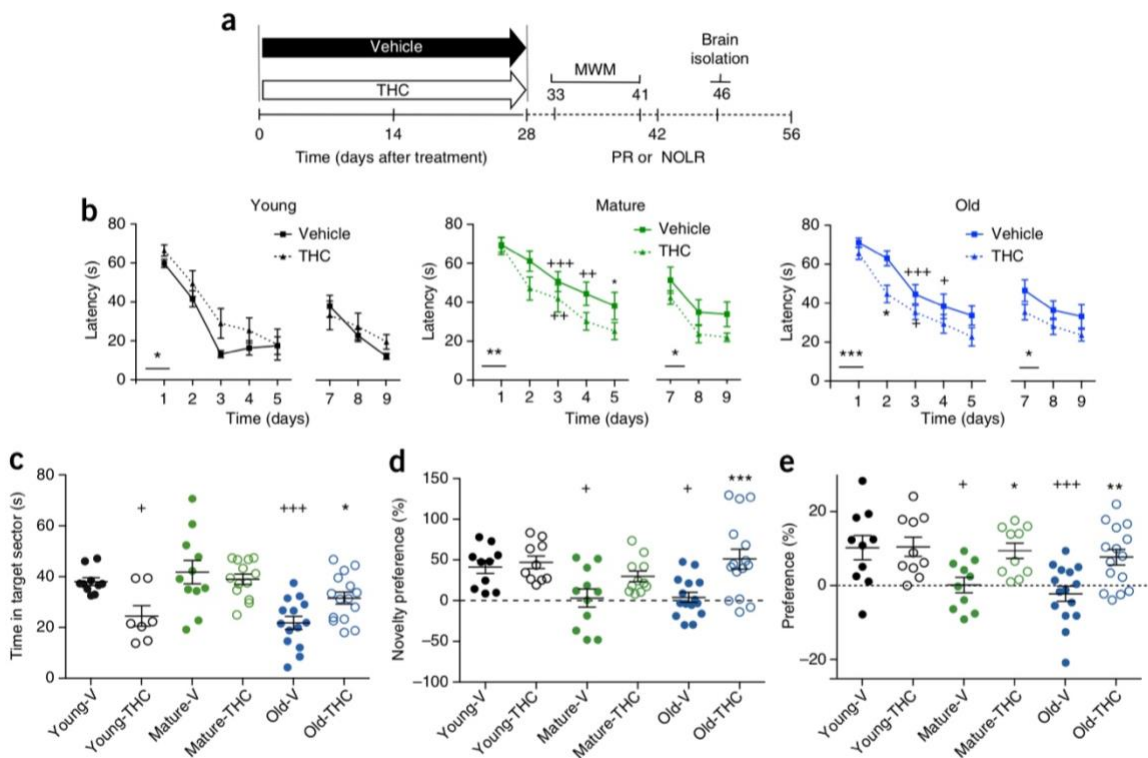


Figure 7. A) Timeline of treatment B) Morris Water Maze C) Probe Reversal Phase Testing D) Novel Object Recognition E) Social Recognition Test. (Bilkei-Gorzo et al. 2017)

REFERENCES

- Pacher, P., Bátkai, S., & Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacological reviews*, 58(3), 389-462.
- Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American chemical society*, 86(8), 1646-1647.
- Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, 346(6284), 561.
- Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, 365(6441), 61.
- Di Marzo, V., Bifulco, M., & De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nature reviews Drug discovery*, 3(9), 771.
- Pagotto, U., Marsicano, G., Cota, D., Lutz, B., & Pasquali, R. (2005). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrine reviews*, 27(1), 73-100.
- Fukuda, S., Kohsaka, H., Takayasu, A., Yokoyama, W., Miyabe, C., Miyabe, Y., ... & Nanki, T. (2014). Cannabinoid receptor 2 as a potential therapeutic target in rheumatoid arthritis. *BMC musculoskeletal disorders*, 15(1), 275.
- French, E. D. (1997). Δ^9 -Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neuroscience letters*, 226(3), 159-162.
- Bilkei-Gorzo, A., Racz, I., Valverde, O., Otto, M., Michel, K., Sarstre, M., & Zimmer, A. (2005). Early age-related cognitive impairment in mice lacking cannabinoid CB1 receptors. *Proceedings of the National Academy of Sciences*, 102(43), 15670-15675.
- Berrendero, F., Romero, J. U. L. I. A. N., Garcia-Gil, L., Suarez, I., De la Cruz, P., Ramos, J. A., & Fernandez-Ruiz, J. J. (1998). Changes in cannabinoid receptor binding and mRNA levels in several brain regions of aged rats. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1407(3), 205-214.

11. Cannich, A., Wotjak, C. T., Kamprath, K., Hermann, H., Lutz, B., & Marsicano, G. (2004). CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. *Learning & memory*, 11(5), 625-632.
12. Kano, M., Ohno-Shosaku, T., Hashimoto-dani, Y., Uchigashima, M., & Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiological reviews*, 89(1), 309-380.
13. Bilkei-Gorzo, A., Albayram, O., Draffehn, A., Michel, K., Piyanova, A., Oppenheimer, H., ... & Bab, I. (2017). A chronic low dose of Δ^9 -tetrahydrocannabinol (THC) restores cognitive function in old mice. *Nature Medicine*, 23(6), 782.
14. Degenhardt, L., Coffey, C., Carlin, J. B., Swift, W., Moore, E., & Patton, G. C. (2010). Outcomes of occasional cannabis use in adolescence: 10-year follow-up study in Victoria, Australia. *The British Journal of Psychiatry*, 196(4), 290-295.
15. Hall, W., & Degenhardt, L. (2007). Prevalence and correlates of cannabis use in developed and developing countries. *Current opinion in Psychiatry*, 20(4), 393-397.
16. Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols*, 1(2), 848.
17. Antunes, M., & Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cognitive processing*, 13(2), 93-110.
18. Schoenbaum, G., Chiba, A. A., & Gallagher, M. (2000). Changes in functional connectivity in orbitofrontal cortex and basolateral amygdala during learning and reversal training. *Journal of Neuroscience*, 20(13), 5179-5189.

Targeting chondroitin sulfate proteoglycans in order to reduce neural inflammation and promote oligodendrogenesis

Damage to the central nervous system results in limited tissue regeneration and permanent injuries such as paralysis. A study based in the University of Manitoba done by Dyck et al shows the cellular mechanism available for targeting to counteract this phenomenon.

Jason Driver

In 2007 the World Health Organization estimated the prevalence of those suffering from neurological disorders to be greater than 1 in 7, with more than 1 billion cases of neurological damage and 6.8 million deaths each year attributed to neurological damage¹. A unique feature about central nervous system damage is that it is generally viewed to be permanent. For the most part, damaged neural connections will remain in that state for the rest of the subject's life². The motivation for this research was to better understand the reasons the body does not recover from spinal cord injury, with the long term hope of applying this knowledge towards building treatments.

The resistance of the adult CNS to regeneration is rooted in its physiology, specifically its extracellular matrix. The matrix inhibits regeneration through three classes of molecules; myelin-associated growth inhibitors, chemorepulsive guidance molecules, and highly sulfated proteoglycans with the greatest contributing factor being the proteoglycans often called CSPGs³.

It has been seen for centuries that the central nervous system resists regeneration, with spinal cord injuries and brain trauma being permanent⁴. As analytical technology has been improved upon and new techniques and machines created, our ability to understand the why behind the central nervous system rejecting regeneration has also improved. The extracellular matrix of the central nervous system differs greatly from the majority of the body, including the peripheral nervous system. Oligodendrocytes and CNS associated astrocytes and microglia all excrete multiple forms of growth inhibiting factors, the most prominent of which being CSPGs⁵. CSPGs consist of a protein core and a sugar chondroitin sulfate side chain; the sugar side chain sulfonation pattern can change influencing how CSPGs bind and the inhibitory factors associated with them. CSPGs inhibit neuroregeneration by binding to growth-inhibitory receptors found at axons, directly activating the growth inhibitory pathway, inhibiting neurotrophic receptors and activating phosphatases⁶. Though, while it is known that CSPGs play an integral role in neural regulation, it is not fully understood how they do so.

One portion that is known, is that leukocyte common antigen-related (LAR), and protein tyrosine phosphatase-sigma (PTP σ) are both CSPG signalling receptors of oligodendrocytes in the CNS⁷. It's been seen that inhibition of PTP σ and LAR receptors promotes oligodendrogenesis, supporting recovery of the spinal cord⁶.

The article in focus from Dyck et al., 2019 goes into more depth on how the LAR PTP σ pathway can be targeted in a potentially clinically beneficial manner⁸.

The study used female Sprague Dawley rats and targeted LAR and PTP σ through LAR peptide (ILP) and intracellular sigma peptide (ISP); two peptides blocking the receptors for LAR and PTP σ . ILP and ISP are designed to bind to a 24-amino acid intracellular wedge domain on oligodendrocytes, modulating the catalytic activity of these receptors. Rats had their spinal cords crushed and solutions of ILP and ISP solutions were injected at the injury site in order to attempt to change the body's inflammatory and regenerative response. Figure 1 shows a cartoon model of the divergence in how the body addresses damage.

The study found a significant decrease in inflammation, pointing to the use of ILP & ISP solutions as an area of more study for recovering from spinal cord injury. The study specifically cited CSPG signaling manipulation, the basis of this research, as a promising approach to promote recovery. Overall, the work done by Dr. Dyck focusing on the microenvironment of spinal cord injuries is ground breaking and provides hope for the future of this research.

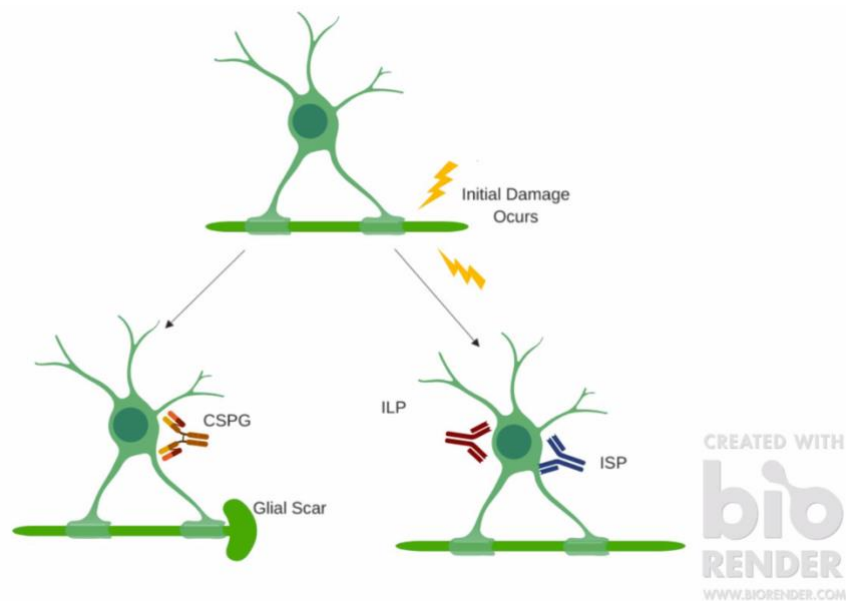


Figure 1. A cartoon model showing the divergence of CNS damage relating to CSPG binding presence. When ILP and ISPs block the receptor for CSPG, inflammation and damage-associated scars do not occur as easily.

REFERENCES

1. Neurological disorders affect millions globally: WHO report. (2007, February 27). Retrieved March 18, 2019, from <https://www.who.int/mediacentre/news/releases/2007/pr04/en/>
2. Purves D, Augustine GJ, Fitzpatrick D, et al., editors. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Recovery from Neural Injury. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK10856/>
3. Sharma, K., Selzer, M. E., & Li, S. (2012). Scar-mediated inhibition and CSPG receptors in the CNS. *Experimental neurology*, 237(2), 370-8.
4. Todd, R. B. (1855). *Clinical lectures on paralysis, disease of the brain, and other affections of the nervous system*. Lindsay & Blakiston.
5. Miller, G. M., & Hsieh-Wilson, L. C. (2015). Sugar-dependent modulation of neuronal development, regeneration, and plasticity by chondroitin sulfate proteoglycans. *Experimental neurology*, 274(Pt B), 115-25.

6. Lutz, A. B., & Barres, B. A. (2014). Contrasting the glial response to axon injury in the central and peripheral nervous systems. *Developmental cell*, 28(1), 7-17.
7. Takahashi, H., & Craig, A. M. (2013). Protein tyrosine phosphatases PTP δ , PTP σ , and LAR: presynaptic hubs for synapse organization. *Trends in neurosciences*, 36(9), 522–534. doi:10.1016/j.tins.2013.06.002
8. Dyck, S., Kataria, H., Akbari-Kelachayeh, K., Silver, J., & Karimi-Abdolrezaee, S. (2019). LAR and PTP σ receptors are negative regulators of oligodendrogenesis and oligodendrocyte integrity in spinal cord injury. *Glia*, 67(1), 125-145.
9. Properzi, F., Asher, R. A., & Fawcett, J. W. (2003). Chondroitin sulphate proteoglycans in the central nervous system: changes and synthesis after injury.
10. Xie, Y., Massa, S. M., Ensslen-Craig, S. E., Major, D. L., Yang, T., Tisi, M. A., ... & Brady-Kalnay, S. M. (2006). Protein-tyrosine Phosphatase (PTP) Wedge Domain Peptides A NOVEL APPROACH FOR INHIBITION OF PTP FUNCTION AND AUGMENTATION OF PROTEIN-TYROSINE KINASE FUNCTION. *Journal of Biological Chemistry*, 281(24), 16482-16492.
11. Dyck, S., Kataria, H., Alizadeh, A., Santhosh, K. T., Lang, B., Silver, J., & Karimi-Abdolrezaee, S. (2018). Perturbing chondroitin sulfate proteoglycan signaling through LAR and PTP σ receptors promotes a beneficial inflammatory response following spinal cord injury. *Journal of neuroinflammation*, 15(1), 90. doi:10.1186/s12974-018-1128-2
12. Dyck, S. M., & Karimi-Abdolrezaee, S. (2018). Role of chondroitin sulfate proteoglycan signaling in regulating neuroinflammation following spinal cord injury. *Neural regeneration research*, 13(12), 2080–2082. doi:10.4103/1673-5374.241452
13. Lau, L. W., Keough, M. B., Haylock-Jacobs, S., Cua, R., Döring, A., Sloka, S., ... & Yong, V. W. (2012). Chondroitin sulfate proteoglycans in demyelinated lesions impair remyelination. *Annals of neurology*, 72(3),419-432.

The brain on cocaine: a closer look at the cellular changes to blame

New research gives insight into the cellular changes that occur during cocaine addiction, a meaningful step towards treating the disorder.

Jonathan Anguiano

Cocaine is a highly addictive drug and has the potential to ruin the lives of those who fall victim to its grasp. In 2017, roughly 966,000 individuals of age 12 or older suffered from a cocaine use disorder¹. Of this population, many will relapse after a period of abstinence. Those who undergo standard treatment programs for cocaine addiction are similarly prone to relapse. The root of cocaine's evil lies in its power over the brain. If better treatments for cocaine addiction are to be developed, one must look to the brain and how cocaine influences its function. In a recent study published in *eNeuro*, Slaker et al.² elucidate the mechanisms underlying cocaine addiction by investigating the cocaine-induced cellular changes that occur in a brain region known as the medial prefrontal cortex (mPFC). Slaker and her research team were interested in this brain region because of past research showing that the mPFC controls behaviors related to cocaine addiction, at least, in rodents³. Additionally, Slaker et al. had conducted a previous study showing that removal of a structure known as the perineuronal net (PNN) in the mPFC could decrease relapse in a rodent model of cocaine addiction⁴. Many studies have revealed PNNs to be involved in learning and memory^{5,6}. In light of this, Slaker et al. concluded from the study that removing PNNs disrupts a rodent's memory of the rewards associated with cocaine, eliminating the drive for cocaine and decreasing relapse. The study described here builds on this research by characterizing the cellular changes in PNN-surrounded neurons. Interestingly, more than 90% of PNNs in the mPFC surround a specialized neuron known as the fast-spiking interneuron (FSI)². FSIs contribute greatly to the function of the mPFC and are themselves involved in learning and memory^{7,8}. FSIs accomplish this via communication with principal neurons, which project to various brain regions to influence behavior. Important to the development of treatments for cocaine addiction, Slaker et al. reveal significant changes in FSIs and PNNs that may contribute to cocaine addiction and relapse.

To replicate cocaine addiction in humans, the researchers administered cocaine to rats over the course of five days. The researchers then assessed how cocaine impacted levels of excitatory and inhibitory input onto FSIs. In general, excitatory input activates a neuron, like stepping on the gas pedal to ramp up the speed of a car. On the other hand, inhibitory input suppresses a neuron, like slamming on the brakes to come to a halt. After five days of cocaine administration, FSIs display signs of increased inhibitory input and an increased responsiveness to inhibitory input. Slaker et al. also measured cocaine-induced changes in FSI electrical properties. FSIs are themselves inhibitory, and measuring such changes in electrical properties gives insight into how cocaine affects the inhibitory control of principal neurons and, therefore, behavior. Slaker et al. found that the FSIs were overall less excitable after cocaine administration, meaning their ability

to inhibit would be greatly reduced. Additionally, the level of PNNs surrounding FSIs was decreased after one day of cocaine administration.

The study conducted by Slaker et al. represents an important advancement in the realm of understanding cocaine addiction. The results described above demonstrate that cocaine alters the excitatory/inhibitory balance. The excitatory/inhibitory balance describes an appropriate level of excitatory and inhibitory responses to a given event⁹. When this balance is disrupted, disease states, such as that of addiction, can ensue. In the mPFC, cocaine appears to decrease the ability of FSIs to inhibit principal neurons. This decrease frees principal neurons from inhibitory control, increasing excitation elicited by principal neurons and possibly leading to relapse (Fig 1). Indeed, previous research has shown that principal neurons display increased excitability in response to repeated cocaine administration^{10,11}.

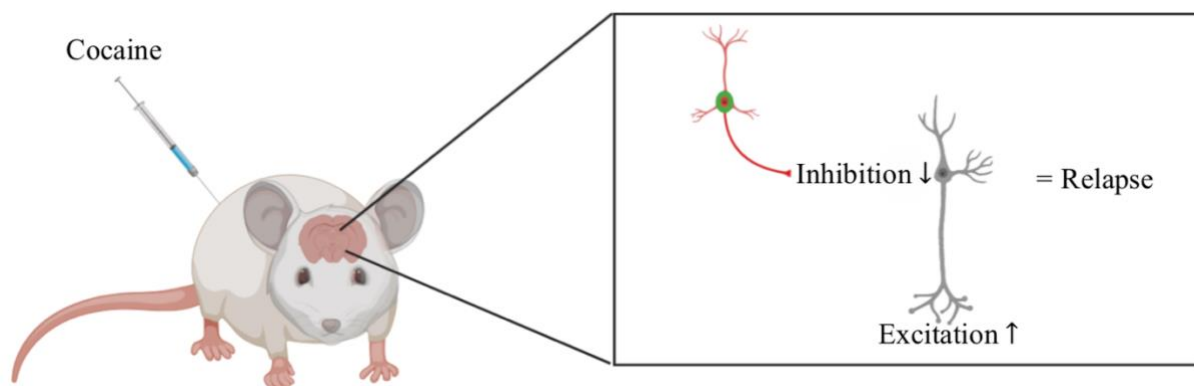


Figure 1. Cocaine administration induces changes in PNN (green) surrounded FSI (red) function, increasing excitatory output by principal neurons (grey) and promoting relapse.

Slaker et al. further suggest that the changes in FSIs may contribute to the chronic nature of drug addiction by altering their function in communication networks². The impact of cocaine on FSI function may be in part mediated by the observed decrease in PNN levels. This is consistent with research showing that a specific component of PNNs, known as brevican, controls FSI function¹². While the research by Slaker et al. does not test treatments for cocaine addiction, it is a necessary first step in preventing and treating the disorder. The FSI and PNN system may one day be targeted in order to reverse or attenuate characteristics of cocaine addiction. For example, transcranial magnetic stimulation (TMS) is already used to alter excitation and inhibition in disorders such as major depressive disorder. If the precise mechanism of cocaine addiction in the mPFC are identified, TMS may be able to target and manipulate activity of said brain region and thereby reverse characteristics of the disorder. Accordingly, future research should attempt to determine how FSIs might be taken advantage of in treating cocaine addiction.

REFERENCES

1. Substance Abuse and Mental Health Services Administration. (2018). *Key substance use and mental health indicators in the United States: Results from the 2017 National Survey on Drug Use and Health* (HHS Publication No. SMA 18-5068, NSDUH Series H-53). Rockville, MD: Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration. Retrieved from <https://www.samhsa.gov/data/>

2. Slaker, M. L., Jorgensen, E. T., Hegarty, D. M., Liu, X., Kong, Y., Zhang, F., . . . Sorg, B. A. (2018). Cocaine Exposure Modulates Perineuronal Nets and Synaptic Excitability of Fast-Spiking Interneurons in the Medial Prefrontal Cortex. *Eneuro* 5. doi:10.1523/eneuro.0221-18.2018
3. McLaughlin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine- seeking behavior in rats. *Psychopharmacology* 168:57–65.
4. Slaker, M., Churchill, L., Todd, R. P., Blacktop, J. M., Zuloaga, D. G., Raber, J., . . . Sorg, B. A. (2015). Removal of Perineuronal Nets in the Medial Prefrontal Cortex Impairs the Acquisition and Reconsolidation of a Cocaine-Induced Conditioned Place Preference Memory. *Journal of Neuroscience* 35:4190-4202. doi:10.1523/jneurosci.3592-14.2015
5. Balmer, T. S., Carels, V. M., Frisch, J. L., & Nick, T. A. (2009). Modulation of perineuronal nets and parvalbumin with developmental song learning. *The Journal of Neuroscience* 29:12878-85.
6. Romberg, C., Yang, S., Melani, R., Andrews, M. R., Horner, A. E., Spillantini, M. G., Bussey, T. J., Fawcett, J. W., Pizzorusso, T., ... Saksida, L. M. (2013). Depletion of perineuronal nets enhances recognition memory and long-term depression in the perirhinal cortex. *The Journal of Neuroscience* 33:7057-65.
7. Lee AT, Gee SM, Vogt D, Patel T, Rubenstein JL, Sohal VS (2014) Pyramidal neurons in prefrontal cortex receive subtype-specific forms of excitation and inhibition. *Neuron* 81:61–68.
8. Hu, H., Gan, J., & Jonas, P. (2014). Fast-spiking, parvalbumin GABAergic interneurons: From cellular design to microcircuit function. *Science* 345:1255263-1255263. doi:10.1126/science.1255263
9. Okun, M., & Lampl, I. (2009). Balance of excitation and inhibition. *Scholarpedia*, 4(8):7467
10. Dong Y, Nasif FJ, Tsui JJ, Ju WY, Cooper DC, Hu XT, Malenka RC, White FJ (2005) Cocaine-induced plasticity of intrinsic membrane properties in prefrontal cortex pyramidal neurons: adaptations in potassium currents. *J Neurosci* 25:936–940.
11. Nasif FJ, Sidiropoulou K, Hu XT, White FJ (2005) Repeated cocaine administration increases membrane excitability of pyramidal neurons in the rat medial prefrontal cortex. *J Pharmacol Exp Ther* 312:1305–1313.
12. Favuzzi E, Marques-Smith A, Deogracias R, Winterflood CM, Sánchez-Aguilera A, Mantoan L, Maeso P, Fernandes C, Ewers H, Rico B (2017) Activity-dependent gating of parvalbumin interneuron function by the perineuronal net protein brevican. *Neuron* 95:639– 655.e10.

Social anxiety isn't just for mammals anymore

A new study, "Social harassment induces anxiety-like behaviour in crayfish", shows a novel crayfish model for acute anxiety in response to harassment that responds well to pharmacological treatment.

Percy Atwell

In humans, anxiety is often brought about by social stressors and acute harassment¹. Social stress can also increase the risk of depression, heart disease, or substance abuse^{2,3,4}. In order to model this form of anxiety accurately, animal models focus on the use of rats and mice, or sometimes hamsters, to create situations of "social defeat" or harassments. These harassed animals may then be administered anxiolytics such as diazepam to attenuate the anxiety-like behavior⁶. Oddly enough, in certain mice strains, instead of displaying anxiety-like activity in a dark/light plus maze the mice show "catatonic-like immobility"⁷. While there exists a range of murine models for examining the efficacy of anxiolytics on attenuating anxiety-like behavior, there is room for improvement. In a study published in *Nature*, Bacqué-Cazenave, Cattaert, Delbecq, and Fossat set out to find another possible model for anxiety. Their paper shows how social harassment in crayfish induces anxiety-like behavior that is analogous to human anxiety. Examining crayfish in 2014, Bacqué-Cazenave and his colleagues found that the crayfish acted anxious after a physical stressor, but the existing body of research on crayfish had only established their use in models of aggression^{8,9}. Returning to crayfish once again, the authors set out this time to determine if social harassment would elicit anxiety-like behaviors; if so, would the crayfish respond to anti-anxiety drugs.

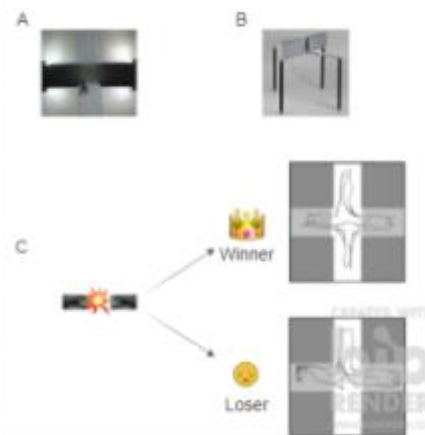


Fig. 1 Light/dark plus maze. **A)** Image of crayfish within the light/dark maze. **B)** Image of mouse within analogous maze. **C)** After social harassment period, crayfish routes within maze tracked with Ethovision software showed winning crayfish exploring the whole maze while losing crayfish stayed within dark arms. Images modified from Bacqué-Cazenave et al. 2017 and Linton Instrumentation.

When two crayfish males are placed together within a small aquarium, once they come face to face, they'll have a fight. These fights always end by one crayfish backflipping away, similar to the fight and defeat that murine models use. Also similar to murine models, the winner will continue to follow around the loser, attacking randomly. These repetitive hostile attacks are considered similar to harassment in humans. After 20 minutes paired together, allowing time for harassment to occur, both winner and loser crayfish were placed in an underwater dark/light plus maze (Fig. 1). The more a crayfish sticks to the dark, the more anxious it is. The crayfish were split into three

groups for the dark/light maze. One group was isolated without social contact to serve as a control, one group was the fight winners, and one group was all the losers. Both isolated and winner crayfish explored the entire maze, but loser crayfish stuck to the dark arms. Then, the losing crayfish were injected with Chlordiazepoxide, an anti-anxiety drug commonly used due to its low rate of intolerable side effects¹⁰. The drug-treated crayfish explored the entire maze, no longer showing an anxious preference for the dark arms. Compared to both saline-injected losers and untreated losers, this was a significant reduction of anxiety-like behavior.

These findings display the validity of crayfish as a model for studying anxiety or psychological harassment, examining either the losing or the winning crayfish in a pair respectively. Not only are crayfish useful for examining the underlying neurological pathways that allow both crustacean and human brains to experience anxiety, but they're sensitive to the same pharmacological treatments. Anxiety-like behavior in crayfish reacted to anxiolytic drugs in the same way that anxiety in humans, allowing for the use of crayfish in drug development research. Finally, while animal models of social stress show a great variety of behavioral changes that can be quantified and linked to anxiety-like activity, it is known that stressors may also affect the brain. Net changes in the amount of various neurotransmitters, especially serotonin, have been observed, and chronic social stress has shown shrinkage of dendritic arbors within the hippocampus in mammalian models^{11,12}. In the future, robust examinations of the effects of stress on serotonin or the hippocampal-like structures within the crayfish brain may provide an even stronger case for the use of crayfish models in the study of stress.

REFERENCES

1. Michopoulos, V., Higgins, M., Toufexis, D., & Wilson, M. E. (2012). Social subordination produces distinct stress-related phenotypes in female rhesus monkeys. *Psychoneuroendocrinology*, *37*(7), 1071-1085.
2. Kessler, R. C. (1997). The effects of stressful life events on depression. *Annual review of psychology*, *48*(1), 191-214.
3. Hemingway, H., Kuper, H., & Marmot, M. (2003). Psychosocial factors in the primary and secondary prevention of coronary heart disease: an updated systematic review of prospective cohort studies. *Evidence-based cardiology*, 181-218.
4. Brady, K. T., & Sonne, S. C. (1999). The role of stress in alcohol use, alcoholism treatment, and relapse. *Alcohol Research*, *23*(4), 263.
5. Blanchard, R. J., McKittrick, C. R., & Blanchard, D. C. (2001). Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiology & behavior*, *73*(3), 261-271.
6. Wolffgramm, J., & Heyne, A. (1991). Social behavior, dominance, and social deprivation of rats determine drug choice. *Pharmacology Biochemistry and Behavior*, *38*(2), 389-399.
7. Kudryavtseva, N. N., Bakshtanovskaya, I. V., & Koryakina, L. A. (1991). Social model of depression in mice of C57BL/6J strain. *Pharmacology Biochemistry and Behavior*, *38*(2), 315-320.
8. Huber, R., Smith, K., Delago, A., Isaksson, K., & Kravitz, E. A. (1997). Serotonin and aggressive motivation in crustaceans: altering the decision to retreat. *Proceedings of the National Academy of Sciences*, *94*(11), 5939-5942.
9. Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Delbecque, J. P., & Cattaert, D. (2014). Anxiety-like behavior in crayfish is controlled by Serotonin. *Science*, *344*(6189), 1293-1297.
10. National Center for Biotechnology Information. PubChem Database. Chlordiazepoxide, CID=2712, <https://pubchem.ncbi.nlm.nih.gov/compound/2712> (accessed on Apr. 18, 2019)
11. McKittrick, C. R., Magariños, A. M., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., & Sakai, R. R. (2000). Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. *Synapse*, *36*(2), 85-94.
12. Magariños, A. M., McEwen, B. S., Flügge, G., & Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *Journal of Neuroscience*, *16*(10), 3534-3540.
13. Bacqué-Cazenave, J., Cattaert, D., Delbecque, J. P., & Fossat, P. (2017). Social harassment induces anxiety-like behaviour in crayfish. *Scientific reports*, *7*, 39935.

Are you one of 70% people that want to learn a foreign language: Have a nap and learn

New foreign words and their translated words could be stored into memory as associations during a midday nap.

Liubov Beregova

Seventy percent of the population is interested in learning another language; however, most people do not have enough time². As opposed to the standard 40 hours workweek, the average American works a 47-hour per week, which is equivalent to working an extra day³. Forty seven percent of workers argue that they do not have enough free time; American mothers only have an estimated 36 minutes of leisure time⁴. If humans cannot find a time during the day to learn a few new words, is it possible to store new information during sleep? This was the research question of a scientist from the University of Bern in Switzerland. Most sleep research focuses on the consolidation (strengthening) and stabilization of memories that are formed during periods of consciousness. However, the examination of learning during sleep is rarely performed. For the first time, Katharina Henke and her colleagues showed that new foreign words and their translated words could be stored into the memory as associations during a midday nap¹. Following waking, when represented with formerly sleep-played foreign words, participants were able to reactivate the sleep-formed associations to access word meanings which suggest that memory formation does not appear to require consciousness.

There are two types of sleep: non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM is considered a deep sleep, which is divided into further stages representing a continuum of relative depth. The interesting thing about deep stages is that our brain cells changing its activity every half-second, depicting as waveforms of varying frequency and amplitude^{6,7}. Those cells transition from activate states (“up-state”) to inactive states (“down-state”) to active again and so on.

To perform the experimental goal, 41 physically and mentally health native German speakers, between age 19 and 32, were recruited. Participants were asked to have 4 hours of sleep the night before the experiment to increase their propensity to sleep. Once subjects arrived at the sleep laboratory, they were outfitted with electroencephalogram (EEG) electrodes and in-ear headphones. Then they were asked to take a nap. The EEG allowed the scientists to monitor the different stages of sleep for each participant. As a subject reached the criterion for slow-wave sleep (up-state) each pair of pseudowords and real German words were repeated four times in sequence, changing the order of presentation each time (pseudoword – a German word, German word – pseudoword). The study introduced to participants as an investigation of “how sleep contributes to the ability to correctly guess the meaning of foreign words from a language one has never encountered before.” Therefore, contributors were unaware that words had been presented to them while they were asleep. That was done to prevent subjects from consciously trying to attend to the sleep-played words in future awake-learning test. The experiment stopped when the

EEG showed signs of arousal depicted as the reappearance of slow-waves. The subject's data were analyzed only if at least 20 sleep-played word pairs were played. After waking up, participants took an implicit memory test. Pseudowords were presented both acoustically and visually to the subjects who were asked to decide the meaning of the word. During this awake-learning test, functional magnetic resonance imaging (fMRI) was used to decode areas of the brain that were involved in the recruitment of the memory.

The researchers were examining whether a sleeping person can form new semantic associations between played foreign words and translated words during the brain cells' up-states (Figure 1). Semantic associations refer to something that is logically related to the meaning of the word. The scientists found that those association can be stored only if the second word of a pair was repeatedly played during an active state of deep sleep. For instance, a sleeping person, heard the word "tofer" paired with its translated word "house" or "guga" – "key", after waking up was able to categorize with a better-than-chance accuracy whether those foreign words denominated something large ("tofer") or small ("guga"). The overall success rate was found to be 60%.

Physiologically speaking, scientist found that language areas of the brain and the hippocampus, the brain's essential memory hub, were activated during retrieval of sleep-learned vocabulary where these brain structures usually mediate wake-learning vocabulary^{8,9,10}.

This study opens up a door to a new world of memory formation regardless of consciousness or unconscious. From now on, sleep will no longer consider as unproductive time and can be used, not only to relax, but also to learn new foreign words. As Henke said, it is essential to examine how deep sleep can be utilized for the acquisition of new information and with what consequences.

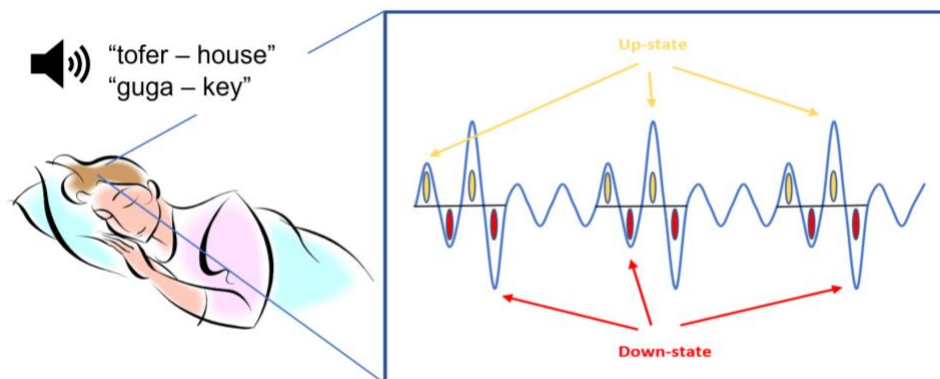


Figure 1. Representation of the EEG study during sleep. The figure represent sleeping person during deep sleep where brain electrical activity recording is performed using electroencephalogram (EEG). Left side of the image shows sleeping person being introduced to a pseudoword and a German translation word. Right side of the image shows electrical activity of the brain which denotes in waveform where slow oscillatory high-amplitude waves appears during deep sleep. Yellow ovals represent highly active phase (up-state) and red ovals indicates passive phase (down-state). A word pairs were played during slow wave peak (Up-state).

REFERENCES

1. Marc Alain Züst, Simon Ruch, Roland Wiest, and Katharina Henke. (2019). Implicit Vocabulary Learning during Sleep Is Bound to Slow-Wave Peaks. *Current Biology*. 29(4):541-553. 10.1016/j.cub.2018.12.038
2. (2016). Why There's A Language Learning Gap In The United States. Here and Now. <https://www.wbur.org/hereandnow/2018/07/16/foreign-language-gap-united-states>

3. Saad, L. (2014). The "40-Hour" Workweek Is Actually Longer - by Seven Hours. Gallup. <https://news.gallup.com/poll/175286/hour-workweek-actually-longer-seven-hours.aspx>
4. Craig, L., Lyn Craig, Mullan, K. (2001). How Mothers and Fathers Share Childcare: A Cross-National Time-Use Comparison. *SAGE Journals*. 76(6):834-861. <https://doi.org/10.1177/0003122411427673>
5. Colten, H. R., Altevogt, B. M. (2006). *Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem*. National Academy Press. <https://www.ncbi.nlm.nih.gov/books/NBK19956/>
6. Ngo, V. H-V., Martinetz, T., Born, J., Mölle, M. (2013). Auditory Closed-Loop Stimulation of the Sleep Slow Oscillation Enhances Memory. *Neuron*. 78(3):413-415. <https://doi.org/10.1016/j.neuron.2013.03.006>
7. (2007). What are Brainwaves? *Brainworks*. <https://brainworksneurotherapy.com/what-are-brainwaves>
8. Pulvermüller, F. (2005). Brain mechanisms linking language and action. *Nature Review Neuroscience*. 6:576–582. <https://www.nature.com/articles/nrn1706>
9. Fortin, J. N., Agster, L. K., Eichenbaum, B. H. (2002). Critical role of the hippocampus in memory for sequences of events. *Nature Neuroscience*. 5:458–462 <https://www.nature.com/articles/nn834>
10. Moscovitch, M., Rosenbaum, R. S., Gilboa, A., Addis, R. D., Westmacott, R., Grady, C., McAndrews, P. M., Levine, B., Black, S., Winocur, G., Nadel, L. (2005). Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. *Journal of Anatomy*. 207(1):35-66 <https://doi.org/10.1111/j.1469-7580.2005.00421.x>

Possible treatment to rehabilitate the brain from addiction

A new study shows that a rehabilitating mechanism known as transcranial magnetic stimulation can be used as a treatment for substance use disorders.

Kawther Elolaimi

In a paper recently published in *Frontiers*, Corinna Bolloni et al., investigate transcranial magnetic stimulation (TMS) mechanisms and usage as a potential treatment for addiction.¹ TMS has been used to improve symptoms of depression and is a noninvasive procedure that uses repetitive magnetic fields to stimulate nerve cells in the brain.² This method has been proven to be innovative, safe and cost effective, however the mechanism and effectiveness for addiction treatment remain a mystery which we hope this study will uncover.^{1,3} This study indicates that TMS can be used as a promising treatment for addiction and hopefully can change the rewiring of the brain to avoid substance usage as shown in the figure below.¹ They discovered that post treatment, drug intake had been reduced in comparison to pre-treatment. Their evidence claims that TMS can indeed produce adaptations to specific brain areas changing the pathway and firing patterns of cells resulting in substantial physical behavioral changes as well as reduced intake.^{1,3} This implies that this treatment can alter the wiring of the brains of those who are addicts to potentially prevent them from continuing their addiction.

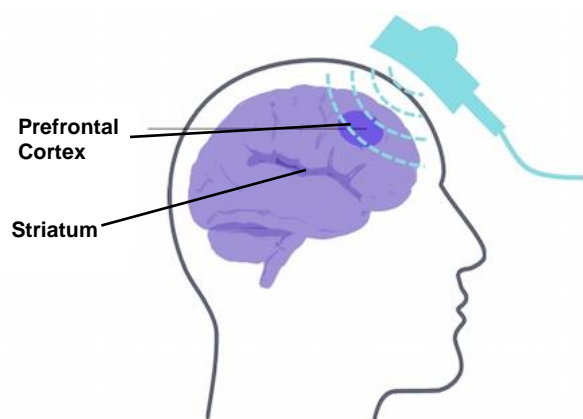
Substance use disorders (SUDs) represent a major public health concern worldwide and are a leading cause of disease and mortality.^{1,3,4} The addiction becomes compulsive and difficult to control despite harmful consequences.^{3,4} There are about 27.7 million users in the western world alone allowing researchers to gain a general consensus on drug addiction and identifying therapeutic options which they consider to be limited.^{1,3,7} Circuits in the brain involved in reward seeking behavior as well as fear processing adapt as a result of chronic use and abuse of drugs as mentioned in the figure below.^{3,5} The pathway of these circuits has become a primary focus of researchers who aim to develop treatment methods to disrupt these cycles. When you constantly keep using something or repeating a certain action, you come prone to it, therefore changing the cycle starting with the brain may alter that behavior or habit allowing you to change or quit it. However, it is unclear exactly which pathways and processes in the brain are associated with the development and progression of addictive disorders.^{5,6,7} In the USA alone, the cost to illicit drugs exceeds 700 billion dollars a year.^{6,7} Currently, TMS has been widely studied to be used as a potential treatment to disrupt cycles in the brain to alter behavior. TMS contains magnetic fields which are generated with a coil placed over the scalp and transmit electrical currents to the brain as demonstrated in the figure below.^{1,2,3} As mentioned, it is a noninvasive and painless procedure with repetitive stimulation that allows for long-term changes in the brain which facilitate or impede specific behaviors depending on the area of the brain that gets stimulated.^{1,2,3}

This study was conducted on ten addicts with an average age of 35 years who on average have been dependent on cocaine for 13 years and consumed an average of 0.5 to 20ng/mg (7.75 ng/mg) of cocaine, cannabis, and/or opioids. A *hair test* was used to measure the amount of drug

intake since it provides long-term information about drug consumption with higher sensitivity and specificity in comparison to urine analysis. The subjects were administered with 12 repetitive TMS sessions 3 times a week for 4 weeks with the intensity of the stimulation set at the lowest intensity required to provoke a neuronal response. During the TMS sessions, the prefrontal cortex was targeted and stimulated bilaterally. Drug intake was dependent of hair analysis at baseline, which was assessed before treatment, after 1 month, after 3 months, and after 6 months for a total of three treatment sessions at the end of each indicated month.

Throughout the course of the six months, the researchers observed decreased drug consumption from the onset to the end of treatment which was six months later. After the first treatment, they immediately started to notice decreased consumption of drug intake from the participants. They used 10 Hz stimulation which was the minimal amount of stimulation required to enhance neuronal responses which activated 1000 pulses. The duration of each treatment session lasted about 10 minutes. The frequency activated a response and significantly reduced the amount of drug consumption in all participants in the 6-month period, however, a greater frequency may prevent drug consumption entirely in a shorter amount of time.

The results of this study supported the claim made from previous studies claiming that TMS can be used as a potential treatment to cure drug addiction. However, additional research is highly encouraged to further understand the correlation between frequency stimulation and drug consumption in hopes to alter the wiring of the brain and break the addiction. Additionally, though not mentioned in detail earlier, some studies have only used unilateral (asymmetric) stimulation in one specific site in the brain while this study conducted bilateral stimulation. They thought this would decrease drug consumption at a faster rate since addiction affects the whole brain and not just one area. They believe that it would be more of a suitable method for clinical studies aimed at reducing drug intake. Though additional studies are needed, the results in this study seem promising and supportive of the treatment option of using TMS to treat addiction. As of now though, exercise contains many health benefits and is also being considered as a treatment option for drug addiction.⁹ In addition, scientists are also developing new pharmaceutical treatments for drug addiction.¹²



This figure shows transcranial magnetic stimulation at the prefrontal cortex. This mechanism aims to disrupt the cycle of addiction and prevent substance use disorders. Addiction affects many brain areas cycling throughout the prefrontal cortex which anticipates the reward, the striatum and surrounding brain structures during intoxication, and finally the amygdala when negative affects occur such as withdrawal.

REFERENCES

1. Bolloni, et al. "Bilateral Transcranial Magnetic Stimulation of the Prefrontal Cortex Reduces Cocaine Intake: A Pilot Study." *Frontiers*, Frontiers, 19 July 2016, www.frontiersin.org/articles/10.3389/fpsy.2016.00133/full.
2. "Transcranial Magnetic Stimulation." *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 27 Nov. 2018, www.mayoclinic.org/tests-procedures/transcranial-magnetic-stimulation/about/pac-20384625.
3. Diana, Marco, et al. "Rehabilitating the Addicted Brain with Transcranial Magnetic Stimulation." *Nature News*, Nature Publishing Group, 29 Sept. 2017, www.nature.com/articles/nrn.2017.113.

4. Substance Use Disorder: MedlinePlus Medical Encyclopedia." *MedlinePlus*, U.S. National Library of Medicine, medlineplus.gov/ency/article/001522.htm.
5. "Biological Evidence for Paradoxical Improvement of Psychiatric Disorder Symptoms by Addictive Drugs." *Trends in Pharmacological Sciences*, Elsevier Current Trends, 4 Apr. 2017, www.sciencedirect.com/science/article/pii/S0165614717300627.
6. Herman, Melissa A, and Marisa Roberto. "The Addicted Brain: Understanding the Neurophysiological Mechanisms of Addictive Disorders." *Frontiers in Integrative Neuroscience*, Frontiers Media S.A., 19 Mar. 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC4365688/.
7. "Neurobiologic Advances from the Brain Disease Model of Addiction | NEJM." *New England Journal of Medicine*, www.nejm.org/doi/full/10.1056/nejmra1511480.
8. Dunlop, Katharine, et al. "Noninvasive Brain Stimulation Treatments for Addiction and Major Depression." *Annals of the New York Academy of Sciences*, John Wiley and Sons Inc., Apr. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5434820/.
9. "Exercise as a Novel Treatment for Drug Addiction: A Neurobiological and Stage-Dependent Hypothesis." *Neuroscience & Biobehavioral Reviews*, Pergamon, 24 June 2013, www.sciencedirect.com/science/article/pii/S0149763413001668.
10. Volkow, Nora, and Ting-Kai Li. "The Neuroscience of Addiction." *Nature News*, Nature Publishing Group, www.nature.com/articles/nn1105-1429.
11. "Drug Abuse as a Problem of Impaired Control: Current Approaches and Findings." *SAGE Journals*, journals.sagepub.com/doi/abs/10.1177/1534582303257007.
12. "The Addicted Brain." *Cambridge Neuroscience*, www.neuroscience.cam.ac.uk/research/comeos/AddictedBrain.php.

No need for surgery

Cook et al. investigated a new form of neuromodulation therapy for individuals with psychiatric disorders. Significant improvements in depression and posttraumatic stress disorder symptoms were observed after acute external trigeminal nerve stimulation treatment.

Tamsen Hutchison

In a paper published on January 28, 2016 in *Neuromodulation*, Cook et al. investigated the effectiveness of an 8-week external trigeminal nerve stimulation intervention on posttraumatic stress disorder (PTSD) and major depressive disorder (MDD) symptoms¹. Recent estimates for the prevalence of PTSD in the U.S. suggest that approximately 3.5% of the population is affected by the disorder². The symptoms associated with PTSD can have a detrimental effect on quality of life, putting individuals at a higher risk of suicide³. Common treatments for PTSD symptoms are medications, such as selective serotonin reuptake inhibitors (SSRIs), or psychosocial interventions, such as cognitive behavioral therapy⁴. However, even when these treatments are combined, the majority of patients do not experience significant improvement in their symptoms⁵. The motivation behind this research was influenced by the lack of effective treatments for patients with co-occurring PTSD and MDD. The researchers found that severity of depression and PTSD symptoms improved over the course of the 8-week external trigeminal nerve stimulation (eTNS) treatment. These results imply that neuromodulation therapy may be an effective new approach for the treatment of psychiatric disorders⁶.

External trigeminal nerve stimulation therapy can be used to send signals to the brain by stimulating the trigeminal nerve with small electrical currents⁷. One of the regions from which the trigeminal nerve transports signals is from the face to the nucleus tractus solitarius (NTS). The NTS is located in the brainstem and is believed to be responsible for integrating and relaying signals to structures that are associated with PTSD⁸. In previous studies, symptoms of fear and depression have been significantly improved by treatment with eTNS^{9,10}.

For this experiment, the researchers recruited 12 individuals (8 women and 4 men) between the ages of 18 and 75 who displayed symptoms of PTSD and MDD. In order to meet this criterion, the participants had to score a minimum of 14 of the 17-item Hamilton Depression Rating Scale (HDRS-17) and at least 50 on the Clinical-Administrated PTSD Scale (CAPS). Prior to the TNS intervention, participants were assessed for baseline PTSD and depression severity using the PTSD Patient Checklist (PCL), HDRS-17, and the Quick Inventory of Depressive Symptomatology (QIDS-C). These measures were then reassessed after 2 weeks, 4 weeks, 6 weeks, and 8 weeks of eTNS therapy. Scores from the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q) were used to assess quality of life at baseline and again at week 8. During the TNS intervention, the participants were instructed to place electrodes on their forehead and to stimulate the supraorbital and supratrochlear nerves (depicted in Figure 1) each night for eight hours. The stimulation used for this experiment had a frequency of 120 Hz, a pulse width of 250 μ sec, and was cycled on and off for 30 seconds at a time.

The results of the 8-week TNS intervention showed significant decreases in the negative symptoms associated with PTSD and MDD. Scores for the PCL averaged at 43.0 at week 8 compared to the 63.3 at baseline, indicating an improvement in PTSD characteristics. Improvement in depression severity was also observed following the 8-week TNS intervention. HDRS-17 scores dropped from a baseline average of 25.3 to 11.9 at week 8, and QIDS-C scores dropped from 17.8 to an 8.8 by the end of the intervention. Scores for quality of life also revealed significant improvement, with an average final score of 50.1 compared to 38.4 at baseline.

The results display evidence to support eTNS therapy for the treatment of individuals with comorbid PTSD and MDD. Neuromodulation treatments using external devices are beneficial because they are convenient, easy to use, and they do not require the patient to undergo any invasive surgical implantations¹¹. This study also found that this method of treatment resulted in mild or no side effects, and that all

participants considered the treatment to be safe and acceptable for clinical use. Future research in the field should focus on the mechanisms that are involved in the treatment of psychiatric disorders through neuromodulation. In addition, future research would benefit from obtaining a larger sample size and from including a control group to avoid a potential placebo effect from occurring.

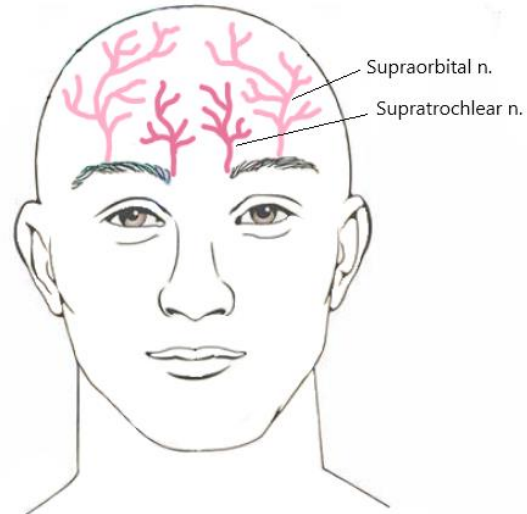


Figure 1. Surface electrode placement for eTNS therapy. During the 8-week eTNS intervention, participants applied stimulation to the supraorbital and supratrochlear branches of the trigeminal nerve for 8 hours each night. This non-invasive neuromodulation therapy has shown to be therapeutic for several psychiatric disorders, including major depressive disorder.

REFERENCES

1. Cook, I. A., Abrams, M., & Leuchter, A. F. (2016). Trigeminal nerve stimulation for comorbid posttraumatic stress disorder and major depressive disorder. *Neuromodulation: Technology at the Neural Interface*, 19: 299-305.
2. Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*, 62: 593-602.
3. Kessler, R. C. (2000). Posttraumatic stress disorder: the burden to the individual and to society. *Journal of Clinical Psychiatry*, 61: 4-14.
4. Ipser, J. C., & Stein, D. J. (2012). Evidence-based pharmacotherapy of post-traumatic stress disorder (PTSD). *Int J Neuropsychopharmacol*, 15: 825-840.
5. Bradley, R., Greene, J., Russ, E., Dutra, L., & Westen, D. (2005). A multidimensional meta-analysis of psychotherapy for PTSD. *Am J Psychiatry*, 162: 214-227.
6. DeGiorgio, C. M., Murray, D., Markovic, D., & Whitehurst, T. (2009). Trigeminal nerve stimulation for epilepsy: long-term feasibility and efficacy. *Neurology*, 72: 936-938.
7. Schrader, L. M., Cook, I. A., Miller, P. R., Maremont, E. R., & DeGiorgio, C. M. (2011). Trigeminal nerve stimulation in major depressive disorder: first proof of concept in an open pilot trial. *Epilepsy Behav*, 22: 475-478.
8. Jean, A. (1991). The nucleus tractus solitarius: neuroanatomic, neurochemical and functional aspects. *Arch Int Physiol Biochim Biophys*, 99: A3-A52.

9. Cook, I. A., Schrader, L. M., Degiorgio, C. M., Miller, P. R., Maremont, E. R., & Leuchter, A. F. (2013). Trigeminal nerve stimulation in major depressive disorder: acute outcomes in an open pilot study. *Epilepsy Behav*, *28*: 221-226.
10. Sui, L., Huang, S., Peng, B., Ren, J., Tian, F., & Wang, Y. (2014). Deep brain stimulation of the amygdala alleviates fear conditioning-induced alterations in synaptic plasticity in the cortical-amygdala pathway and fear memory. *J neural Transmission*, *121*: 773-782.
11. Shiozawa, P., da Silva, M. E., Netto, G. T., Tair, I., & Cordeiro, Q. (2015). Effect of a 10-day trigeminal nerve stimulation (TNS) protocol for treating major depressive disorder: a phase II, sham-controlled, randomized clinical trial. *Epilepsy Behav*, *44*: 23-26.

Deep brain stimulation improves cognitive control in depressed patients

Maxwell Neideigh

New research suggests that increasing “theta” rhythms, frequencies in the brain easily observed in EEG recordings, provides neurosurgeons and psychiatrists with the consistent, and reliable feedback needed to calibrate the placement and intensity of Deep Brain Stimulation. Deep brain stimulation is a method used by neurosurgeons to treat a myriad of neurodegenerative diseases, that involves the placement of stimulating electrodes to specific brain regions. The greatest success thus far in the field has been in treating Parkinson’s disease, where patients under the influence of DBS experience an overall reduction in the amount of tremors they would normally have as a result of the disease. Present day research has extended the reach of DBS not only as a treatment for tremors, but as a therapy to treat psychiatric disorders like major depressive disorder (MDD) and obsessive compulsive disorder (OCD).

Assistant professor of psychiatry at the University of Minnesota Medical School Alike Widge explains that, one way to alleviate symptoms associated with depression is to induce synchronized Theta brain wave activity in a brain region called the ventral striatum¹. In rats, theta wave rhythmicity can be observed in the hippocampus, but can also be seen in a number of other cortical and subcortical brain structures. Ventral Striatum theta waves carry a frequency with a range of 6–10 Hz, and are predominantly seen when a rat is engaged in locomotion like walking back and forth in its cage, when a new smell enters its nose, and when the rat is in its deepest phase of the sleep cycle called REM sleep².

Widge explains that both MDD and OCD patients are characterized as having a lack of cognitive control which is needed to interfere with negative thinking patterns and obsessive impulses³. When it comes to MDD and OCD, evidence for patient improvement is much more subtle than in the case with Parkinson’s disease, where neurosurgeons can see a reduction in tremors almost immediately following calibration of the electrodes and stimulator⁴. One hypothesis for how DBS can help depressive symptoms presented by Alik Widge, the lead and corresponding author on the paper, “Deep brain stimulation of the internal capsule enhances human cognitive control and prefrontal cortex function,” published on April 4th, involves reinforcing theta rhythms in the prefrontal cortex. DBS has been shown to have an impact on what Widge’s team of researchers would call “cognitive control,” an ability primarily attributed to the prefrontal cortex, and an ability most MDD and OCD patients struggle with.

In the April 4th paper published in Nature Communications researchers used 14 patients who have had previous DBS treatment for their depression. Each participant was faced with a background image displaying emotionally evocative content, and then were told to complete a conflict task involving a series of numbers. Participants brain waves were recorded during the conflict tasks once with DBS turned on and once with DBS turned off (Figure 1c).

When Theta rhythms in the cortex increased as a result of DBS, depressed patients chose the correct answer to the conflict task faster and more accurately than when the DBS was absent.

Exploring further into the field of DBS neurosurgeons may discover more effective sites for stimulation to treat the symptoms associated with depression like habitual negative thinking by reinforcing theta brain wave activity and improving cognitive control. Obsessive compulsive disorder is another psychiatric disorder that is characterized by a person having uncontrollable, reoccurring thoughts or obsessions, that can be interfered with through DBS. Furthermore, DBS may provide patients who are chronically stuck in compulsive or negative thinking patterns an alternative to traditional antidepressant medication like serotonin specific reuptake inhibitors (SSRI's), which are only slightly effective at treating these symptoms in the long terms.

Cognitive control tasks that use DBS typically involve participants viewing an emotionally arousing image while trying to pick numerical values in a sequence of three that doesn't fit (Fig. 1a). The images purpose is to place an emotional "distractor" on to the patient, simulating a real world situation that requires cognitive control to overcome. Participants performed the cognitive control task under the influence of DBS and without it. Emotionally arousing images present distractors to the participants, inhibiting their ability to react quickly and accurately to the series of numbers.

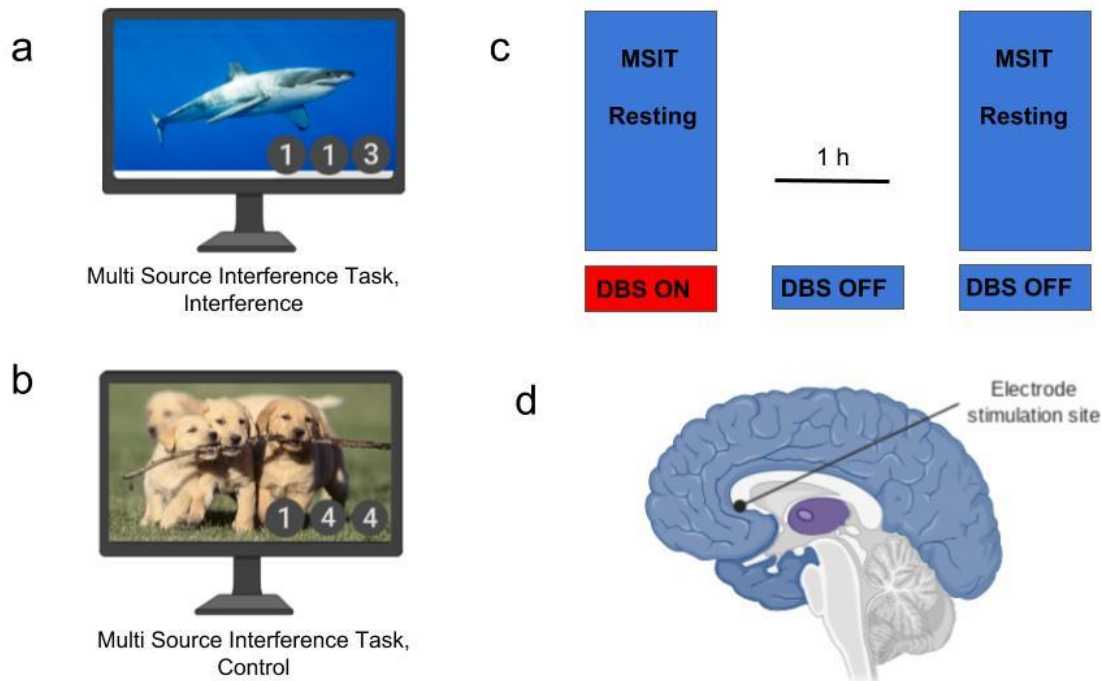


Figure 1. Experiment Design and Behavior. a,b.) Visual demonstration on participants perspective during multi-source interference task. Emotionally arousing images are displayed in conjunction with a series of three numerical values, one of which different from the rest. Distractors like the shark are known to increase cognitive load on participants. c.) Participants performed the interference task under the influence of deep brain stimulation, then again following one hour without the stimulation. d.) Brain region being stimulated during MSIT.

Deep brain stimulation improves performance on cognitive control tasks and increases theta oscillations in the medial, and lateral prefrontal cortex. With DBS on, subjects in-fact made their choice quicker demonstrating their ability to overcome the interference of the emotional picture, without sacrificing accuracy.

Previous studies indicate deep brain stimulation (DBS) has proven to be an effective therapeutic option for alleviating tremors associated with Parkinson's Disease and now more recently,

restoring cognitive control. The current study demonstrates that DBS to an FDA approved target for psychiatric disabilities, is shown to impact a particular symptom, that lies at the root of multiple psychiatric illnesses.

These findings suggest that DBS may provide patients who are chronically stuck in compulsive or negative thinking patterns an alternative to prescription medication to treat their symptoms. DBS therapies that focus on activating theta brain waves can reinforce particular suppressed neural networks that may be implicated in a range of diseases related to mental health.

REFERENCES

1. S. Widge, S. Zorowitz, I. Basu, A. C. Paulk, S. S. Cash, E. N. Eskandar, T. Deckersbach, E. K. Miller & D. D. Dougherty. *Nature Communications* volume 10, Article number: 1536 (2019) doi:10.1038/s41467-019-09557-4
2. Kirk IJ (1998). "Frequency modulation of hippocampal theta by the supramammillary nucleus, and other hypothalamo-hippocampal interactions: mechanisms and functional implications". *Neurosci Biobehav Rev.* 22(2): 291–302. doi:10.1016/S0149-7634(97)00015-8. PMID 9579319
3. Klimkeit, EI; Mattingley, JB; Sheppard, DM; Farrow, M; Bradshaw, JL (2004). "Examining the development of attention and executive functions in children with a novel paradigm". *Child Neuropsychology.* 10 (3): 201–211. doi:10.1080/09297040409609811. PMID 1559049
4. McIntyre CC, Thakor NV (2002). "Uncovering the mechanisms of deep brain stimulation for Parkinson's disease through functional imaging, neural recording, and neural modeling". *Critical Reviews in Biomedical Engineering.* 30 (4–6): 249–81. doi:10.1615/critrevbiomedeng.v30.i456.20
5. Gartlehner G, Hansen RA, Morgan LC, Thaler K, Lux L, Van Noord M, Mager U, Thieda P, Gaynes BN, Wilkins T, Strobelberger M, Lloyd S, Reichenpfader U, Lohr KN (December 2011). "Comparative benefits and harms of second-generation antidepressants for treating major depressive disorder: an updated meta-analysis". *Annals of Internal Medicine.* 155 (11): 772–85. doi:10.7326/0003-4819-155-11-201112060-00009. PMID 22147715.
6. Accolla EA, Aust S, Merkl A, Schneider GH, Kühn AA, Bajbouj M, Draganski B (April 2016). "Deep brain stimulation of the posterior gyrus rectus region for treatment resistant depression". *Journal of Affective Disorders.* 194: 33–7. doi:10.1016/j.jad.2016.01.022
7. Sainsbury, RS; Heynen A; Montoya CP (1987). "Behavioral correlates of hippocampal type 2 theta in the rat". *Physiol Behav.* 39 (4): 513–519. doi:10.1016/0031-9384(87)90382-9. PMID 3575499.
8. Tauscher-Wisniewski S, Nilsson M, Caldwell C, Plewes J, Allen AJ (October 2007). "Meta-analysis of aggression and/or hostility-related events in children and adolescents treated with fluoxetine compared with placebo". *Journal of Child and Adolescent Psychopharmacology.* 17 (5): 713–8. doi:10.1089/cap.2006.0138. PMID 17979590
9. Volkmann J, Herzog J, Kopper F, Deuschl G (2002). "Introduction to the programming of deep brain stimulators". *Movement Disorders.* 17 Suppl 3: S181–7. doi:10.1002/mds.10162
10. Weaver, Frances M. (2009). "Bilateral Deep Brain Stimulation vs Best Medical Therapy for Patients With Advanced Parkinson Disease<subtitle>A Randomized Controlled Trial</subtitle>". *JAMA.* 301 (1): 63. doi:10.1001/jama.2008.929

Women versus men, what do you want to eat? We might have an answer

A recent study showed that when it comes to choosing food when unconsciously eating for hungry or pleasure, females prefer high calorie meals while males prefer low calorie meals.

Zenette McCoy

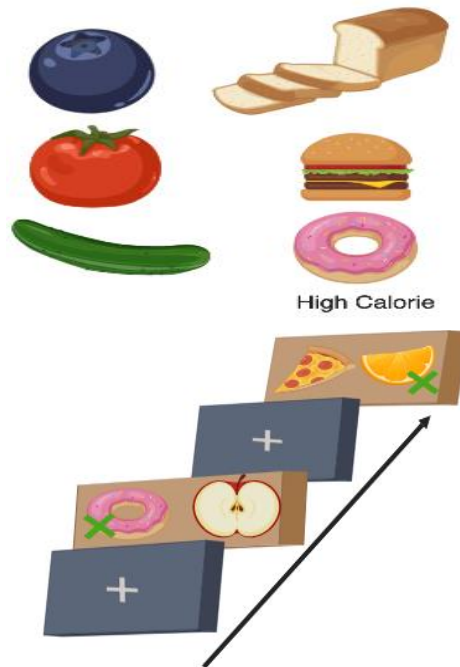
Everyone knows the never-ending argument between couples, friends, or family members of the opposite sex: what do you want to eat? It has become such a trend that it has been turned into memes and a running joke in the 21st century. The common scenario occurs as such: male asks the female what they want to eat and after going back and forth about food options, the male finally picks a place to only be told by the female they don't want to eat there. Oddly enough, an article by Manippa and colleagues found that there is science behind this common scenario. They came to conclusion that forced food choices vary between sexes. However, in order for Manippa and colleagues to come to this conclusion, previous studies need to be evaluated in order to gain a better understanding of food choices between males and females¹.

In the last decade more studies about food behaviors and food choices have become a higher priority due to the large availability of food in western countries^{1,2,3}. This results in individuals to shift from eating for survival to eating for pleasure^{1,2,3}. This style of eating has led people to choose higher calorie foods which could lead to negative effects such as weight gain and obesity^{3,4,5}. These studies in the last decade found that not only is psycho-physiology a factor, but the sex of the consumer affects eating behavior as well^{1,2,3}. In a study conducted by Shingleton and colleagues, they looked at the difference between males and females and binge eating^{4,5}. The study looked at the difference in men's and women's outlook on their body image and how this affected their eating disorder treatment^{4,5,6,7}. They found that women were more concerned with body weight/shape compared to men. During the initial eating disorder examination, they found women tested higher with severe concerns of body weight/shape as a majority of men tested much lower^{4,5,7}. However, some men tested higher with severe concerns, but it was not a significant population⁷. The treatment took significantly longer for women and men that tested higher with severe concerns with body weight/shape⁴. As males who tested low for concerns with body weight/shape treatment was significantly lower⁴. This trend of women being more concerned with weight and body shape than men is common⁴.

A study conducted by Uccula and Nuvoli found that women tend to be more invested in food choices resulting in women to overestimate their weight and decrease amount of meals (also limiting themselves to healthier food options such as higher intakes of dietary fibers and low intake of fats and salts)^{1,8}. When these decisions and choices were evaluated by looking at the brain regions between men and women, women showed significantly more activation in these areas compared to men: the prefrontal cortex, insula and middle/posterior cingulate^{1,8}. All of these areas are involved in behavioral control and self-referential cognition⁸. In other studies, evaluating brain region correlation to food choices found that the superior temporal sulcus (STS) was activated

during forced food choices (between high and low calorie foods)^{1,8}. This is the common situation we all know of when individuals are forced to pick a place to eat^{1,8}. However, there were no studies done seeing if there was a difference in forced food choice between males and females^{1,8}.

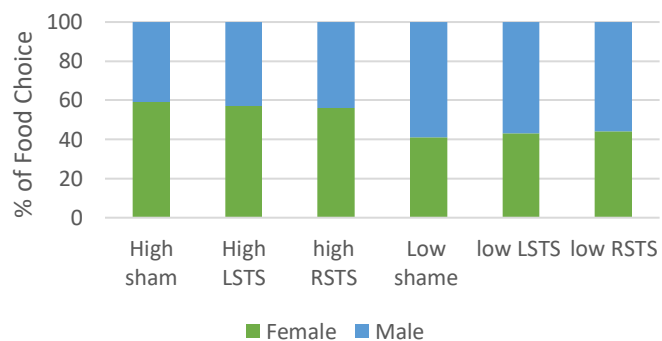
Due to no studies being done about the differences in male and female STS activation during forced food choices, Manippa and colleagues took the initiative to investigate this question. In their study, they decided to use high frequency transcranial magnetic stimulation on the STS (right and left) as individuals (men or women) had to choose between high and low calorie foods¹. Transcranial magnetic stimulation is a magnet that creates a magnetic field in a focused area that produces small electrical currents to activate desired brain regions^{9,10}. The method Manippa and colleagues used was transcranial random-noise stimulation (tRNA) which is random amplitudes of electrical current applied to the participant's scalp^{1,9,10,11}.



In their study there was a total of 17 males and 18 females, and they were assigned right and left STS and control (participants received no tRNA)¹. There was a total of two trials, half of the participants (both male and female) did one trial during the morning and other half in the afternoon (all participants had to eat the same meal before the trial)¹. Individuals had to look at a total of 183 products (being high or low calorie foods. Low calorie foods were vegetables and fruit/ healthier food options as high calorie food were doughnuts and other high fat/carbohydrate foods) and they were shown the images for a total of 3 sec after looking at a fixated cross for 1.5 secs¹.

After each image the participants were asked the following questions and had to rate each item from 0-9: “How much do you like the product?” (0 = not at all/ 9 = very much), “How many calories do you think this product consists of?” (0 = very few calories/ 9 = many calories), and “How healthy do you think this product is?” (0 = not healthy/ 9 = very healthy)¹.

Percent Differences Between Males and Females Food Choice Based on High and Low Calorie Food



temporal sulcus. (A) The definition of what was considered low and high calorie food. **(B)** the procedure of participants selecting a food choice in 3 seconds. The green X represent the choice selected **(C)** The results, showing that males had a higher percentage of food choices that were low calorie as females had a higher percent choice for higher calorie foods. Sham = control, LSTS = left superior temporal sulcus, RSTS = right superior temporal sulcus

These choices were used to independently create food pairing for each participant to be used during the tRNA task₁. During the tRNA task, the selected photos for each participant were presented in a random order₁. Each participant was told to choose the product they most wanted to eat₁. The first choice that comes to mind is the best option. Each participant was shown the image for 3 seconds (s) and had 3 s to make a choice. If participants did not make a choice in 3 s it was counted as a miss choice.

They found that women significantly chose higher calorie foods in control, right STS stimulation, and left STS stimulation compared to males. Even though males significantly choose lower calorie food than females, there was a significant difference between males left STS compared to the males' sham and right STS stimulation. When analyzing the miss choices in males and females they found that there were significantly more miss choices during the right STS stimulation compared to the control and left STS₁.

In conclusion they found that females tend to choose higher calorie food when forced to make food decisions as males tend to want lower calorie food. These results also show that there is neurological factor in deciding food choices when forced to pick food without thinking heavily about food consumption results. This is due to a larger activation in the STS in females than males. Based on these articles you can see that males and females have different ideas on food intake and food choices. Food arguments arise due to these differences, so next time when making a choice on what to eat, try to think of a middle ground that will meet everyone's conscious and subconscious needs.

REFERENCES

1. Manippa, V., Padulo, C., van der Laan, L. N., & Brancucci, A. (2017). Gender Differences in Food Choice: Effects of Superior Temporal Sulcus Stimulation. *Frontiers in human neuroscience*, 11, 597. doi:10.3389/fnhum.2017.00597
2. Leblanc, V., Hudon, A. M., Royer, M. M., Corneau, L., Dodin, S., Bégin, C., & Lemieux, S. (2015). Differences between men and women in dietary intakes and metabolic profile in response to a 12-week nutritional intervention promoting the Mediterranean diet. *Journal of nutritional science*, 4, e13. doi:10.1017/jns.2015.2
3. Leblanc, V., Bégin, C., Corneau, L., Dodin, S. & Lemieux, S. (2015) Gender differences in dietary intakes: what is the contribution of motivational variables? doi:10.1111/jhn.12213
4. Shingleton, R. M., Thompson-Brenner, H., Thompson, D. R., Pratt, E. M., & Franko, D. L. (2015). Gender differences in clinical trials of binge eating disorder: An analysis of aggregated data. *Journal of consulting and clinical psychology*, 83(2), 382–386. doi:10.1037/a0038849
5. Smith, K. E., Mason, T. B., Murray, S. B., Griffiths, S., Leonard, R. C., Wetterneck, C. T., ... Lavender, J. M. (2017). Male clinical norms and sex differences on the Eating Disorder Inventory (EDI) and Eating Disorder Examination Questionnaire (EDE-Q). *The International journal of eating disorders*, 50(7), 769–775. doi:10.1002/eat.22716
6. Wardle J., Haase A., Steptoe A., Nillapun M., Jonwutiwes K., Bellisle F., (2017) Gender differences in food choice: The contribution of health beliefs and dieting, *Annals of Behavioral Medicine*, Volume 27, Issue 2, Pages 107–116, doi: 10.207/s15324796
7. Zayas, LV, Wang, SB, Coniglio, K, et al. Gender differences in eating disorder psychopathology across DSM-5 severity categories of anorexia nervosa and bulimia nervosa. *Int J Eat Disord*. 2018; 51: 1098– 1102. doi:10.1002/eat.22941
8. Uccula A., Nuvoli G. (2016) Body Perception and Meal Type Across Age and Gender in a Mediterranean Island (Sardinia), *Psychology, Health, & Medicine*, doi: 10.1080/13548506.2017.1307997
9. Basil, B., Mahmud, J., Mathews, M., Rodriguez, C., & Adetunji, B. (2005). Is there evidence for effectiveness of transcranial magnetic stimulation in the treatment of psychiatric disorders?. *Psychiatry (Edgmont (Pa. : Township))*, 2(11), 64–69.
10. Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of TMS Consensus Group (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology*, 120(12), 2008–2039. doi: 10.1016/j.clinph.2009.08.016
11. Korzhova, J. , Bakulin, I. , Sinitsyn, D. , Poydasheva, A. , Suponeva, N. , Zakharova, M. and Piradov, M. (2019), High-frequency repetitive transcranial magnetic stimulation and intermittent theta-burst stimulation for spasticity management in secondary progressive multiple sclerosis. *Eur J Neurol*, 26: 680-e44. doi:10.1111/ene.13877

Amyloid- β beta get outta here

A study found that local injection of gene editing proteins significantly improved the commonly observed neuroanatomical and behavioral changes associated with Alzheimer's disease. Moreover, these findings advance the field of research investigating gene therapy as a novel method of treatment for neurodegenerative diseases.

Jordan Donaldson

Every 66 seconds someone is diagnosed with Alzheimer's disease¹. Alzheimer's is a disease which causes degeneration of the brain through a complex process where plaques accumulate within the brain causing memory dysfunction amongst other deficits, leading to death in many cases². Furthermore, it is estimated that by 2050, diagnoses will increase to every 33 seconds¹. Currently, Alzheimer's disease is the 6th most common cause of death and has already deaths caused by stroke, heart disease, and prostate cancer combined¹. This results in over \$236 billion dollars a year in health care fees¹. These statistics express the necessity for rapid development of treatments for Alzheimer's disease. Fortunately, Hanseul Park and colleagues published in Nature Neuroscience this year investigating how gene therapy may be a treatment for people with Alzheimer's disease. They found that their novel delivery technique of gene editing proteins significantly decreased toxic levels of the proteins clumps associated with Alzheimer's disease. These findings suggest a new delivery method to the brain for many neurodegenerative diseases, resulting in more effective and precise prevention or treatment.

Alzheimer's disease is an irreversible neurodegenerative disease resulting in memory dysfunction and hippocampal degeneration due to the accumulation of plaques called β -amyloid plaques and tau tangles, amongst other etiologies^{3,4,5}. These β -amyloid plaques accumulate after the metabolism of β -amyloid precursor protein (APP) by γ - and β -secretases⁶ (Figure 1). Therefore, targeting β -secretases activity serves as an important therapeutic target for preventing the accumulation of β -amyloid. Although β -amyloid accumulation does not account for the entirety of the neurodegenerative properties of Alzheimer's disease, it has been shown to be one of the greatest contributions to the neurodegeneration⁷. Current complications with developing treatments is delivery to the brain. This is because the vascular system of the brain forms what is known as the blood-brain-barrier (BBB)⁸. The BBB selectively allows compounds into the internal structures of the brain. Many drugs are unable to pass the BBB and therefore, treatments, no matter how effective in cell culture, are not effective *in vivo*⁸. Thus, recent studies have been testing novel methods of treatment delivery across the BBB.

The study most recently published has explored the efficacy of amphiphilic nanoparticles. These nanoparticles contain the gene editing proteins collectively known as the amphiphilic-CRISPR-Cas9 construct or nanoparticles for short² (Figure 1). An amphiphilic molecule is one that has a side of the membrane which is water soluble and the other side of the membrane is water insoluble, meaning that the particle is able to pass through water-insoluble membranes while holding water soluble components within the vesicle⁹ (Figure 1). Hanseul utilized the properties of the amphiphilic nanoparticles to deliver his drug across the BBB. The nanoparticle he

developed contained a CRISPR Cas9 construct which targeted beta-secretase 1 (*Bace1*) and silenced the gene^{6,10,11} (Figure 1). The nanoparticles were injected into the hippocampus of mice which were genetically modified to reflect the pathophysiology of Alzheimer's disease². This method of administration allowed for direct administration of the construct in a controlled and localized manner. Through use of these novel methods, they found astonishing results.

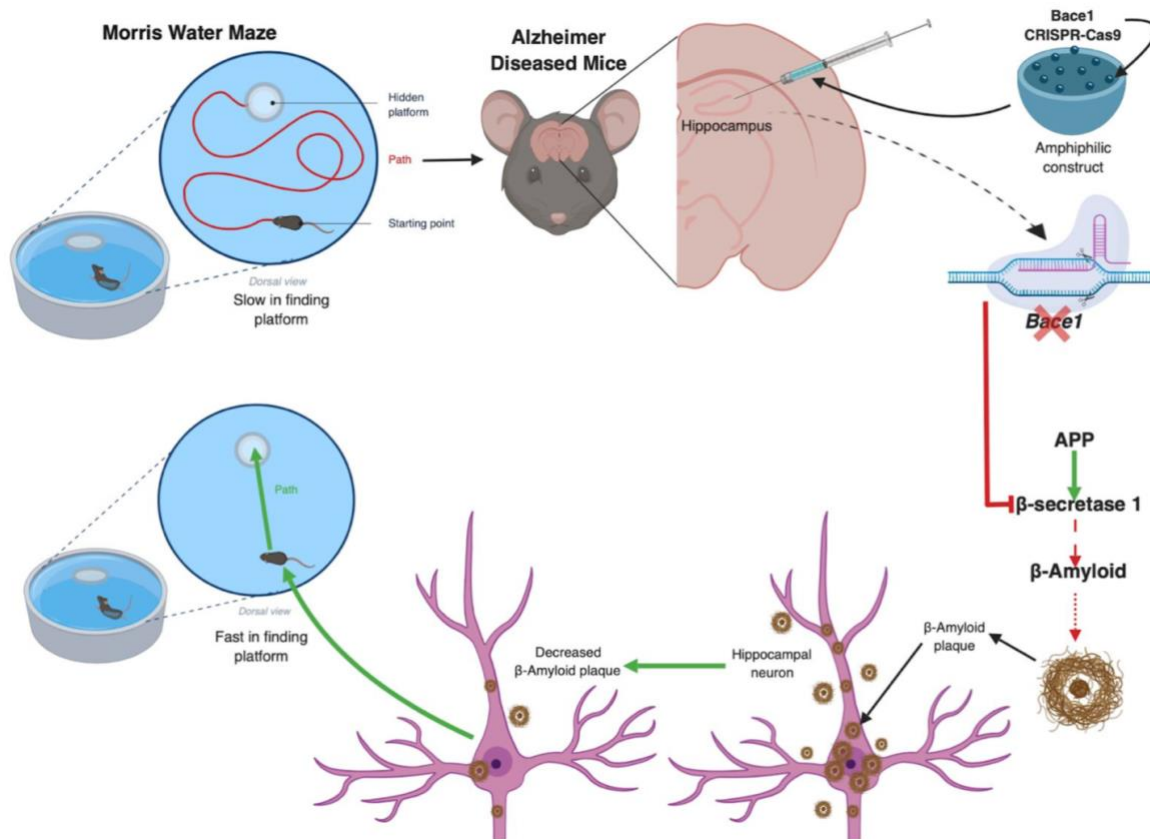


Figure 1: Nanoparticle injection into the hippocampus of Alzheimer diseased mice significantly reduced β -amyloid plaques and improved time to find platform with Morris Water Maze Test. Use of amphiphilic-CRISPR-Cas9 construct to genetically silence the function of β -secretase 1 expression significantly reduced the conversion of APP to β -amyloid leading to a reduction in β -amyloid plaque accumulation. APP = amyloid precursor protein. CRISPR = clustered regularly interspaced short palindromic repeats. Cas9 = CRISPR Associated protein 9.

Upon injection of *Bace1*-Cas9 construct into the hippocampus they reported a 70% reduction of *Bace1* expression². Then, they wanted to evaluate the phenotypic result from the reduction of *Bace1*. Hanseul Park had the mice perform various memory tasks such as the water maze and spontaneous alternation Y-maze test which both demonstrated a significant improvement in memory and learning in the nanoparticle treated mice compared to the Alzheimer's diseased mice (Figure 1). These results are extremely important and clinically relevant as the mice only had to receive one low dose injection with significant reduction in both the plaque accumulation and memory deficits. Although these results are very reassuring for the advance of CRISPR-Cas9 applications, there are remaining concerns yet to be completely evaluated.

One of the concerns by the field is that there will be chronic off-site binding of the CRISPR-Cas9 system which would cause random mutations with unknown effects. Park addressed this concern in his paper by using whole-genome sequencing, whole-exome sequencing and Digenome-

sequencing and found that there was a low percent difference between the sham and nanoparticle treated mice². This thorough examination reassures immediate concerns, however, there have yet to be studies on the chronic effects of the system. Although Park reassured the reader by a study he performed, where he found that the Cas9 system was undetectable in the brain three weeks post-injection². Lastly, after the injection there was no observed increase in inflammation, cell death or microglia recruitment².

In conclusion, this work greatly advances the reality of using gene therapy as a possible treatment for neurodegenerative disorders. As Park stated, the novel delivery system may also be used to treat localized neurodegenerative disorders. This opens the future field of research not only to the capabilities at attenuating the effects of Alzheimer's disease but also other diseases such as Parkinson's disease. This study greatly increases the likelihood of remediation of this extremely serious and prevalent disease. Future studies will likely evaluate the effects in higher-order mammals prior to human trials.

REFERENCES

1. Alzheimers Dement "Alzheimer's disease facts and figures." *Alzheimer's Association*. 2016; 124: 459–509.
2. Park, Hanseul, et al. "In vivo neuronal gene editing via CRISPR–Cas9 amphiphilic nanocomplexes alleviates deficits in mouse models of Alzheimer's disease." *Nature neuroscience* (2019): 1.
3. Fukumoto H, Cheung BS, Hyman BT, Irizarry MC. β -Secretase Protein and Activity Are Increased in the Neocortex in Alzheimer Disease. *Arch Neurol*. 2002;59(9):1381–1389.
4. Serrano-Pozo, Alberto, et al. "Neuropathological alterations in Alzheimer disease." *Cold Spring Harbor perspectives in medicine* 1.1 (2011): a006189.
5. Yang, Li-Bang, et al. "Elevated β -secretase expression and enzymatic activity detected in sporadic Alzheimer disease." *Nature medicine* 9.1 (2003): 3.
6. Zilai Wang, Li Yang and Hui Zheng, " Role of APP and A β 946; in Synaptic Physiology", *Current Alzheimer Research* (2012) 9: 217.
7. Murphy, M Paul, and Harry LeVine 3rd. "Alzheimer's disease and the amyloid-beta peptide." *Journal of Alzheimer's disease : JAD* vol. 19,1 (2010): 311-23.
8. Pardridge, William M. "Drug transport across the blood-brain barrier." *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* vol. 32,11 (2012): 1959-72. doi:10.1038/jcbfm.2012.126
9. Dehsorkhi, Ashkan et al. "Self-assembling amphiphilic peptides." *Journal of peptide science : an official publication of the European Peptide Society* vol. 20,7 (2014): 453-67.
10. Kang, E. L., Biscaro, B., Piazza, F., & Tesco, G. (2012). BACE1 Protein Endocytosis and Trafficking Are Differentially Regulated by Ubiquitination at Lysine 501 and the Di-leucine Motif in the Carboxyl Terminus. *Journal of Biological Chemistry*, 287(51), 42867–42880. doi:10.1074/jbc.m112.407072
11. Steven L. Roberds, John Anderson, Guriqbal Basi, Michael J. Bienkowski, Daniel G. Branstetter, Karen S. Chen, Stephen Freedman, Normand L. Frigon, Dora Games, Kang Hu, Kelly Johnson-Wood, Karl E. Kappenman, Thomas T. Kawabe, Ismail Kola, Ralf Kuehn, Michael Lee, Weiqun Liu, Ruth Motter, Nanette F. Nichols, Michael Power, David W. Robertson, Dale Schenk, Michael Schoor, George M. Shopp, Mary E. Shuck, Sukanto Sinha, Kjell A. Svensson, Gwen Tatsuno, Hartmut Tintrop, John Wijsman, Sarah Wright, Lisa McConlogue, "BACE knockout mice are healthy despite lacking the primary β -secretase activity in brain: implications for Alzheimer's disease therapeutics", *Human Molecular Genetics*, Volume 10, Issue 12, 1 June 2001, Pages 1317–1324

Why it doesn't hurt to get enough sleep

Researchers find that even subtle day-to-day changes in sleep patterns can significantly alter the perception of pain.

Cheyenne Lewis

The relationship between sleep and pain can be a vicious cycle – what happens as a problem in one area leads to problems in the other¹. Those who must deal with chronic or acute pain report fragmentations in their sleep cycle such that they are unexpectedly woken up in the middle of the night. These fragmentations, referred to as microarousals, are recurrent brief awakenings, often fifteen seconds in duration, during which a person will suddenly move from deep sleep to wakefulness². These microarousals are not always linked to pain, however, as they are associated with a variety of conditions including elevated blood pressure, high cholesterol, and stress³. Yet, several studies have found that even if microarousals are not caused by pain, they can ultimately lead to pain^{4,5}. More specifically, they can alter pain sensitivity.

Following repeated sleep disturbance, there is not only a reduction in duration, but in sleep quality. There is a loss in the most important phase of sleep: rapid eye movement (REM) sleep. Different from the stages of non-REM sleep, REM sleep is considered paradoxical in that physiological changes are similar to a person's waking state with increases in breathing and heart rate and, of course, rapid eye movements. The chemical and electrical imbalances associated with REM brain wave activity are believed to have origin within the brain stem following large electrical bursts of energy. REM sleep is most often associated with memory formation and learning, and in losing precious REM sleep, there is the result of sleep debt. Sleep debt is what follows extended periods of wakefulness and occurs when the stages of sleep cannot fully cycle throughout the night⁶. There is a biological "cost" that accumulates, and sometimes the result is hyperalgesia^{7,8}: an increased sensitivity to pain.

Specific neural networks within the brain are responsible for pain intensity as well as encoding higher-order evaluative processes following painful stimuli (Figure 1). Together, these regions constitute the pain-reactivity network. Activity within the somatosensory cortex creates a scale as a function of pain intensity that increases in increments between no detectable pain and maximum pain threshold⁹. The cingulate cortex as well as the insula are responsible for the higher order

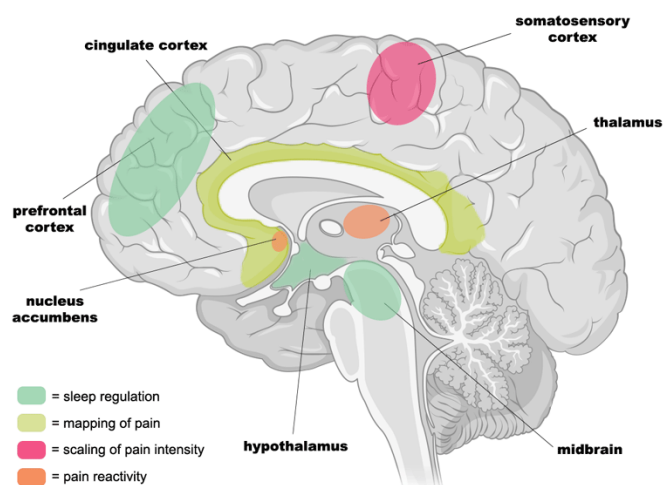


Figure 1. Anatomy of sleep and the pain reactivity network. Following sleep deprivation, various cortical and limbic regions change activation patterns in relation to both the perception and processing of painful stimuli.

processing of internal state changes in response to pain¹⁰ while the nucleus accumbens encodes for emotional value and saliency¹¹. Both of which are important for pain modulation. Changes in activity of these regions are hypothesized to account for increases in pain that follow sleep deprivation.

One recent study published in the *Journal of Neuroscience* explored the bidirectionality of sleep and pain to find out whether minor changes in sleep, in addition to full deprivation, can have significant effects on pain sensitivity. Krause and colleagues looked at changes within the pain-reactivity network by (1) measuring brain activity following acute sleep deprivation in a lab setting, and (2) using an online study following typical nightly changes within the general population.

The laboratory study involved 25 healthy participants who were free of sleep-related disorders, chronic pain, and injuries. Prior to the first session of the experiment, each participant underwent a quantitative sensory test to measure baseline thermal sensitivity between a temperature considered “warm, not yet painful” and one considered “painfully hot”. This step was particularly important to establish each participant’s thermal pain scale and ensure that observations of neural changes caused by sleep deprivation were not due to subjective differences in the experience of pain in response to objectively different thermal temperatures. During the sleep deprivation session, participants arrived at the laboratory late at night and were monitored while participating in low-stress activities such as internet surfing, reading, and movies of low emotionality. In the sleep-rested session, participants were prepared for an 8-hour night of sleep. At around 8:30 A.M. for both sessions, participants performed the thermal pain sensitivity task in the fMRI scanner to observe alterations in reactivity and if their perceived level of pain had changed. In the online phase of the study, a separate group of 236 participants were assessed over 2 nights and 2 days following their normal sleeping schedule. Throughout this phase, they quantified a number of factors related to sleep by filling out a sleep diary. Following this, they completed a questionnaire at the end of the day to rate their physical pain experience on a scale of 0-100 with 100 signifying unimaginable pain.

The researchers hypothesized that following sleep loss, activity within the somatosensory cortex, if directly correlated, would increase in scale with the degree of perceived pain. Decreases in activation would occur in the thalamus and nucleus accumbens assuming the threshold of pain had shifted, indicating a failure to modulate pain. Decreases in activity of the insula and cingulate would indicate improper integration of higher-order evaluation. And after analyzing the fMRI data, this is exactly what they had observed for the in-laboratory phase of the study. For the online portion, they found that subtle changes in sleep in the general population was a predictor of day-to-day changes in self-reported pain intensity in that insufficient sleep in terms of efficiency and quality correlated with higher ratings of experienced pain.

Together, these findings suggest that...

- (1) acute sleep deprivation alters the range for classifying a stimulus as painful by lowering thresholds
- (2) sleep loss alters the pain-reactivity network by increasing pain reactivity and halting higher order processes that are in charge of valuing the painful stimulus
- (3) the same effects can be seen following both acute sleep deprivation and minor changes in sleep

This study is important in offering a new avenue for pain treatment, suggesting that modest improvements in sleep quality have the potential to significantly reduce feelings of pain. For many people, small changes that ensure quality, rather than quantity, are more realistic. Even if there

isn't enough time in the day to get the full recommended hours of sleep, finding ways to ensure quality, particularly in REM sleep, will at least have some protective effects against the dangerous cycle of pain and sleep loss.

REFERENCES

1. Anderson M.L, Araujo P., Frange C. & Tufik S. (2018) Sleep Disturbance and Pain: A Tale of Two Common Problems, *Chest*, 154(5): 1249-1259
2. Martin S.E., Englemen H.M., Kingshott R.N. & Douglas N.J. (1997) Microarousals in patients with sleep apnoea/hypopnoea syndrome, *Journal of Sleep Research*, 6: 276-280
3. Ekstedt M., Åkerstedt T. & Söderström M. (2004) Microarousals During Sleep Are Associated With Increased Levels of Lipids, Cortisol, and Blood Pressure, *Psychomatic Medicine*, 66(6): 925-931
4. Krause A.J., Prather A.A., Wager T.D., Linquist M.A. & Walker M.P. (2019) The Pain of Sleep Loss: A Brain Characterization in Humans, *The Journal of Neuroscience*, 39(12):2291-2300
5. Smith M.T., Haythornthwaite J.A. (2004) How do sleep disturbance and chronic pain inter-relate? Insights from the longitudinal and cognitive-behavioral clinical trials literature, *Sleep Medicine Reviews*, 8(2): 119-132
6. Desseilles M., Dang-Vu T., Schwartz S., Peigneux P. & Maquet P. (2009) "Neuroimaging in Sleep and Sleep Disorders". *Sleep Disorders Medicine (Third Edition)*. Philadelphia, PA: W.B. Saunders
7. Roehrs T., Hyde M., Blaisdell B., Greenwald M. & Roth T. (2006) Sleep Loss and REM Loss are Hyperalgesic, *Sleep*, 29(2): 145-151
8. Lautenbacher S., Kundermann B. & Krieg J.C. (2005) Sleep deprivation and pain perception, *Sleep Medicine Reviews*, 10(5): 357-369
9. Bushnell M.C., Duncan G.H., Hofbauer R.K, Ha B., Chen J.I. & Carrier B. (1999) Pain perception: Is there a role for primary somatosensory cortex?, *PNAS*, 96(14): 7705-7709
10. Vogt B.A. & Sikes R.W. (2000) The medial pain system, cingulate cortex, and parallel processing of nociceptive information, *The Biological Basis for Mind Body Interactions*, 122: 223-235
11. Baliki M.N., Geha P.Y., Fields H.L. & Apkarian A.V. (2010) Predicting Value of Pain and Analgesia: Nucleus Accumbens Response to Noxious Stimuli Changes in the Presence of Chronic Pain, *Neuron*, 66(1): 149-160

Puzzled? Turn on the gamma waves

External amplification of gamma wave frequencies applied to the temporal lobe can increase one's ability to find a solution to a problem, inducing a Eureka! moment.

Janee Meengs

From puzzles to jokes, we have all been left in the proverbial dust when it comes to understanding. Some of us remain without a clue, but most of us experience a Eureka moment. Eureka moments are described as abstract leaps, a sudden moment of clarity that allows us to see a problem as we never had before¹. In an article recently published in *Nature*, Santarnecchi and colleagues attempt to use functional magnetic resonance imaging (fMRI) and brain activity recordings using an electroencephalogram (EEG), to try and capture the moment when unconscious turns to conscious by stimulating the right temporal and right parietal brain regions. In this study, the authors found that when stimulation is applied at frequencies related to gamma waves, increased accuracy was associated with the number of correct answers achieved with insight problem solving on a compound remote association problem. The significance of this finding is that the previously correlated studies now have physiological evidence that links the gamma waves to the neural processing required to solve problems with insight rather than logical reasoning.

Insight problem solving is the sudden and unexpected understanding of a problem leading to its solution². Research has attempted to find what causes this Eureka moment, with various theories about its neural basis¹. What is known is that unconscious processing must occur prior to conscious knowledge. Therefore, the solution to a problem is subconsciously known before consciously indicating the answer³. Previous research has identified the high frequency waves, gamma waves, emitted by our brains as we reach this Aha moment and a specific region of the brain where this takes place^{1,3}. However, some studies only focused on the differences between insight problem solving and non-insight problem solving, where non-insight is defined as solving the problem in a step-wise logical manner¹. As shown in this study, electrical activity can be used to record brain activity. Electrical activity recorded from the brain is shown in five types of brain waves: alpha, beta, delta, gamma and theta⁴. Alpha waves are present during a relaxed and wakeful state. Beta waves occur when attention is directed towards a certain task. Delta waves are slow and generally occur during dreamless sleep. Theta waves also occur during sleep, but are present during the deeper sleep stages. Lastly, gamma waves are high frequency waves associated with information processing⁵.

Brain stimulation of the temporal lobe at gamma wave frequencies can increase accuracy of solutions gained through insight problem solving

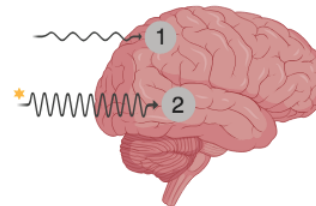


Figure 8. Brain stimulation was applied at the parietal-occipital region (1) and the temporal region (2). The star indicates the area stimulated to improve accuracy of CRA puzzles.

Santarneccchi and the other researchers used EEG and fMRI to visualize these brain waves during their study. EEG is ideal because it is a non-invasive method that can record brain activity. Using electrodes placed on the scalp, electrical brain activity can be recorded in response to environmental stimuli. One drawback of this mechanism is its inability to be specific about which brain areas are activated. By coupling the EEG with the fMRI, more precise spatial information can be gathered about activated areas since the fMRI records changes in blood oxygen fluctuation related to brain activity⁶.

Participants in the study performed compound remote association (CRA) tasks and rebus puzzles while receiving a fake stimulation or gradual 2 mA stimulation was applied for two minutes to the temporal and parietal regions (Figure 1). A CRA problem is a verbal problem where three words are given and the object is to find a single word that is associated to all three words^{3,7}. For example, the given words are play, hog and floor and one solution is ground. A rebus puzzle is where words are used to cryptically represent another word or saying⁸. An example of that would be the word "MIND" physically over the word "MATTER" and the solution is the phrase Mind Over Matter. To test the repetitive and timed motion of the brain wave activity seen in previous research that indicated a switch in wave pattern, the stimulations were given in the same manner. Following each CRA or rebus puzzle the participant indicated whether they had achieved the answer through insight or non-insight reasoning.

Participants correctly solved 54% of the rebus puzzles and 55% of the CRA problems. Of the correct answers, approximately 63% and 52% participants reported using insight problem solving in the CRA and rebus puzzles. The condition in which stimulation was used over the temporal lobe had a significant effect on accuracy during the CRA problem task at gamma wave frequencies. The effect during the rebus task was not significant and there was no significant difference from the sham condition at frequencies more closely associated with the alpha waves. EEG results showed a significant effect of the stimulation at gamma wave frequencies and trended towards significance at alpha wave frequencies.

The implications of this study provide possible causal link between a switch in brain wave patterns and the Eureka moment we experience during insight problem solving. Future research show include controls for other confounding factors like mood and age that could have an effect on the solution style implemented in CRA problems. The data here are somewhat convincing but many of the results were not statistically significant suggesting more evidence needs to be found to fully show that a burst of gamma waves in the temporal lobe lead to the switch of information from unconscious to conscious thought.

REFERENCES

1. Santarneccchi, E., Sprugnoli, G., Bricolo, E., Costantini, G., Liew, S., Musaeus, C.S., Salvi, C., Pascual-Leone, A., Rossi, A. and Rossi, S. (2019). Gamma tACS Over the Temporal Lobe Increases the Occurrence of Eureka Moments. *Scientific Reports* 9: 1-12
2. Webb, M.E., Little, D.R. and Cropper, S.J. (2016). Insight is Not in the Problem: Investigating Insight in Problem Solving Across Task Types. *Frontiers in Psychology* 7: 1-13
3. Jung-Beeman, M., Bowden, E.M., Haberman, J., Frymiare, J.L., Arambel-Liu, S., Greenblatt, R., Reber, P.J. and Kounios, J. (2004). Neural Activity When People Solve Verbal Problems with Insight. *PLOS Biology* 2: 500-510
4. Herrmann, N. (1997). What is the Function of Various Brainwaves? *Scientific American* [Online] Retrieved from <https://www.scientificamerican.com/article/what-is-the-function-of-t-1997-12-22/>
5. Brainworks. (2007). What are Brainwaves? Retrieved April 13, 2019 from <https://brainworksneurotherapy.com/what-are-brainwaves>
6. Antonenko, P., Paas, F., Grabner, R. and van Gog, T. (2010). Using Electroencephalography to Measure Cognitive Load. *Educational Psychology Review* 22: 425-438
7. Bowden, E.M. and Jung-Beeman, M. (2003). Normative Data for 144 Compound Remote Associative Problems. *Behavior Research Methods, Instruments and Computers* 35:634-639

8. Salvi, C., Constantini, G., Bricolo, E., Perigini, M. and Beeman, M. (2015). Validation of Italian Rebus Puzzles and Compound Remote Associate Problems. *Behavior Research Methods* 48: 664-685

Don't go to bed angry! Good for your relationship and for your frontal cortex

A new study found that people who have greater alpha wave power in their right frontal cortex during REM sleep and evening wakefulness experience more anger in dreams.

Gavin Lockard

It is still unclear how neural processes regarding mood and emotion differ in wakeful versus dreaming states. Sikka et al. recently published a paper in 2019 in *The Journal of Neuroscience* titled “EEG Frontal Alpha Asymmetry and Dream Affect: Alpha Oscillations Over the Right Frontal Cortex During REM Sleep and Pre-Sleep Wakefulness Predict Anger in REM Sleep Dreams”¹. Sikka et al. used electroencephalography on humans during evening resting wakefulness and REM sleep and observed increases in alpha waves in the right frontal cortex compared to the left frontal cortex. This is known as frontal alpha asymmetry (FAA). Participants were awakened in the middle of their REM sleep and provided a dream report. Results demonstrated that FAA during evening wakefulness and REM sleep correlate with increased ratings of dream anger. FAA may be important in regulating emotions both during wakefulness and during dreams.

Humans experience emotions both in waking and dreaming states^{2,3}. Neural processes that underlie these emotions are expected to be similar in both waking and dreaming states^{4,5,6,7}. There is evidence for activation of brain regions during REM sleep that are involved in mood and emotions^{8,9,10}. The connection between emotional dream experiences that were reported upon awakening and neural activity during pre-awakening sleep has not been studied well. Electroencephalographic (EEG) FAA measures the difference in alpha waves on the right frontal cortex compared to the left frontal cortex.

The methods of the Sikka et al. study are outlined in the following. 17 human participants (with seven men) spent two nights in the sleep laboratory, separated by a week. On both nights, EEG electrodes were attached, and baseline recordings were taken. Participants then fell asleep, and sleep stages were scored visually^{11,12}. Every time that REM sleep lasted for more than five minutes, a tone was used to awaken the participants. The participants then provided an oral report on their dream, described the last image they saw in their dream, and rated their emotions using a computer program. Finally, the participants were awoken for the final time and after one last dream report, morning baseline EEG recordings were taken.

Sikka et al. found that participants experienced more intense emotions during dreaming than during evening or morning wakefulness. Evening FAA and REM FAA were highly correlated with each other. They also found that the relationship between REM sleep FAA and anger during dreaming were driven by increased alpha power in the right frontal cortex (Figure 1). This was found to be specific to alpha waves only.

Sikka et al. demonstrated in this study that emotions during dreaming and wakeful states are highly similar and can even be measured using a quantitative technique, being EEG. Similar brain regions are activated in wake vs. dream, and similar emotional experiences are reported. This implies that memory consolidation is an important process during REM sleep, and subjective experiences during the day with emotional tags are relived in dreams.

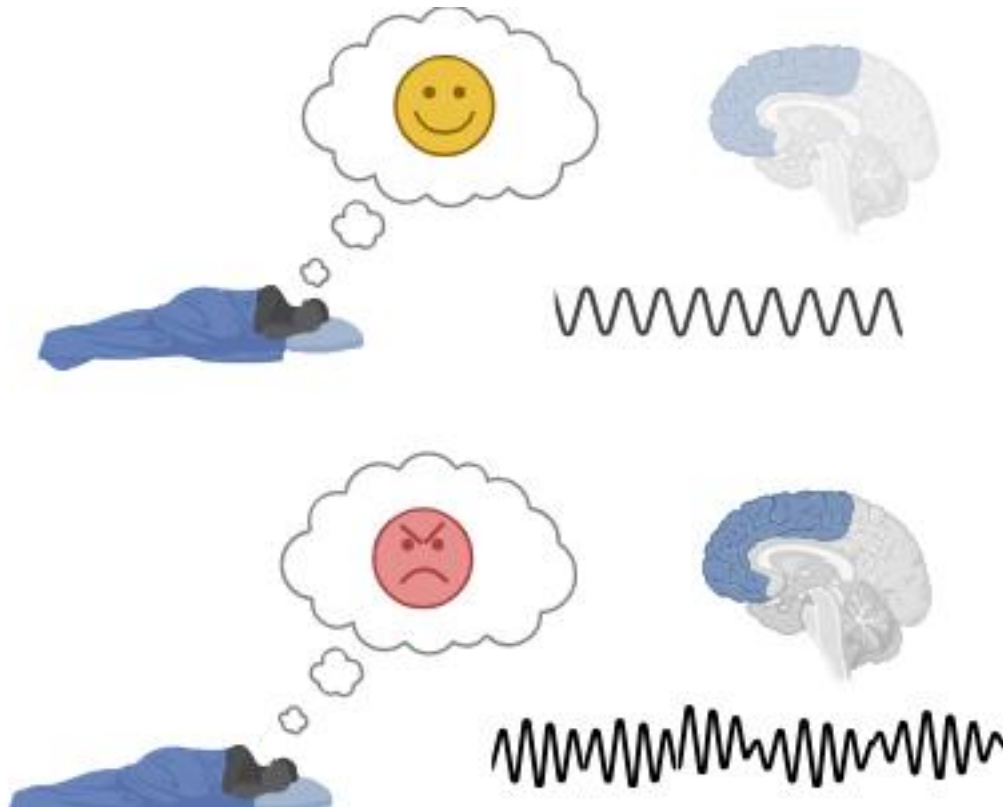


Figure 9. This figure demonstrates that the relationship between REM sleep FAA and anger during dreaming were driven by increased alpha power in the right frontal cortex. People dreaming with non-negative emotions had reduced FAA.

REFERENCES

1. Sikka, P., Revonsuo, A., Noreika, V., & Valli, K. (2019). EEG Frontal Alpha Asymmetry and Dream Affect: Alpha Oscillations Over the Right Frontal Cortex During REM Sleep and Pre-Sleep Wakefulness Predict Anger in REM Sleep Dreams. *Journal of Neuroscience*, 2884-18.
2. Hobson JA, Pace-Schott EF, Stickgold R (2000) Dreaming and the brain: toward a cognitive neuroscience of conscious states. *Behav Brain Sci* 23:793-842.
3. Desseilles M, Dang-Vu TT, Sterpenich V, Schwartz S (2011) Cognitive and emotional processes during dreaming: a neuroimaging view. *Conscious Cogn* 20:998-1008.
4. De Gennaro L, Marzano C, Cipolli C, Ferrara M (2012) How we remember the stuff that dreams are made of: neurobiological approaches to the brain mechanisms of dream recall. *Behav Brain Res* 226:592-596.
5. Fox KCR, Nijboer S, Solomonova E, Domhoff GW, Christoff K (2013) Dreaming as mind wandering: evidence from functional neuroimaging and first-person content reports. *Front Hum Neurosci* 7:412.
6. Wamsley EJ (2013) Dreaming, waking conscious experience, and the resting brain: report of subjective experience as a tool in the cognitive neuroscience. *Front Psychol* 4:637.
7. Domhoff WG, Fox KR (2015) Dreaming and the default network: a review, synthesis, and counterintuitive research proposal. *Conscious Cogn* 33:342-353.
8. Maquet P, Pétters J-M, Aerts J, Delfiore G, Degueldre C, Luxen A, Franck G (1996) Functional neuroanatomy of human rapid-eye-movement sleep and dreaming. *Nature* 383:163-166.

9. Nofzinger AE, Mintun MA, Wiseman M, Kupfer DJ, Moore RY (1997) Forebrain activation in REM sleep: an FDG PET study. *Brain Res* 770:192-201.
10. Braun RA, Balkin TJ, Wesensten NJ, Gwady F, Carson RE, Varga M, Baldwin P, Belenky G, Herscovitch P (1998) Dissociated pattern of activity in visual cortices and their projections during human rapid eye movement sleep. *Science* 279:91-95.
11. Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, D.C.: Government Printing Office.
12. Iber C, Ancoli-Israel S, Chesson A, Quan SF, for the American Academy of Sleep Medicine (2007) The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. 1st ed. Westchester, IL: American Academy of Sleep Medicine.

Special K – Not just a cereal: ketamine as a novel treatment of depression

Traditional antidepressants have delayed therapeutic onset and are ineffective in up to 30% of treatment resistant forms of depression. Ketamine may provide a novel fast acting mechanism of reversing depression by changing the structure and signaling in the brain.

Daniel Salazar

It is not yet fully understood how ketamine, a commonly used anesthetic, reverses depression and anxiety symptoms. Moda-Sava and colleagues recently published a paper in *Science Journals*, aiming to understand how chronic stress affects the connectivity of the brain, neuronal structure, and behavior in mice. Strikingly, they discovered that ketamine is able to reverse depression symptoms and improve connectivity of the brain within 4 hours. After 24 hours, ketamine induces the regrowth and addition of structural components of neurons. Furthermore, they observed that this regrowth and addition of new structural components of neurons attributed to the sustainable remission of depression behavior in mice. This research provides application of ketamine as a powerful clinical tool in treating depression and sustaining recovery.

DEPRESSION AND KETAMINE

Depression is a complex mental disorder which results from a combination of genetic and environmental factors. Growing research has shown that depression may arise from misfiring neurons¹. Specifically, the excitatory neurons in areas of the brain important in executive function and memory formation¹. This is formally known as the “Glutamate Hypothesis” of depression. Depression is also associated with loss of dendritic spines². Dendritic spines are tiny protrusions coming off of neurons. Additionally, another hallmark of depression is chronic stress³. Thus, scientists use stress-induced-depression rodent models to understand the underlying mechanisms of depression and to develop future treatments. Unfortunately, up to 30% of people who suffer from depression do not respond to traditional treatments such as SSRIs and cognitive behavioral therapy^{3,4}. Moreover, traditional antidepressants often take weeks to take effect⁴. Severe symptoms of depression include suicidal ideation and self harm. Thus, therapeutic onset of antidepressant medication is extremely important in these circumstances⁵. In humans, ketamine has shown promise in acutely reversing depression symptoms within 24 hours⁶. However, the way in which ketamine acts on the brain to reverse depression is not yet fully understood.

METHODS

Moda-sava and colleagues aimed to understand the role of ketamine on depression-like behavior in mice. They monitored apical spine density in the mPFC before and after chronic stress exposure as well as after intraperitoneal ketamine injections. Chronic stress was applied with 21

days of corticosterone administration in the drinking water OR 21 days of restrained stress test. These researchers were able to monitor the structural changes in dendritic spines using microprisms and optic fibers implanted in the mPFC of the mice. Two photon microscopy was used to image the dendrites before and after exposure to chronic stress and ketamine administration. Additionally, connectivity within mPFC microcircuits was measured using 2 photon calcium imaging. Behavioral tests included sucrose preference test, exploration of the open arms in the elevated plus maze, and mobility during the tail suspension test.

KETAMINE REVERSES DEPRESSION INDUCED BEHAVIOR AND CHANGES IN THE BRAIN

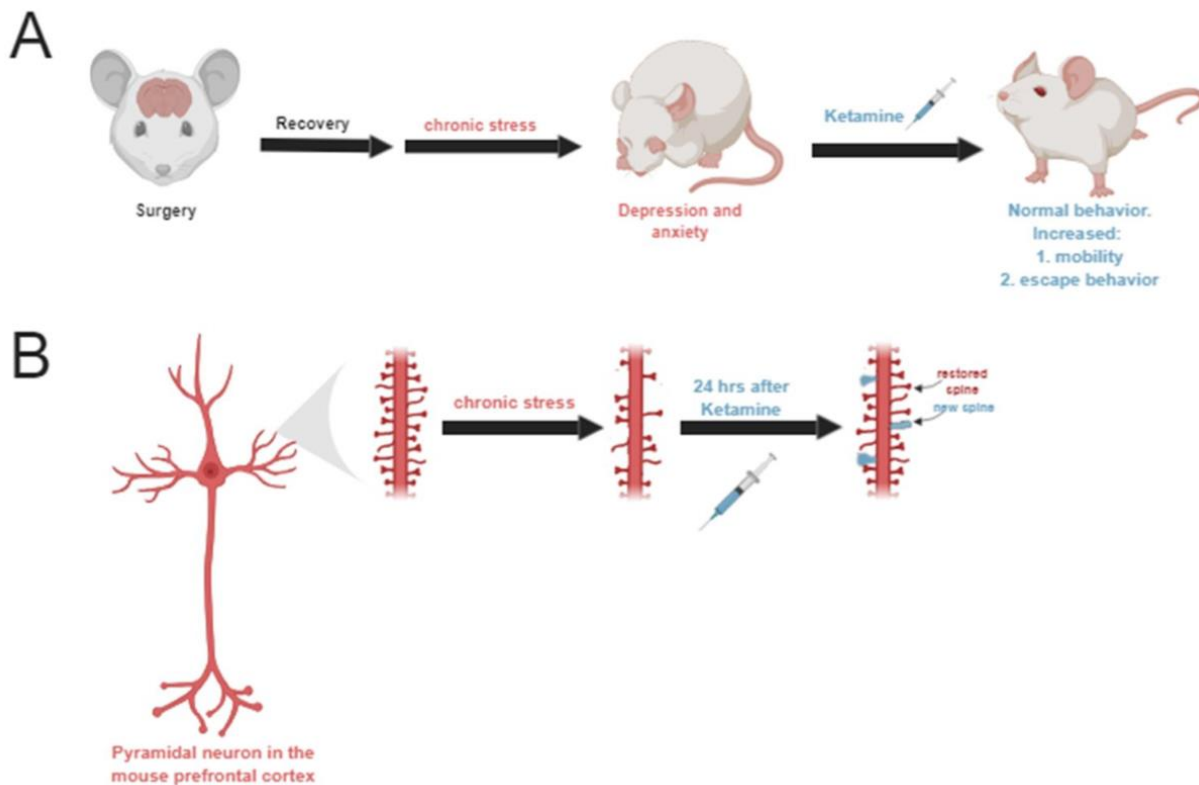


Figure. Behavioral and structural changes of pyramidal dendritic spines after exposure to chronic stress and ketamine. (A) General timeline of the experiment. Surgery with implantation of prism allowing for a 2 week recovery time. Next, the mice were subjected to 21 d of chronic stress treatment (Corticosterone administration OR repeated restraint stress). Finally, the mice were given a ketamine injection which reversed depression and anxiety symptoms within 4 hours. **(B)** Changes observed in the apical dendrites of pyramidal neurons found in the mouse medial PFC following 21 d of chronic stress and ketamine administration.

Unsurprisingly, 21 days of chronic stress led to the development of depression and anxiety-like behavior in mice. Two photon microscopy revealed that dendritic spine density had also decreased following chronic stress. Remarkably, ketamine administration reversed depression-like behavior within 4 hours after the injections (Figure A). Connectivity and synapse formation also improved within the mPFC within 4 hours after ketamine. Interestingly, there was no significant changes in spine densities until after 24 hours post ketamine injections. At this 24 hour mark, they saw an increase in dendritic spine re-growth as well as the addition of new spines (Figure B). Next, Moda-Sava and his team wanted to test whether ketamine provoked stochastic

or spine specific regrowth/addition. They discovered that deletion of new spines led to relapse in behavior and deletion of random spines had no significant effect on behavior.

SIGNIFICANCE AND FUTURE STUDIES

These findings suggests that spinogenesis plays a vital role in the sustained recovery of depression. Study author Dr. Liston states, "Our results suggest that interventions aimed at enhancing synapse formation and prolonging their survival could be useful for maintaining the antidepressant effects of ketamine in the days and weeks after treatment." Future studies should focus on developing treatments that prolong the life of synapses in conjunction to ketamine treatment. Other glutamatergic system targeted therapies should also be further explored in the future. Ketamine may serve as a powerful clinical tool in treating depression and importantly in elucidating the complexities underlying mental illness.

REFERENCES

1. Sanacora, G., Treccani, G., & Popoli, M. (2012, January). Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/21827775>
2. Qiao, H., An, S., Xu, C., & Ma, X. (2017, May 15). Role of proBDNF and BDNF in dendritic spine plasticity and depressive-like behaviors induced by an animal model of depression. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28284898>
3. Seo, J., Wei, J., Qin, L., Kim, Y., Yan, Z., & Greengard, P. (2017, October). Cellular and molecular basis for stress-induced depression. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/27457815>
4. Al-Harbi, K. S. (2012). Treatment-resistant depression: Therapeutic trends, challenges, and future directions. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3363299/>
5. Health Quality Ontario. (2017, November 13). Psychotherapy for Major Depressive Disorder and Generalized Anxiety Disorder: A Health Technology Assessment. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5709536/>
6. Lener, M. S., Kadriu, B., & Zarate, C. A. (2017, March). Ketamine and Beyond: Investigations into the Potential of Glutamatergic Agents to Treat Depression. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5342919/>

Salt on the brain: a possible explanation for the irresistibility of salty foods

A new study combines several genetic tools to identify a neural circuit in mice that synthesizes information about taste and internal state to modulate appetite for sodium. Similar techniques could be used to probe a number of other peripheral circuits.

Jacob Pennington

Mouth-watering cravings for salty foods like french fries or mozzarella sticks are something most people can probably identify with, but the source of these cravings in the brain has long remained unclear¹. Several studies have identified brain regions that are associated with appetite for sodium – one kind of important salt – but what exactly those regions do is not known. In a study recently published in *Nature*, Lee et al. sought to investigate the role of one such brain region by switching its activity in mice on or off while monitoring sodium appetite. The researchers found that stimulation of this region tended to increase appetite for sodium and that consuming sodium subsequently decreased the region's activity but only if the mice were able to taste the sodium. The comprehensive strategy used in this study could be applied to other sensory modalities and internal drives to solidly advance our understanding of how the brain responds to daily needs.

BACKGROUND

Neurons in the pre-locus coeruleus (pre-LC) express c-FOS, a marker for increased activity, in response to a prolonged low-sodium diet². Relatedly, a population of HSD2-positive neurons in the nucleus of the solitary tract (NTS) drives appetite for sodium when stimulated, and this region projects to the aforementioned pre-LC neurons³. Both of these regions are therefore likely involved in balancing sodium concentrations in the body by driving sodium appetite, a “hard-wired” drive akin to thirst that can impact blood pressure and other health metrics^{4,5,6}. The study by Lee et al. focuses on the pre-LC region, but also demonstrates vital forward projections from the NTS to the pre-LC region (results omitted for brevity).

METHODS

To assess the causes and results of pre-LC activation, the authors first measured c-FOS expression in the pre-LC under sodium-depleted, water-depleted, sodium-rescued, and control conditions⁷. Then the authors used an adeno-associated viral (AAV) delivery system to cause pre-LC neurons to be responsive to light stimulation via Cre-dependent channelrhodopsin^{8,9}. Two variations on this procedure were used to allow either optogenetic excitation or inhibition of the pre-LC, and the authors observed the effects of both types of stimulation on sodium appetite and behavior. The authors also monitored pre-LC activity, measured via calcium fluorescence due to GCaMP6s expression, before and after sodium intake. This measurement was repeated with the addition of amiloride to block taste sensation of sodium^{10,11}. The authors also measured activity before and after injection of sodium directly into the mice's stomachs.

RESULTS

A significant number of pre-LC neurons expressed c-FOS in the sodium-depleted condition but not in other conditions, indicating that the neurons became active in response to a lack of dietary sodium. Optogenetic excitation of the pre-LC resulted in increased sodium consumption, aversion to stimulation in a place preference task, and increased effort to avoid stimulation in a lever-pressing task. The researchers interpreted this to mean that pre-LC activity both induced appetite for sodium and was perceived as unpleasant. Optogenetic inhibition of pre-LC activity instead resulted in decreased sodium consumption. Ingestion of sodium by deprived mice quickly suppressed pre-LC activity under normal conditions, but not when the mice were also treated with amiloride. Gastric injection of sodium did not affect pre-LC activity. These results indicated that sodium's impact on pre-LC activity was directly tied to the sensation of tasting the sodium.

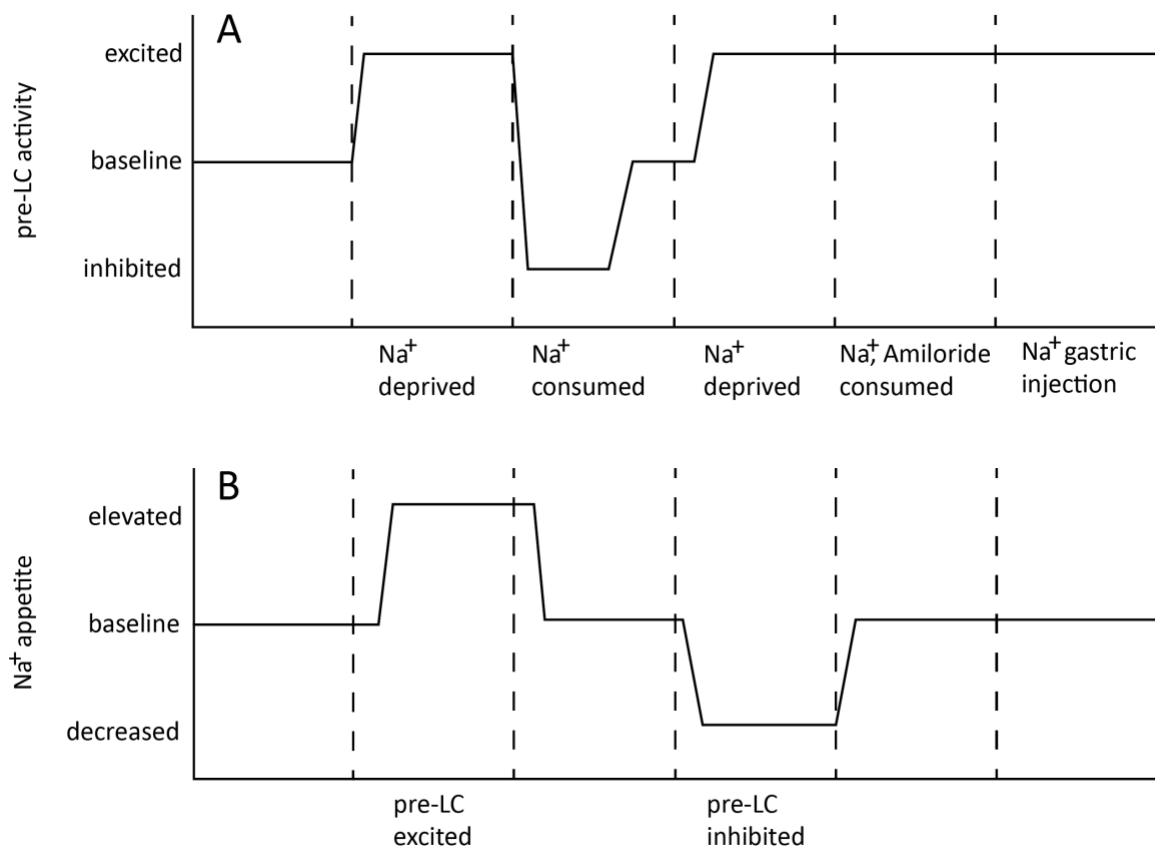


Figure 1. Schematic summary of observed effects. A. Level of Ca₂₊ fluorescence in pre-LC region. A prolonged low-sodium diet resulted in increased pre-LC activity. Subsequent oral consumption of sodium inhibited pre-LC activity, but gastric injection of sodium did not. Treatment with Amiloride blocked consumption-mediated inhibition. B. Sodium appetite (measured in lick rate). Appetite increased in response to optogenetic excitation of the pre-LC region, and decreased in response to optogenetic inhibition.

CONCLUSION

The results presented by Lee et al. clearly demonstrate a causal interaction between sodium taste and pre-LC neural activity, solidifying the region's role as a taste-mediated regulator of sodium

appetite. Further, the authors found that peripheral impacts on pre-LC neurons were eliminated if the NTS was inhibited, placing these results (summarized in Figure 1) in the context of a well-defined circuit. The fact that pre-LC activity, and sodium appetite in turn, is significantly modulated by sodium taste (but not consumption) has interesting implications for the moderation of sodium intake in humans. Of more general significance from this study is its demonstration of how calcium fluorescence, optogenetics, behavioral measures, and genetic markers can be combined to directly probe the function of neural circuits.

REFERENCES

1. Lee, S., Augustine, V., Zhao, Y., Ebisu, H., Ho, B., Kong, D., and Oka, Y. (2019). Chemosensory modulation of neural circuits for sodium appetite. *Nature*, 568: 93-97. doi: 10.1038/s41586-019-1053-2.
2. Geerling, J., Stein, M., Miller, R., Shin, J., Gray, P., and Loewy, A. (2011). FoxP2 expression defines dorsolateral pontine neurons activated by sodium deprivation. *Brain Research*, 1375: 19-27. doi: 10.1016/j.brainres.2010.11.028.
3. Jarvie, B. and Palmiter, R. (2017). HSD2 neurons in the hindbrain drive sodium appetite. *Nature Neuroscience*, 20(2): 167-169. doi: 10.1038/nn.4451.
4. Farquhar, W., Edwards, D., Jurkowitz, C., and Weintraub, W. (2015). Dietary Sodium and Health: More Than Just Blood Pressure. *Journal of the American College of Cardiology*, 65(10): 1042-1050. doi: 10.1016/j.jacc.2014.12.039.
5. Geerling, J. and Loewy, A. (2008). Central regulation of sodium appetite. *Experimental Physiology*, 93(2): 177-209. doi: 10.1113/expphysiol.2007.039891.
6. Johnson, A. and Thunhorst, R. (1997). The Neuroendocrinology of Thirst and Salt Appetite: Visceral Sensory Signals and Mechanisms of Central Integration. *Frontiers in Neuroendocrinology*, 18: 292-353. doi: 10.1006/frne.1997.0153.
7. Leeyup, C. (2015). A Brief Introduction to the Transduction of Neural Activity into Fos Signal. *Development & Reproduction*, 19(2): 61-67. doi: 10.12717/DR.2015.19.2.061.
8. Naso, M., Tomkowicz, B., Perry, W., and Strohl, W. (2017). Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs*, 31: 317-334. doi: 10.1007/s40259-017-0234-5.
9. Deisseroth, K. and Hegemann, P. (2017). The form and function of channelrhodopsin. *Science*, 357(6356). doi: 10.1126/science.aan5544.
10. Eylam, S. and Spector, A. (2003). Oral Amiloride Treatment Decreases Taste Sensitivity to Sodium Salts in C57BL/6J and DBA/2J Mice. *Chemical Senses*, 28(5): 447-458.
11. Ossebaard, C., Polet, I., Smith, D. (1996). Amiloride Effects on Taste Quality: Comparison of Single and Multiple Response Category Procedures. *Chemical Senses*, 22(3): 267-275.

Are pesticides safe to use with proper protections?

Lillian Hughes

Pesticides are handy in day to day life, in order to keep slugs out of the garden bed, or to keep a football field green. While people who only use pesticides once or twice a year may not notice negative effects, do people who regularly use pesticides notice detrimental effects? While many pesticides are deemed safe for human contact under safety procedures, as time passes, more and more pesticides are being considered unsafe, even under safety procedures.

A recent study done by Lee et al., discusses that when pesticides are used in an outside location they can easily contaminate inside buildings¹. Not only can pesticides be tracked into human living quarters, but pesticides can also make their way into rivers and affect animal habitats. Pesticides can be a danger to both humans and animals.

Certain classes of pesticides are designed to kill pests by causing mitochondrial dysfunctions. Mitochondria are the 'powerhouse of the cell' so they are vital for cell function. Since pests are small most can be killed at a much smaller dose than humans. While a small dose due to the pesticide moving inside may not cause a large effect, the small effect can build up over time.

Deltamethrin is pesticide that is derived from chrysanthemums and therefore a natural pesticide². While deltamethrin is deemed to be a safe pesticide, a review written by Lu et al., insinuates that deltamethrin may have dangerous effects on mitochondrial function, even as far as leading to Parkinson's Disease. This is not the only pesticide that has been connected to Parkinson's Disease. For example, two pesticides that were once commonly used, rotenone and paraquat, are now being used in Parkinson's Disease models due to their damage to the mitochondria.

The study specifically looked at the epidemiology of deltamethrin on various wildlife. Deltamethrin induced symptoms such as "Oxidative DNA damage" (in rainbow trout³, carp⁴), "increased carbonyl levels" (In freshwater fish⁵), "DNA Fragmentation" (in Male albino rats⁶), and "DNA damage" (Female Wistar Rats⁷).

Deltamethrin has been shown to activate the P66SHC pathway, which is connected to Reactive Oxygen Species (ROS). Figure 1 illustrates the biological pathway that deltamethrin activates. Deltamethrin increases the activity of P66SHC by phosphorylation. The P66SHC pathway increases ROS by moving into the mitochondria, then interacting with cytochrome C⁸. Cytochrome C then acts to increase ROS production. ROS are dangerous to the cell since they can cause DNA damage, and apoptosis⁹.

While deltamethrin may not have a deadly effect in a small dosage, people who are constantly exposed to pesticides, such as farmers, may be at risk. Farmers would not have an opportunity to decrease their exposure time, especially if the pesticide were to be deemed safe. People outside of the science community do not scour the information on a can of pesticide. Safety is not always the first concern of people, allowing overexposure to occur.

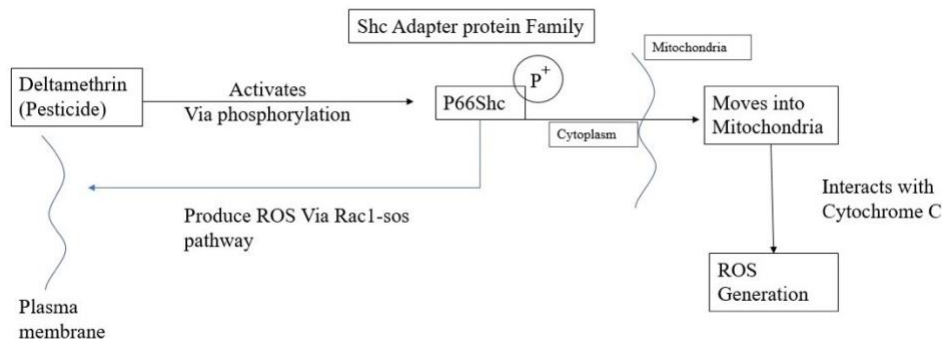


Figure 1.

It is vital to determine if deltamethrin is safe for reoccurring use. It is also important to stop laypeople from misusing pesticides. While pesticides can decrease disease and pests while increasing crop yield, they are currently dangerous for humans and wildlife.

REFERENCES

1. Lee et al., "Indoor contamination from pesticides used for outdoor insect control", *Science of The Total Environment*, Volume 625, 2018, Pages 994-1002, ISSN 0048-9697, <https://doi.org/10.1016/j.scitotenv.2018.01.010>.
2. National Center for Biotechnology Information. PubChem Database. Deltamethrin, CID=40585, <https://pubchem.ncbi.nlm.nih.gov/compound/40585> (accessed on Apr. 17, 2019)
3. Alak et al., "Assessment of 8-hydroxy-2-deoxyguanosine activity, gene expression and antioxidant enzyme activity on rainbow trout (*Oncorhynchus mykiss*) tissues exposed to biopesticide", *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, Volume 203, 2017, Pages 51-58, ISSN 1532-0456, <https://doi.org/10.1016/j.cbpc.2017.10.007>.
4. Arslan et al., "Acute toxication of deltamethrin results in activation of iNOS, 8-OHdG and up-regulation of caspase 3, iNOS gene expression in common carp (*Cyprinus carpio* L.), *Aquatic Toxicology*, Volume 187, 2017, Pages 90-99, ISSN 0166-445X, <https://doi.org/10.1016/j.aquatox.2017.03.014>.
5. Sayeed et al., "Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch", *Ecotoxicology and Environmental Safety*, Volume 56, Issue 2, 2003, Pages 295-301, ISSN 0147-6513, [https://doi.org/10.1016/S0147-6513\(03\)00009-5](https://doi.org/10.1016/S0147-6513(03)00009-5).
6. Ogaly et al., "Influence of green tea extract on oxidative damage and apoptosis induced by deltamethrin in rat brain", *Neurotoxicology and Teratology*, Volume 50, 2015, Pages 23-31, ISSN 0892-0362, <https://doi.org/10.1016/j.ntt.2015.05.005>.
7. Chargui et al., "Oxidative Stress, Biochemical and Histopathological Alterations in the Liver and Kidney of Female Rats Exposed to Low Doses of Deltamethrin (DM): A Molecular Assessment", *Biomedical and Environmental Sciences*, Volume 25, Issue 6, 2012, Pages 672-683, ISSN 0895-3988, <https://doi.org/10.3967/0895-3988.2012.06.009>.
8. Giorgio et al., "Electron Transfer between Cytochrome c and p66Shc Generates Reactive Oxygen Species that Trigger Mitochondrial Apoptosis", *Cell*, Volume 122, Issue 2, 2005, Pages 221-233, ISSN 0092-8674, <https://doi.org/10.1016/j.cell.2005.05.011>.
9. Wojtala et al., "Modulation of mitochondrial dysfunction-related oxidative stress in fibroblasts of patients with Leigh syndrome by inhibition of prooxidative p66Shc pathway", *Mitochondrion*, Volume 37, 2017, Pages 62-79, ISSN 1567-7249, <https://doi.org/10.1016/j.mito.2017.07.002>.
10. Giorgi et al., "Chapter Six - Mitochondria and Reactive Oxygen Species in Aging and Age-Related Diseases", Editor(s): Carlos López-Otín, Lorenzo Galluzzi, *International Review of Cell and Molecular Biology*, Academic Press, Volume 340, 2018, Pages 209-344, ISSN 1937-6448, ISBN 9780128157367, <https://doi.org/10.1016/bs.ircmb.2018.05.006>.
11. Ingersoll et al., "p66Shc regulates migration of castration-resistant prostate cancer cells", *Cellular Signalling*, Volume 46, 2018, Pages 1-14, ISSN 0898-6568, <https://doi.org/10.1016/j.cellsig.2018.02.008>.

DALE FORTIN, PhD

Dale Fortin is currently a neuroscience instructor at Washington State University Vancouver as well as a senior research associate at the Vollum Institute at Oregon Health & Science University. Fortin's teaching style is considered a breath of fresh air for many students as they not only have the opportunity to immerse themselves in the intricacies of neuroscience, but they are encouraged to improve their skills and build on their learning. Students are invited to discuss topics openly, combining critical thinking with statistical analysis to solve complex problems. With a professor like Fortin, it is no surprise that students quickly pick up on his passion and enthusiasm.



In May of 2018, Fortin was awarded the Students' Award for Teaching Excellence for not only his dedication to students, but for inspiring his students to put out their best work. For future scientists who find themselves taking a class with Fortin, many feel they have acquired new skills they can then apply to their own in-class research. There are many opportunities to focus assignments around individual interests allowing students to not only explore their passions, but learn about new topics as peers present and discuss their own work. Fortin's teaching skills foster a safe environment for students to grow and explore new topics.

Having dabbled in many areas of research ranging from synaptic plasticity to endocannabinoids, Fortin's recent work explores the removal of perineuronal nets. These perineuronal nets form during development and are comprised of specialized extracellular matrix structures. Perineuronal nets serve multiple functions, and there is speculation that they regulate chemical signaling between brain cells. To study how alterations in these structures contribute to disorders such as schizophrenia and epilepsy, this research utilizes gene silencing approaches to target the knockdown of specific proteins that are imperative to the formation of perineuronal nets. In targeting the knockdown of these proteins, the goal is develop a more specific test for their involvement in a range of disorders and diseases.

In order to share the wealth of knowledge and encourage younger minds to partake in science, Fortin has organized several outreach opportunities that allow WSU Vancouver students to share some of their favorite things about neuroscience. Not only does he bring equipment on these endeavors, such as microscopes and electromyographs, but his favorite wow factor is bringing in a real human brain!

Bring more bugs into the lab! A review on the use of social invertebrate species as models in the study of learning and memory

Percy Atwell

INTRODUCTION

The study of mammalian learning and memory is a field relying on the use of various animal models, due in part to the unethical nature of human experimentation and nonhuman models providing faster development and simpler or more accessible nervous systems. However, nonhuman primates, the closest mammalian analogue to humans, are expensive to care for and take a long time to reach adulthood¹. More common are small animal models such as mice and rats, which are less expensive to acquire and raise, and have a vast spectrum of genetic modifications to create robust models of specific disorders². However, using only a select few models over and over in research can create artificial boundaries, so common model species should be complemented by studies using uncommon models. To this end, various invertebrate species are suitable models for research. The animals themselves require small setups and minimal care. In addition to this low-intensity maintenance, invertebrate brains have structural analogues that link them to brain structures in many other species, including humans. Most notable is the link between a highly distinct region in invertebrates, the mushroom bodies in bees or hemiellipsoid bodies in crabs, and the cerebral cortex in vertebrates^{3,4}. This provides an anatomical basis for the use of these animals in neurological studies on learning. In addition to analogous brain structures, invertebrates have biomarkers useful for cognitive studies of anxiety-like and depression-like behaviors. Crabs and bees have biogenic amines such as serotonin and dopamine, as well as a third amine, octopamine, that is analogous to human noradrenaline. Assessment of these biogenic amines in invertebrates has shown changes in response to various stimuli that simulate the same changes in humans⁵. A study on social harassment in crayfish resulted in a rise in hemolymphic serotonin levels and anxiety-like behaviors; both were attenuated with a common antianxiety drug⁶. In humans, stressful situations such as harassment result in abnormal, fluctuating serotonin levels and short-term anxiety⁷. Both depression or anxiety, along with other mood disorders, have an impact on behavior, cognition, and learning in humans, so having measurable biomarkers of analogous chemical reactions is useful in learning studies that examine emotional impacts⁸. The crayfish, with many features analogous to results seen in human studies, are useful models for anxiety and depression. With plenty of reason for using invertebrate models, current studies in a wide range of fields utilize the fruit flies *D. melanogaster* and the nematodes *C. elegans*. There are uses for *D. melanogaster* in genetics, development, and aging, as well as learning and behavior⁹. Similarly, *C. elegans* are used in neural development research, as they have accessible and plain nervous systems¹⁰. However, both of these species are limited in their uses. The simple nervous system of *C. elegans* is incapable of firing action potentials in the same way more complex neurons can¹¹. Conversely, while fruit flies have a wide

range of uses within studies on learning, due to their fast speeds and small size in conjunction with their small setups, factoring in the social behaviors of a fruit fly colony is difficult. The mechanisms that regulate their social grouping activity are not easily accessed or recorded, making the social habits of *D. melanogaster* difficult to classify and control for in an experiment¹². However, the social behaviors of a model organism are an important variable to account for, especially when humans are also a social species¹³. In order to include social behaviors, a wider range of invertebrate species with well-studied and defined social behaviors should be used. There are previous studies done that used social invertebrates to examine a wide range of certain traits, all associated with learning and memory¹⁴. The body of research provides a range of evidence that these social invertebrates display long-term memory in conjunction with associative learning, egocentric comprehension of their environment, and context-dependant learning^{15,16,17}. This review article will discuss and encourage the use of three groups of social invertebrates that are viable models for the study of learning and memory, specifically honeybees of the genus *Apis*, homing ants of the genus *Cataglyphis*, and the herbivorous crab *Neohelice granulata*, also referred to as *Chasmagnathus granulata*.

BEES, ASSOCIATIVE LEARNING, AND SLEEP DEPRIVATION

Invertebrates go through stages of sleep, wakefulness, and sudden arousal. Crayfish show slow brain waves during sleep periods that are similar to REM slow brain waves in vertebrates; scorpions display low responsiveness and heart rate in varying stages during sleep-like behaviors^{18,19}. Honeybees, however, show evidence of a capability to sleep that is more similar to avian or mammalian sleep patterns. The bees have fluctuations in overall neuronal responses to stimuli that synchronize with a typical circadian rhythm and their sleep-wake cycles can be reliably monitored by recordings of their antennal movements^{20, 21}. In mammals, sleep is essential for memory consolidation, a two-stage system redistributing initially recorded memories to long-term storage throughout the pallium²². Sleep is also essential to avian memory consolidation, although it follows an unknown, alternate consolidation pathway that is thought to end within the avian pallium²³. Do honeybees, with their circadian rhythms and mushroom bodies functioning as analogs to mammalian sleep patterns and vertebrate cortices, also utilize sleep for memory consolidation²⁴?

To show the link between learning and sleep in bees, Hussaini et al. approached this question using a sleep deprivation study, exploring the effects of not sleeping on learning in bees. Two types of learning were examined, acquisition learning and extinction learning. By pairing a sugar reward with a neutral odor, the bees were trained into a proboscis extension response to the odor. This is an example of acquisition learning, a strong memory trace. After one night of sleep deprivation post training, the bees were tested to determine if they still responded to the odor without the sugar. Interestingly enough, the sleep deprivation did not attenuate the proboscis extension response. Repeating the initial training, the bees then underwent extinction training the following day, exposing them to the odor without the reward. This is an example of extinction learning, a weaker memory trace where the bee suppresses the previously learned memory, something that is considered as a new memory of an inhibitory response²⁵. This time, the sleep deprived bees performed significantly worse on retention trials than rested bees after extinction trials. The way each form of learning was differentially affected by sleep deprivation was also seen in mammals. In some mammalian studies, strong conditioning is not affected by sleep deprivation, while the weaker extinction memory or spatial learning is affected^{26, 27}. Sleep is essential to forming new memories in bees, especially during weak forms of learning such as extinction learning.

ANTS, EGOCENTRIC MAPPING, AND NAVIGATIONAL MEMORY

For successful navigation, organisms need some way of recalling their spatial orientation in relation to the world around them. In mammals, each individual forms a cognitive map using their hippocampus to store landmark memories, while different parts of their cortex form egocentric memories.²⁸ In this way, humans constantly update their internal maps with information of where their body is within the environment²⁹. This kind of goal-directed navigation is also seen within invertebrates, especially in species that forage outward from a home nest such as honeybees or ants³⁰. The homing desert ant, *Cataglyphis*, is an exceptional model for navigational learning and memory. In addition to using visual landmarks, these ants have an additional way of navigating when the desert shifts and landmarks change. They create an egocentric map, combining information from their internal odometer and the polarization of the sky above them in order to determine their exact location relative to their nestbox³¹.

To show clearly the desert ant's ability to form egocentric, updating maps over the more common landmark memory, Andel and Wehner teach ants to follow obvious landmarks from their nest-box to a feeder, then displace the ants to a landmark-free pathway¹⁶. The ants did not search for missing landmarks, but neither did they simply repeat the pathway back to the nest without interruption. They walked directly towards the nest-box for a short distance, then began systematic searching patterns. Each ant searched further in certain directions and in alternate patterns, although most navigated successfully back to the nest-box in the end. Then, ants would be allowed to run all the way home along the landmark path, then picked up and placed back at the feeder in order to perform a second run home. Once they reached the nest-box a second or third time, the ants were displaced into the landmark-free pathway. Each ant started their path following the landmarks down the wrong way, but they soon corrected their paths towards home. The further away the ants had been displaced, the sooner the ant would begin searching for familiar ground. In humans, the same kind of procedural egocentric mapping is created simultaneously with landmark memory formation. If landmarks are not available, or the landmarks are moved, humans will be able to correctly retrace their route back home based on the navigational, rather than landmark memory³². This same response is seen by Andel and Wehner. Once the ant's landmark memories no longer match the route home, they prioritize their internal maps for navigational tasks.

CRABS, LONG-TERM HABITUATION, AND CONTEXT-DEPENDANT LEARNING

The herbivorous crab *Neohelice granulata* is the sixth most studied crab species, with a toolbox of varied behavioral, pharmacological, electrophysiological and molecular methods established for examining neurophysiological changes in the crab's responses to various stimuli^{33,34}. A common experimental paradigm is training crabs to suppress their flee response from a simulated overhead predator. The pattern and timing of this training can result in a dramatic difference in habituation. Exposing the crab to 15 training trials in one minute results in short-term habituation, while repeating 15 trials over the span of several hours results in long-term habituation that can last for at least five days without any additional training¹⁵. In this way, long-term or short-term potentiation of the crab's flee response may be activated without using invasive stimuli. The crabs do not simply memorize the stimuli and respond to that exact stimulus, which indicates simpler stimulus-specific learning. Instead, the trained responses of the crabs are attenuated when exposed to the stimulus within a new, unfamiliar environment³⁵. By only trusting the visual stimulus

in certain environments, these crabs pair a specific stimulus with a specific context in a display of context-dependant learning. Training the crab to generalize or differentiate similar stimuli in the same escape response paradigm has shown changes within the crab's lobula giant neurons, brain areas responsible for long-term memory storage of visual stimuli in crustaceans³⁶. Hemiellipsoid bodies, brain structures similar to the vertebrate cortex, also appear to be involved in the crab's ability to associate place memories with the stimulus they are exposed to^{3,36}. In this way, the way crabs learn utilizes one brain structure for storage of the memories and a cortex analogue for associating place memory to a stimuli. This is similar to the dual-process model of recognition memory, a suggested theory of the way humans form associations between place memories and cues. While this theory is still highly debated, animal studies using rodents imply that the process of recognizing stimuli is subdivided neuroanatomically between the cortex for processing and association and another brain region, likely the hippocampus, for generalizing or differentiating a presented stimulus^{37,38}. These crabs may be a very simple animal model that supports the dual-process model of recognition memory, with their hemiellipsoid bodies and lobula giant neurons functioning as the two regions involved in the recognition process.

CONCLUSION

The simplistic nature of an invertebrate brain compared to a mammal brain allows for easier pinpointing of the underlying mechanisms when studying learning and memory, while also similar enough in structure to display analogous functions. While the invertebrate species discussed in this review display evidence of complex long-term memory formation, each species of interest lends themselves to specific neuroscientific specialties. Honeybees display clear and trackable sleep patterns useful in studies on the memory traces that are affected by sleep deprivation, as well as associative learning with sugar rewards. Homing ants display an egocentric comprehension of their environment, ideal for studies into spatial memory and the retrieval of navigational memories. *Neohelice* crabs are capable of context-dependant learning, with established methods of recording the neurophysiological changes the crab undergoes. Each of these species have unique traits beneficial to neuroscientific studies on learning and memory, and should be used to precede or supplement the use of vertebrate models. Future research should examine invertebrate and murine models simultaneously in order to determine the similarity and accuracy of their relations to the human disorders they are being used to investigate.

REFERENCES

1. Jennings, M., Prescott, M. J., & Joint Working Group on Refinement (Primates). (2009). Refinements in husbandry, care and common procedures for non-human primates: Ninth report of the BVA/WF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*, 43(1_suppl), 1-47.
2. Brenowitz, E. A., & Zakon, H. H. (2015). Emerging from the bottleneck: benefits of the comparative approach to modern neuroscience. *Trends in neurosciences*, 38(5), 273-278.
3. Maza, F. J., Sztarker, J., Shkedy, A., Peszano, V. N., Locatelli, F. F., & Delorenzi, A. (2016). Context-dependent memory traces in the crab's mushroom bodies: Functional support for a common origin of high-order memory centers. *Proceedings of the National Academy of Sciences*, 113(49), E7957-E7965.
4. Tomer, R., Denes, A. S., Tessmar-Raible, K., & Arendt, D. (2010). Profiling by image registration reveals common origin of annelid mushroom bodies and vertebrate pallium. *Cell*, 142(5), 800-809.
5. Bateson, M., Desire, S., Gartside, S. E., & Wright, G. A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Current biology*, 21(12), 1070-1073.
6. Bacqué-Cazenave, J., Cattaert, D., Delbecq, J. P., & Fossat, P. (2017). Social harassment induces anxiety-like behaviour in crayfish. *Scientific reports*, 7, 39935.
7. Stein, D. J., & Stahl, S. (2000). Serotonin and anxiety: current models. *International clinical psychopharmacology*, 15, S1-6.
8. Murphy, F., Smith, K., Cowen, P., Robbins, T., & Sahakian, B. (2002). The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology*, 163(1), 42-53.
9. Jennings, B. H. (2011). *Drosophila*—a versatile model in biology & medicine. *Materials today*, 14(5), 190-195.

10. Kosinski, R. A., & Zaremba, M. (2007). DYNAMICS OF THE MODEL OF THE CAENORHABDITIS ELEGANS NEURAL NETWORK. *Acta Physica Polonica B*, 38(6).
11. Clare, J. J., Tate, S. N., Nobbs, M., & Romanos, M. A. (2000). Voltage-gated sodium channels as therapeutic targets. *Drug discovery today*, 5(11), 506-520.
12. Simon, A. F., Chou, M. T., Salazar, E. D., Nicholson, T., Saini, N., Metchev, S., & Krantz, D. E. (2012). A simple assay to study social behavior in *Drosophila*: measurement of social space within a group 1. *Genes, Brain and Behavior*, 11(2), 243-252.
13. Young, S. N. (2008). The neurobiology of human social behaviour: an important but neglected topic. *Journal of psychiatry & neuroscience: JPN*, 33(5), 391.
14. Glanzman, D. L. (2010). Common mechanisms of synaptic plasticity in vertebrates and invertebrates. *Current Biology*, 20(1), R31-R36.
15. Hussaini, S. A., Bogusch, L., Landgraf, T., & Menzel, R. (2009). Sleep deprivation affects extinction but not acquisition memory in honeybees. *Learning & memory*, 16(11), 698-705.
16. Andel, D., & Wehner, R. (2004). Path integration in desert ants, *Cataglyphis*: how to make a homing ant run away from home. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1547), 1485-1489.
17. Hermitte, G., Pedreira, M. E., Tomsic, D., & Maldonado, H. (1999). Context Shift and Protein Synthesis Inhibition Disrupt Long-Term Habituation after Spaced, but Not Massed, Training in the Crab *Chasmagnathus*. *Neurobiology of learning and memory*, 71(1), 34-49.
18. Mendoza-Angeles, K., Cabrera, A., Hernández-Falcón, J., & Ramón, F. (2007). Slow waves during sleep in crayfish: A time-frequency analysis. *Journal of neuroscience methods*, 162(1-2), 264-271.
19. Tobler, I., & Stalder, J. (1988). Rest in the scorpion—a sleep-like state?. *Journal of Comparative Physiology A*, 163(2), 227-235.
20. Kaiser, W., & Steiner-Kaiser, J. (1983). Neuronal correlates of sleep, wakefulness and arousal in a diurnal insect. *Nature*, 301(5902), 707.
21. Sauer, S., Kinkelin, M., Herrmann, E., & Kaiser, W. (2003). The dynamics of sleep-like behaviour in honey bees. *Journal of Comparative Physiology A*, 189(8), 599-607.
22. Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological reviews*, 93(2), 681-766.
23. Rattenborg, N. C., Martinez-Gonzalez, D., Roth, T. C., & Pravosudov, V. V. (2011). Hippocampal memory consolidation during sleep: a comparison of mammals and birds. *Biological Reviews*, 86(3), 658-691.
24. Menzel, R. (2013). In search of the engram in the honeybee brain. In *Handbook of Behavioral Neuroscience* (Vol. 22, pp. 397-415). Elsevier.
25. Eisenhardt, D., & Menzel, R. (2007). Extinction learning, reconsolidation and the internal reinforcement hypothesis. *Neurobiology of learning and memory*, 87(2), 167-173.
26. Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., ... & Hu, B. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience*, 144(4), 1186-1192.
27. Silvestri, A. J. (2005). REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiology & behavior*, 84(3), 343-349.
28. Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological review*, 99(2), 195.
29. Save, E., & Moghaddam, M. (1996). Effects of lesions of the associative parietal cortex on the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat. *Behavioral neuroscience*, 110(1), 74.
30. Goldschmidt, D., Manoonpong, P., & Dasgupta, S. (2017). A neurocomputational model of goal-directed navigation in insect-inspired artificial agents. *Frontiers in neurorobotics*, 11, 20.
31. Wehner, R., & Srinivasan, M. V. (2003). Path integration in insects. *The neurobiology of spatial behaviour*, 9-30.
32. Broadbent, H. J., Farran, E. K., & Tolmie, A. (2015). Sequential egocentric navigation and reliance on landmarks in Williams syndrome and typical development. *Frontiers in psychology*, 6, 216.
33. Spivak, E. D. (2010). The crab *Neohelice* (= *Chasmagnathus*) *granulata*: an emergent animal model from emergent countries. *Helgoland Marine Research*, 64(3), 149.
34. Tomsic, D., & Romano, A. (2013). *Invertebrate Learning and Memory: Chapter 26. A Multidisciplinary Approach to Learning and Memory in the Crab Neohelice (Chasmagnathus) granulata* (Vol. 22). Elsevier Inc. Chapters.
35. Tomsic, D., Pedreira, M. E., Romano, A., Hermitte, G., & Maldonado, H. (1998). Context-us association as a determinant of long-term habituation in the crab *Chasmagnathus*. *Animal Learning & Behavior*, 26(2), 196-209.
36. Sztarker, J., & Tomsic, D. (2011). Brain modularity in arthropods: individual neurons that support "what" but not "where" memories. *Journal of Neuroscience*, 31(22), 8175-8180.
37. Mandler, G. (2008). Familiarity breeds attempts: A critical review of dual-process theories of recognition. *Perspectives on Psychological Science*, 3(5), 390-399.
38. Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus?. *Nature Reviews Neuroscience*, 2(1), 51.

Frequency discrimination in the primary auditory cortex and the influence of behavioral training

Kawther Elolaimi

INTRODUCTION

Sensorineural hearing loss accounts for about 90% of reported hearing loss¹ affecting 35 million Americans.² The root cause is from inner ear hair cell damage as well as from vestibulocochlear nerve damage which disrupts the transmission of sound from the inner ear to the brain.³ From the inner ear, sound is transmitted to the auditory cortex which is located on the superior temporal gyrus in the temporal lobe⁴ and is essential for hearing and understanding speech.⁵ The auditory cortex is composed of two main regions, primary auditory cortex and the secondary auditory cortex. The primary auditory cortex (A1) is composed of neurons which function to decode the tonotopic and spatial representation of a sound stimulus as well as differentiate frequencies into identifiable sound.^{6,7} The secondary auditory cortex (AII) is essential for sound detection and localization as well as auditory memory^{6,7} and language comprehension.⁴ Impairment of the auditory cortex to discriminate sounds is often an effect of hearing loss since frequency discrimination is important for speech and complex sound recognition. Studies using behavioral training on ferrets, monkeys, and cats have been conducted to demonstrate the importance of the auditory cortex for processing temporal sequences of sound.^{4,8,9,10,11,12} Without the auditory cortex, these animals lose the ability to discriminate between two complex sounds which have similar frequency components that differ in temporal sequence.⁴ Studies with human patients with either bilateral damage to the auditory cortex or unilateral temporal lobe excisions revealed severe problems in processing of temporal order of sounds as well as the dependence of spatial processing on cortical areas within right superior temporal cortex.^{4,13,14} Additional noise detection studies have been conducted to support the usage of animals, especially ferrets, as an appropriate animal model to study temporal processing.¹⁵ During behavioral training, A1 activity was recorded in response to the sound stimulus and the behavioral state of the animals.⁸ Additional studies have experimented on the plasticity of A1 and whether certain target sounds could potentially be encoded into long-term sensory memory due to receptive field changes.¹⁶ Different studies proposed new mechanisms to enhance brain plasticity via vagus nerve stimulation to induce the speed of learning of behavioral training tasks.¹⁹ This review article will discuss how behavioral training influences the primary auditory cortex, including the systems' neuroplasticity and sound signaling processes as well as offer suggestions for future research developments.

BEHAVIORAL TRAINING INFLUENCING THE PLASTICITY OF THE PRIMARY AUDITORY CORTEX

Operant conditioning was used for behavioral training in cats, ferrets, and monkeys.^{8,9,10,11,12,16} Ferrets were trained to detect a target tone of any frequency and were expected to perform a

behavioral task, licking, at the targeted sound stimulus.^{8,9,16} The goal was to investigate whether task performance could reform cortical receptive field properties in accordance to specific task demands and sensory cues.^{8,9,16} In addition, to determine the plasticity in A1 and whether certain target sounds would become long-term sensory memory due to receptive field changes in ferrets.^{8,9,16} The findings determined that the most common shape change during task performance was during the time the target frequency was played which sharpened temporal dynamics due to either enhancement of an excitatory field of the spectrotemporal response field (STRF) or by a weakening of its inhibitory sidebands.^{8,9,16} Additionally, some receptive field changes persisted hours after task completion, therefore they may contribute to long-term memory.^{8,9,16} There was also indication that AI changed in population activity structure to extract task-relevant information during behavior.^{8,9,16} Besides ferrets, studies on monkeys have demonstrated an increase in cortical area representation of restricted frequency range in AI that encodes the sound frequencies used for the frequency discrimination task.¹² These results also demonstrate modification in the tonotopic organization of AI in the adult primates post frequency discrimination training.¹² Modifications consisted of increased cortical area representation of restricted frequency ranges in A1 in addition to the modifications observed in previous studies.¹² Such modifications include the alteration of A1 subsequent to restricted cochlear lesions and that the topographic reorganization is correlated with changes in the perceptual acuity of the animal.¹² Contrary to ferrets, the monkeys were deprived from food and not water while their behavioral response was to pull a lever to obtain food in comparison to the ferrets who were deprived water and had to lick from a sprout at the appropriate targeted frequency.^{8,9,12,16} Though the behavioral tasks slightly differed, consistent results were obtained from both species stating that behavioral training improves overall temporal processes.

USING BEHAVIORAL TRAINING TO BETTER UNDERSTAND AND IMPROVE CURRENT ENGINEERED SENSORY SYSTEMS FOR SIGNALING PROCESSES

Many people who have sensorineural hearing loss rely on hearing aids or cochlear implants to detected sound. Hearing aids only amplify sounds while cochlear implants send sound signals to the brain.¹⁸ Cochlear implants are small complex electronic devices that provide people with a sense of sounds by replacing the function of the damaged parts of the inner ear.¹⁸ The implant consists of a small portion that is surgically placed in the inner ear and an external portion that sits behind the ear.¹⁸ Bilateral implantations have been introduced within the last decade with hopes of improving both speech perception in background noise and sound localization.¹⁷ There is evidence suggesting that binaural perception is possible with two implants, however, research results in humans are variable therefore, ferrets have been used to explore potential contributing factors to these variable, inconsistent outcomes.¹⁷ The idea of using ferrets as an animal model has opened broad possibilities of using them to study potential protective effects of bilateral cochlear implantation on the developing central auditory pathway and develop novel neuroprosthetic therapies for use in humans.¹⁷ Besides ferrets, behavioral training has been used on the temporal processes of deaf cats that received cochlear prostheses and showed how stimulation enhanced their temporal processing.¹¹ All the cats were implanted and separated into three groups depending on when they received electric stimulation in relation to the study; no stimulation prior to study, stimulation prior to study, stimulation as well as behavioral training prior to study, and a control group.¹¹ The results showed that training with behavioral stimulation significantly enhanced all temporal processing parameters to normal levels.¹¹ Unlike the studies conducted on ferrets and primates, this study was conducted on neonatally deafened cats via the systemic administration of neomycin sulfate beginning the day after birth and continuing 16-25

days until profound hearing loss was confirmed based on the absence of auditory brainstem responses to clicks. Three years later, the cats were then implanted with cochlear implants and the following week, electrophysiological experiments began. Like the ferrets and primates, signs of neuroplasticity were observed after behavioral training due to the enhancement of temporal processing parameters to normal levels as previously mentioned.

CONCLUSION

Bilateral damage to the auditory cortex is not necessarily associated with permanent discrepancies in the ability to detect sound, however extensive damage to the auditory cortex does induce a condition called auditory agnosia which is characterized by the inability to perceptually identify the meaning of both verbal and nonverbal sounds.⁷ Additionally, people with damage to the auditory cortex may experience difficulty in perceiving the order of simple sounds, suggesting a fundamental disorder in processing sound across time.⁷ Hearing impairment may be caused by a variety of different factors and can affect people at different times and ages; some may be affected prenatally, others after birth, and some later in life with age.¹ Some causes of hearing loss can be due to prematurity, lack of oxygen during birth, genetics, diseases, infections, noise, aging, head or acoustic trauma, medications, etc.¹ Though with current research and engineered sensory systems of signaling processes, hearing may be preserved with behavioral training. Claims have been made proclaiming that task performance/ behavioral training can adaptively reshape cortical receptive field properties with sensory cues and specific task demands.¹⁶ Additionally, as mentioned above, animal research has proven that behavioral training influences the plasticity of AI while other studies claim that jugular vein stimulation induces synaptic plasticity.^{8,9,12,16} For future studies, it would be interesting to see whether vagus nerve stimulation does in fact induce synaptic plasticity and whether that would induce the speed of learning for animals during behavioral training. Correspondingly, from the study mentioned in the introduction regarding inconsistent data using bilateral implantations in humans, I'd like to see the success of bilateral implantations in humans with consistent results and eventually the development of novel neuroprosthetic therapies for use in humans. Cochlear implants by far have been very effective in restoring hearing by electrical stimulation of the auditory nerve by bypassing damaged hair cells. However, the distance between electrodes and neurons varies from one location to another in the implanted inner ear which can affect the size of the stimulated neuronal population.²⁰ Additionally, cochlear implants are not efficient at restoring music and voice quality due to the broad activation patterns which are adequate for speech patterns but do not have sufficient spectral resolution to convey harmonic pitch.^{20,21} Developing methods to advance the cochlear implant to adjust to different activation patterns and spectral resolution to accommodate for normal speech patterns as well as harmonic pitches could be a potential objective for future researchers. Additionally, I would like to see more research conducted regarding behavioral training enhancing temporal processing. Does it only apply to animals that become deafened due to drug administration or does it also apply to animals that are born deaf or even become deaf later in life? Personally, I feel like this would be a more appropriate representation of deafness in humans since humans are typically either born with hearing loss or gain it later in life due to traumatic injury or a variety of other reasons that result in hair cell damage. Hopefully, these studies on ferrets, primates, and cats will result in improved and high-quality sensory systems for signaling processes.

REFERENCES

1. Clason, Debbie. "Common Causes of Sensorineural Hearing Loss." *Healthy Hearing*, 6 Feb. 2018, www.healthyhearing.com/report/50276-Common-causes-of-sensorineural-hearing-loss.
2. Kochkin, Sergei. "MarkeTrak VIII: 25-Year Trends in the Hearing Health Market." *MarkeTrak VIII: 25-Year Trends in the Hearing Health Market*, The Hearing Review, 1 Oct. 2009

3. "Sensorineural Hearing Loss." *American Speech-Language-Hearing Association*, ASHA, www.asha.org/public/hearing/Sensorineural-Hearing-Loss/.
4. Purves, Dale. "The Auditory Cortex." *Neuroscience. 2nd Edition.*, U.S. National Library of Medicine, 1 Jan. 1970, www.ncbi.nlm.nih.gov/books/NBK10900/.
5. SpinalCord.com. "Temporal Lobe." *SpinalCord.com*, www.spinalcord.com/temporal-lobe.
6. Gil-Loyzaga, Pablo. "Journey into the World of Hearing." *Cochlea*, 9 Dec. 2016, www.cochlea.eu/en/auditory-brain/thalamo-cortex/auditory-cortex-physiology.
7. "Auditory Cortex." *Brain Auditory Pathways - Auditory Cortex*, psych.athabascau.ca/html/Psych402/Biotutorials/26/cortex.shtml.
8. Bagur, Sophie, et al. "Go/No-Go task engagement enhances population representation of target stimuli in primary auditory cortex." *Nature communicators*, 28 June 2018, www.nature.com/naturecommunications
9. Schwartz, Zachary, and Stephen V David. "Focal Suppression of Distractor Sounds by Selective Attention in Auditory Cortex." *OUP Academic*, Oxford University Press, 9 Nov. 2017, academic.oup.com/cercor/article/28/1/323/4608048.
10. Populin, Luis C., and Tom C. T. Yin. "Behavioral Studies of Sound Localization in the Cat." *Journal of Neuroscience*, Society for Neuroscience, 15 Mar. 1998, www.jneurosci.org/content/18/6/2147.
11. Vollmer, Maike & E Beitel, Ralph. (2011). Behavioral training restores temporal processing in auditory cortex of long-deaf cats. *Journal of neurophysiology*. 106. 2423-36. 10.1152/jn.00565.2011.
12. Recanzone, G. H., and M. M. Merzenich. *Plasticity in the Frequency Representation of Primary Auditory Cortex Following Discrimination Training in Adult Owl Monkeys*. The Journal of Neuroscience, Jan. 1993.
13. Johnsrude, Ingrid S., et al. "Functional Specificity in the Right Human Auditory Cortex for Perceiving Pitch Direction." *OUP Academic*, Oxford University Press, 1 Jan. 2000, academic.oup.com/brain/article/123/1/155/269199.
14. Zatorre, Robert J., and Virginia B. Penhune. "Spatial Localization after Excision of Human Auditory Cortex." *Journal of Neuroscience*, Society for Neuroscience, 15 Aug. 2001, www.jneurosci.org/content/21/16/6321.
15. Gold, J. R., Nodal, F. R., Peters, F., King, A. J., & Bajo, V. M. (2015). Auditory gap-in-noise detection behavior in ferrets and humans. *Behavioral neuroscience*, 129(4), 473-90.
16. Fritz, Jonathan, et al. "Rapid Task-Related Plasticity of Spectrotemporal Receptive Fields in Primary Auditory Cortex." *Nature News*, Nature Publishing Group, 28 Oct. 2003, www.nature.com/articles/nn1141.
17. Hartley, D. E., Vongpaisal, T., Xu, J., Shepherd, R. K., King, A. J., & Isaiah, A. (2010). Bilateral cochlear implantation in the ferret: a novel animal model for behavioral studies. *Journal of neuroscience methods*, 190(2), 214-28.
18. "Cochlear Implants." *National Institute of Deafness and Other Communication Disorders*, U.S. Department of Health and Human Services, 15 June 2018, www.nidcd.nih.gov/health/cochlear-implants.
19. "Vagus Nerve Stimulation Enhances Brain Plasticity." *Psychology Today*, Sussex Publishers, www.psychologytoday.com/us/blog/the-athletes-way/201805/vagus-nerve-stimulation-enhances-brain-plasticity.
20. "Measures to Improve Auditory Prosthesis Function | Mcubed." *Mcubed.umich.edu*, mcubed.umich.edu/projects/measures-improve-auditory-prosthesis-function.
21. Shannon, Robert V. "Advances in Auditory Prostheses." *Current Opinion in Neurology*, U.S. National Library of Medicine, Feb. 2012, www.ncbi.nlm.nih.gov/pmc/articles/PMC4123811/.

Aminoglycoside-induced hair cell death and potential therapies

Gavin Lockard

INTRODUCTION

Hearing loss is the third most common disorder in the United States, being more prevalent than diabetes or cancer¹. Our ability to hear is dependent on hair cells that can translate the sounds we hear into electrical impulses that can be interpreted by the brain. When sound waves enter the cochlea located in the inner ear, they deflect the apical portion of hair cells also known as the stereocilia. Stereocilia are connected to one another via tip links. Excitatory deflection results in the “tilting” of the stereocilia in a domino effect, which “pulls open” the mechanotransduction (MET) channels located at the tips of the stereocilia. Potassium ions flux inside hair cells through the open MET channel, resulting in inward depolarizing currents. This can stimulate the release of glutamate onto innervating afferent neurons, resulting in postsynaptic excitatory action potentials^{2,3}.

Hearing loss can occur due to multiple factors including noise, genetics, age, and antibiotics, such as aminoglycosides. Aminoglycosides are highly effective at treating tuberculosis and Gram-negative bacterial infections associated with cystic fibrosis and sepsis⁴. However, aminoglycosides have the unfavorable effect of ototoxicity and nephrotoxicity, damage to hair cells in the inner ear and to proximal tubule cells in the kidney, respectively. Cochlear hair cells are responsible for hearing and vestibular hair cells are responsible for balance. 20% of patients taking lifesaving aminoglycosides suffer permanent hearing loss and/or balance disorders^{5,6,7}. Hearing loss results in socioeconomic burdens of nearly 1.4 million dollars throughout the lifetime of a child born deaf, and greater than \$350,000 for an afflicted adult⁸. Despite this, aminoglycosides are commonly used worldwide, with there being nine aminoglycosides approved by the US Food and Drug Administration. The popular use of aminoglycosides despite side effects is due to their quick bactericidal activity, chemical stability, low costs, and low occurrences of bacterial resistance^{9,10}.

Nearly all cells take up aminoglycosides, but inner ear hair cells are preferentially loaded¹¹. Most cells quickly sequester and remove the aminoglycosides from their cytoplasm, but this is not the case for hair cells, which hold onto aminoglycosides for increased amounts of time¹². The retention of aminoglycosides and the cells' higher metabolic rates are likely the reasons for the cells' increased susceptibility to aminoglycoside-induced damage¹². This review will focus on the entry of aminoglycosides into hair cells, and the potential therapies that include the attenuation of aminoglycoside-induced hair cell death.

ENTRY OF AMINOGLYCOSIDES INTO HAIR CELLS

Aminoglycosides are rapidly taken up by hair cells primarily through MET channels¹³. The liquid surrounding hair cells, called endolymph, has a strong positive membrane potential, whereas the cytoplasm of hair cells has a strong negative membrane potential¹⁴. This creates a strong electrochemical force that drives cations, including aminoglycosides, through open non-selective cation channels, such as MET channels¹⁴. MET channels are complexes composed of TMC (transmembrane-channel) proteins forming the pore¹⁵, myosin VIIA in the hair bundles¹⁶, and Cadherin 23/Protocadherin 15 proteins forming the tip links¹⁷, amongst other proteins that perform other functions within the MET complex. When any of these are mutated, MET channel conductance is decreased, which reduces aminoglycoside uptake^{15,16,17}. Other evidence of aminoglycoside entry through the MET channel includes that fluorescently conjugated gentamicin (Gentamicin-conjugated Texas Red, or GTTR) has been localized at the apical membranes of hair cells in stereocilia, before being taken up into hair cells¹⁸. Gentamicin is an aminoglycoside, and its localization in the stereocilia suggests gentamicin enters through MET channels.

Additionally, aminoglycosides are endocytosed at the apical membranes of hair cells. Aminoglycosides are then sequestered within lysosomes^{19,20}. The rapid sequestering into lysosomes mitigates aminoglycoside-induced hair cell death. However, interrupting endocytosis surprisingly increases aminoglycoside-induced hair cell death²⁰. This is because endocytic and nonendocytic pathways (MET channels) compete. Therefore, aminoglycoside entry will not be substantially reduced if endocytosis is prevented, since nonendocytic pathways will still allow a plethora of aminoglycosides inside the cell²⁰. There are other mechanisms of aminoglycoside entry to the hair cell, but the MET channel and endocytosis are the most well-studied.

MECHANISMS OF AMINOGLYCOSIDE-INDUCED OTOTOXICITY

Aminoglycosides are effective bactericidal agents that interrupt genetic translation by inducing ribosomal misreading in bacteria^{21,22}. Despite their effectiveness in treating bacterial infections, aminoglycosides are often ototoxic via many different mechanisms.

Aminoglycosides bind to cytosolic proteins, such as calreticulin and CLIMP-63, inhibiting normal function²³. Calreticulin is a chaperone protein within the endoplasmic reticulum^{24,25} responsible for protein folding and degradation²⁶. Calreticulin is highly expressed in hair cells' stereocilia²⁵. Aminoglycosides act as competitive antagonists, preventing calcium from binding to calreticulin, resulting in a loss of calreticulin function²⁵. This disruption likely results in cytotoxicity^{27,28}. CLIMP-63 is another protein within the endoplasmic reticulum²³, responsible for anchoring microtubules to the endoplasmic reticulum²⁹. Aminoglycosides bind to CLIMP-63, and CLIMP-63 then binds to 14-3-3 proteins²³. 14-3-3 proteins then bind to and inactivate MDMX, a negative regulator of p53. p53 is a well-known tumor suppressor protein³⁰. In total, aminoglycoside binding to CLIMP-63 leads to p53-dependent apoptosis. When p53 inhibitors were used, hair cell death was significantly attenuated upon exposure to both neomycin and gentamicin, which are two different aminoglycosides with some different cell death pathways³¹. This is evidence for p53-dependent hair cell death following aminoglycoside exposure.

Aminoglycosides mobilize the transfer of cytotoxic levels of calcium from the endoplasmic reticulum to mitochondria via IP₃ (inositol triphosphate) receptors³². This increases mitochondrial calcium, resulting in increased oxidative phosphorylation and consequent accumulation of cytotoxic levels of reactive oxygen species³³. The cytotoxic accumulation of reactive oxygen

species leads to the release of cytochrome C from the mitochondria into the cytosol, which activates caspases and subsequent apoptosis³⁴. Another mechanism of aminoglycoside-induced hair cell death includes the activation of the stress-induced c-Jun N-terminal kinase pathway³⁵. Finally, one more potential mechanism of damage includes excess glutamate signaling leading to an overabundance of calcium influx through NMDA channels^{36,37}. These references discuss glutamate excitotoxicity, but do not look specifically at post-aminoglycoside exposure.

ATTENUATION OF AMINOGLYCOSIDE-INDUCED HAIR CELL DEATH

Aminoglycoside-induced hair cell death can be attenuated via prevention of uptake or interruption of intracellular mechanisms that lead to apoptosis. Prevention of uptake has been studied thoroughly. A screen of 1,040 FDA-approved drugs identified eight compounds that protect against exposure to both neomycin and gentamicin³⁸. All eight drugs shared a similar quinoline structure, and are thought to reduce aminoglycoside uptake into hair cells³⁸. In another study, four plant alkaloid compounds with bisbenzoquinoline structures (chemically similar to structures in the Ou et al., 2012 study) were identified from a screen of 502 natural compounds to be otoprotective against aminoglycoside exposure³⁹. These include berbamine, E6 berbamine, hernandezine, isotetrandrine – with E6 berbamine being most protective³⁹. All four compounds reduced GTTR and FM 1-43x (dye that indicates MET channel functionality) uptake, suggesting a blocking of aminoglycoside uptake through the MET channel³⁹. In a following study, berbamine was examined further, along with another otoprotectant compound d-Tubocurarine (dTC), in zebrafish and neonatal mice⁴⁰. Berbamine and dTC reduce aminoglycoside uptake and protect zebrafish hair cells from neomycin- and gentamicin-induced hair cell death, and protect mouse cochlear hair cells from gentamicin-induced hair cell death⁴⁰. In another study looking at additional mechanisms of protection, two lead otoprotectants were found to protect hair cells from glutamate excitotoxicity by blocking calcium entry through NMDA channels⁴¹. Three other lead otoprotectants work in various ways, with one blocking the MET channel but not GTTR uptake, one reducing GTTR uptake but not blocking the MET channel, and one that does not do either⁴¹. These results indicate protection downstream of aminoglycoside entry⁴¹. In total, these papers all identified protective compounds that reduce aminoglycoside uptake, and they may have intracellular impacts as well.

If uptake of aminoglycosides is not prevented, attenuation of aminoglycoside-induced hair cell death can still occur through the interruption of intracellular apoptotic mechanisms. Aminoglycosides, such as apramycin, have been developed with minimal affinity for eukaryotic mitochondrial ribosomes, while maintaining their bactericidal activity⁴². Another study modified the amine groups on sisomicin, a precursor of gentamicin, and they developed a drug called N1MS that is significantly less ototoxic, but remains bactericidal⁴³. Antioxidants such as N-acetylcysteine, D-methionine, and edaravone have been used to reduce the buildup of cytotoxic levels of reactive oxygen species in mammals post-aminoglycoside exposure^{44,45,46}. CEP-1347, an inhibitor of JNK signaling, has been used to attenuate neomycin- and gentamicin-induced hair cell death³⁵. Knowing that p53-dependent apoptosis is an ultimate cause of hair cell death, mitochondrial-specific p53 inhibition was found to render significant hair cell protection upon exposure to two common aminoglycosides, neomycin and gentamicin³¹. Despite the intriguing previous finding by Coffin et al., p53 inhibitors could not practically be used in humans since p53 is an essential and ubiquitous protein important for tumor prevention³⁰. Otoprotection can occur downstream of aminoglycoside entry via multiple different interventions.

CONCLUSION

Aminoglycosides are highly effective bactericidal agents, but have pronounced ototoxic effects. Despite less toxic alternative antibiotics, aminoglycosides are still used frequently due to their low cost, chemical stability, and low occurrences of bacterial resistance. Therefore, attenuating aminoglycoside-induced hair cell death is of great importance. Work has been done to identify methods of aminoglycoside entry into hair cells, mechanisms of aminoglycoside ototoxicity, and attenuation of the aminoglycoside-induced hair cell death. Promising results have led to identifications of many otoprotective agents that may decrease the risk of permanent hearing loss when patients take lifesaving aminoglycosides

Knowing that aminoglycoside-induced hair cell death can be attenuated via prevention of uptake or interruption of intracellular apoptotic mechanisms, different treatment paradigms with different dosing schedules have been developed. Future work by researchers should focus on the reduction of aminoglycoside uptake. Previous research implied a physical blockage of the MET channel pore, but this needs to be confirmed. Work then needs to be done to identify the chemical functional groups of the different otoprotective agents to determine why they protect hair cells and reduce aminoglycoside uptake at different efficacies. Further work should then identify the binding affinities of the protective agents to the MET channels, and the half-lives of the drugs in mammals; this information would help develop the dosing schedule for how often the protective drugs would need to be taken. The interactions between the otoprotective agents and MET channels could make the difference on patients deciding between treating their life-threatening bacterial infections or suffering permanent hearing loss.

REFERENCES

1. Blackwell DL, Lucas JW, Clarke TC. Summary health statistics for US adults: National Health Interview Survey, 2012. *Vital health statistics, series 10*, no. 260. Atlanta, GA: National Center for Health Statistics, CDC; 2014. http://www.cdc.gov/nchs/data/series/sr_10/sr10_260.pdf
2. Nordang, L., Cestreicher, E., Arnold, W., and Anniko, M. (2000). Glutamate is the afferent neurotransmitter in the human cochlea. *Acta Otolaryngol.* 120, 359–362. doi: 10.1080/000164800750000568
3. Oestreicher, E., Wolfgang, A., and Felix, D. (2002). Neurotransmission of the cochlear inner hair cell synapse—implications for inner ear therapy. *Adv. Otorhinolaryngol.* 59, 131–139. doi: 10.1159/000059245
4. Forge, A., and Schacht, J. (2000). Aminoglycoside antibiotics. *Audiol. Neurootol.* 5, 3–22. doi: 10.1159/000013861
5. Ariano, R. E., Zelenitsky, S. A., and Kassum, D. A. (2008). Aminoglycoside-induced vestibular injury: maintaining a sense of balance. *Ann. Pharmacother.* 42, 1282–1289. doi: 10.1345/aph.1L001
6. Al-Malky, G., Dawson, S. J., Sirimanna, T., Bagkeris, E., and Suri, R. (2015). High-frequency audiometry reveals high prevalence of aminoglycoside ototoxicity in children with cystic fibrosis. *J. Cyst. Fibros.* 14, 248–254. doi: 10.1016/j.jcf.2014.07.009
7. Garinis, A. C., Cross, C. P., Srikanth, P., Carroll, K., Feeney, M. P., Keefe, D. H., et al. (2017a). The cumulative effects of intravenous antibiotic treatments on hearing in patients with cystic fibrosis. *J. Cyst. Fibros.* 16, 401–409. doi: 10.1016/j.jcf.2017.01.006
8. Mohr, P. E., Feldman, J. J., Dunbar, J. L., McConkey-Robbins, A., Niparko, J. K., Rittenhouse, R. K., et al. (2000). The societal costs of severe to profound hearing loss in the United States. *Int. J. Technol. Assess. Health Care* 16, 1120–1135. doi: 10.1017/s0266462300103162
9. Puopolo, K. M., and Eichenwald, E. C. (2010). No change in the incidence of ampicillin-resistant, neonatal, early-onset sepsis over 18 years. *Pediatrics* 125, e1031–e1038. doi: 10.1542/peds.2009-1573
10. Tsai, M. H., Chu, S. M., Hsu, J. F., Lien, R., Huang, H. R., Chiang, M. C., et al. (2014). Risk factors and outcomes for multidrug-resistant Gram-negative bacteremia in the NICU. *Pediatrics* 133, e322–e329. doi: 10.1542/peds.2013-1248
11. Humes, H. D. (1999). Insights into ototoxicity. Analogies to nephrotoxicity. *Ann. N Y Acad. Sci.* 884, 15–18. doi: 10.1111/j.1749-6632.1999.tb00278.x
12. Dai, C. F., Mangiardi, D., Cotanche, D. A., and Steyger, P. S. (2006). Uptake of fluorescent gentamicin by vertebrate sensory cells *in vivo*. *Hear. Res.* 213, 64–78. doi: 10.1016/j.heares.2005.11.011

13. Marcotti, W., van Netten, S. M., and Kros, C. J. (2005). The aminoglycoside antibiotic dihydrostreptomycin rapidly enters mouse outer hair cells through the mechano-electrical transducer channels. *J. Physiol.* 567, 505–521. doi: <https://doi.org/10.1113/jphysiol.2005.085951>
14. Pickles, J. O. (2012). *An Introduction to the Physiology of Hearing*. Bingley, United Kingdom: Emerald Group Publishing Limited.
15. Pan B, Akyuz N, Liu XP, Asai Y, Nist-Lund C, Kurima K, et al. TMC1 Forms the Pore of Mechanosensory Transduction Channels in Vertebrate Inner Ear Hair Cells. *Neuron*. 2018;99(4):736–53.e6. Epub 2018/08/24. 10.1016/j.neuron.2018.07.033
16. Kros, C. J., Marcotti, W., van Netten, S. M., Self, T. J., Libby, R. T., Brown, S. D., et al. (2002). Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations. *Nat. Neurosci.* 5, 41–47. doi: 10.1038/nn784
17. Vu, A. A., Nadaraja, G. S., Huth, M. E., Luk, L., Kim, J., Chai, R., et al. (2013). Integrity and regeneration of mechanotransduction machinery regulate aminoglycoside entry and sensory cell death. *PLoS One* 8:e54794. doi: 10.1371/journal.pone.0054794
18. Steyger, P. S., Peters, S., Rehling, J. et al. (2003). Uptake of Gentamicin by Bullfrog Saccular Hair Cells *in vitro*. *JARO*. 4:565.
19. Hashino, E., Shero, M., and Salvi, R. J. (1997). Lysosomal targeting and accumulation of aminoglycoside antibiotics in sensory hair cells. *Brain Res.* 777, 75–85. doi: 10.1016/s0006-8993(97)00977-3
20. Hailey, D. W., Esterberg, R., Linbo, T. H., Rubel, E. W., Raible, D. W. (2017). Fluorescent aminoglycosides reveal intracellular trafficking routes in mechanosensory hair cells. *J Clin Invest.* 127 (2), 472-486. doi: <https://doi.org/10.1172/JCI85052>
21. Cox, E. C., White, J. R., and Flaks, J. G. (1964). Streptomycin action and the ribosome. *Proc. Natl. Acad. Sci. U S A* 51, 703–709. doi: 10.1073/pnas.51.4.703
22. Davies, J., and Davis, B. D. (1968). Misreading of ribonucleic acid code words induced by aminoglycoside antibiotics. The effect of drug concentration. *J. Biol. Chem.* 243, 3312–3316.
23. Karasawa, T., Wang, Q., David, L. L., and Steyger, P. S. (2010). CLIMP-63 is a gentamicin-binding protein that is involved in drug-induced cytotoxicity. *Cell Death Dis.* 1:e102. doi: 10.1038/cddis.2010.80
24. Horibe, T., Matsui, H., Tanaka, M., Nagai, H., Yamaguchi, Y., Kato, K., et al. (2004). Gentamicin binds to the lectin site of calreticulin and inhibits its chaperone activity. *Biochem. Biophys. Res. Commun.* 323, 281–287. doi: 10.1016/j.bbrc.2004.08.099
25. Karasawa, T., Wang, Q., David, L. L., and Steyger, P. S. (2011). Calreticulin binds to gentamicin and reduces drug-induced ototoxicity. *Toxicol. Sci.* 124, 378–387. doi: 10.1093/toxsci/kfr196
26. Williams, D. B. (2006). Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum. *J. Cell Sci.* 119, 615–623. doi: 10.1242/jcs.02856
27. Bastianutto, C., Clementi, E., Codazzi, F., Podini, P., De Giorgi, F., Rizzuto, R., et al. (1995). Overexpression of calreticulin increases the Ca²⁺ capacity of rapidly exchanging Ca²⁺ stores and reveals aspects of their luminal microenvironment and function. *J. Cell Biol.* 130, 847–855. doi: 10.1083/jcb.130.4.847
28. Mesaeli, N., Nakamura, K., Zvaritch, E., Dickie, P., Dziak, E., Krause, K. H., et al. (1999). Calreticulin is essential for cardiac development. *J. Cell Biol.* 144, 857–868. doi: 10.1083/jcb.144.5.857
29. Sandoz, P. A., and van der Goot, F. G. (2015). How many lives does CLIMP-63 have? *Biochem. Soc. Trans.* 43, 222–228. doi: 10.1042/BST20140272
30. Okamoto, K., Kashima, K., Pereg, Y., Ishida, M., Yamazaki, S., Nota, A., et al. (2005). DNA damage-induced phosphorylation of MdmX at serine 367 activates p53 by targeting MdmX for Mdm2-dependent degradation. *Mol. Cell Biol.* 25, 9608–9620. doi: 10.1128/mcb.25.21.9608-9620.2005
31. Coffin, A. B., Rubel, E. W., Raible, D. W. (2013). Bax, Bcl2, and p53 Differentially Regulate Neomycin- and Gentamicin-Induced Hair Cell Death in the Zebrafish Lateral Line. *JARO*. 14 (5); 645-659.
32. Esterberg, R., Hailey, D. W., Coffin, A. B., Raible, D. W., and Rubel, E. W. (2013). Disruption of intracellular calcium regulation is integral to aminoglycoside-induced hair cell death. *J. Neurosci.* 33, 7513–7525. doi: 10.1523/JNEUROSCI.4559-12.2013
33. Esterberg, R., Linbo, T., Pickett, S. B., Wu, P., Ou, H. C., Rubel, E. W., et al. (2016). Mitochondrial calcium uptake underlies ROS generation during aminoglycoside-induced hair cell death. *J. Clin. Invest.* 126, 3556–3566. doi: 10.1172/JCI84939
34. Matsui, J. I., Gale, J. E., and Warchol, M. E. (2004). Critical signaling events during the aminoglycoside-induced death of sensory hair cells *in vitro*. *J. Neurobiol.* 61, 250–266. doi: 10.1002/neu.20054
35. Ylikoski, J., Xing-Qun, L., Virkkala, J., and Pirvola, U. (2002). Blockade of c-Jun N-terminal kinase pathway attenuates gentamicin-induced cochlear and vestibular hair cell death. *Hear. Res.* 163, 71–81. doi: 10.1016/s0378-5955(01)00380-x
36. Choi, D. W. (1992). Excitotoxic cell death. *J. Neurobiol.* 23, 1261–1276.
37. Pullan, L. M., Stumpo, R. J., Powel, R. J., Paschetto, K. A. & Britt, M. (1992). Neomycin is an agonist at a polyamine site on the N-methyl-D-aspartate receptor. *J. Neurochem.* 59, 2087–2093.

38. Ou, H. C., Keating, S., Wu, P., Simon, J. A., Raible, D. W., and Rubel, E. W. (2012). Quinoline ring derivatives protect against aminoglycoside-induced hair cell death in the zebrafish lateral line. *J. Assoc. Res. Otolaryngol.* 13, 759–770. doi: <https://doi.org/10.1007/s10162-012-0353-0>
39. Kruger, M., Boney, R., Ordoobadi, A. J., Sommers, T. F., Trapani, J. G., Coffin, A. B. (2016). Natural bizbenzoquinoline derivatives protect zebrafish lateral line sensory hair cells from aminoglycoside toxicity. *Front Cell Neurosci.* 10:83. doi: <https://doi.org/10.3389/fncel.2016.00083>
40. Kirkwood, N. K., O'Reilly, M., Derudas, M., Kenyon, E. J., Huckvale, R., van Netten, S. M., Ward, S. E., Richardson, G. P., Kros, C. J. (2017). d-Tubocurarine and berbamine: Alkaloids that are permeant blockers of the hair cell's mechano-electrical transducer channel and protect from aminoglycoside toxicity. *Front Cell Neurosci.* 11 (262). doi: <https://doi.org/10.3389/fncel.2017.00262>
41. Kenyon, E. J., Kirkwood, N. K., Kitcher, S. R., O'Reilly, M., Derudas, M., Cantillon, D. M., Goodyear, R. J., Secker, A., Baxendale, S., Bull, J. C., Waddell, S. J., Whitfield, T. T., Ward, S. E., Kros, C. J., Richardson, G. P. (2017). Identification of ion-channel modulators that protect against aminoglycoside-induced hair cell death. *JCI Insight.* 2 (24). doi: <https://dx.doi.org/10.1172%2Fjci.insight.96773>
42. Matt, T., Ng, C. L., Lang, K., Sha, S. H., Akbergenov, R., Shcherbakov, D., et al. (2012). Dissociation of antibacterial activity and aminoglycoside ototoxicity in the 4-monosubstituted 2-deoxystreptamine apramycin. *Proc. Natl. Acad. Sci. U S A* 109, 10984–10989. doi: 10.1073/pnas.1204073109
43. Huth, M. E., Han, K. H., Sotoudeh, K., Hsieh, Y. J., Effertz, T., Vu, A. A., et al. (2015). Designer aminoglycosides prevent cochlear hair cell loss and hearing loss. *J. Clin. Invest.* 125, 583–592. doi: 10.1172/JCI177424
44. Somdaş, M. A., Korkmaz, F., Gürgen, S. G., Sagit, M., and Akçadağ, A. (2015). N-acetylcysteine prevents gentamicin ototoxicity in a rat model. *J. Int. Adv. Otol.* 11, 12–18. doi: 10.5152/iao.2015.650
45. Campbell, K. C., Martin, S. M., Meech, R. P., Hargrove, T. L., Verhulst, S. J., and Fox, D. J. (2016). D-methionine (D-met) significantly reduces kanamycin-induced ototoxicity in pigmented guinea pigs. *Int. J. Audiol.* 55, 273–278. doi: 10.3109/14992027.2016.1143980
46. Turan, M., Çiğer, E., Arslanoğlu, S., Börekci, H., and Önal, K. (2017). Could edaravone prevent gentamicin ototoxicity? An experimental study. *Hum. Exp. Toxicol.* 36, 123–127. doi: 10.1177/0960327116639360
47. Francis, S. P., Katz, J., Fanning, K. D., Harris, K. A., Nicholas, B. D., Lacy, M., et al. (2013). A novel role of cytosolic protein synthesis inhibition in aminoglycoside ototoxicity. *J. Neurosci.* 33, 3079–3093. doi: 10.1523/JNEUROSCI.3430-12.2013
48. Majumder, P., Moore, P. A., Richardson, G. P., and Gale, J. E. (2017). Protecting mammalian hair cells from aminoglycoside-toxicity: assessing Phenoxybenzamine's potential. *Front. Cell. Neurosci.* 11:94. doi: 10.3389/fncel.2017.00094

A cold case: pediatric high grade glioma

Zenette McCoy

INTRODUCTION

Glioblastoma multiforme (GBM) are a grade IV primary malignant glioma (commonly, grade IV astrocytoma) that are lethal due to the extreme heterogeneity of the disease and sparse treatment options. There are about 26,000 cases in the United States (US) yearly and about 15,000 cases will result in mortality; in the US, GBM account for 52% of primary tumors¹. About 15% of these cases occurs in children between 0-15 years old and 85% in adolescents, adults, and elderly¹. GBM (GBM in pediatrics are called high grade glioma, HGG) in children are much rarer to occur compared to adults¹. However, gliomas, grade I, II, and III cancer that is formed from glial cells, makes up 40-50% of central nervous system tumors in children^{1,2}. Children life expectancy is about 5 years after diagnosis and receiving intensive treatment for HGG. Even with intensive treatment, less than 20% of children will survive 5 years after diagnosis^{1,2}.

Research in the last decade has found treatment targets in GBM patients. One of the targets is a protein called epidermal growth factor receptor (EGFR). Currently, it is known that EGFR and EGFR mutants are commonly seen in many types of tumors and is rarely seen in normal tissue, making it a key detector for cancer. This is important to GBM because EGFR and an EGFR mutant (known as epidermal growth factor receptor variant III) are expressed in 24-67% of GBM patients³. The mutation occurs in the EGFR gene which results in a deletion of 267 amino acids from the extracellular domain of the protein from exon 2 to 7⁴. The key role EGFR in GBM, is thought to be involved in pathogenesis, longevity, and recurrence of the tumors⁵.

The EGFR mutant and the over expression of the wild-type (wt) EGFR has been thoroughly studied in adults. However, this mutation and amplification of wtEGFR is very understudied in pediatrics due to the rarity of HGG. Instead the deletion of EGFR in HGG has more publications due to the high prevalence; the deletion is found in 10% - 80% of HGG (specifically in HGG that forms in the hindbrain)^{6,7}. To gain better insight into the effects of EGFR deletion and mutation in pediatrics, this review will be evaluating how the presents or absence of EGFR affects prognosis and treatment of GBM.

EGFR WILD-TYPE AND MUTANT PREVALENCE IN GLIOBLASTOMA

EGFR helps induce cell proliferation and has trophic effects on other cells. In glioblastoma multiforme a mutation of EGFR can occur. This mutation is known as epidermal growth factor receptor variant III (EGFRvIII); the mutation results in half of the gene being deleted⁴. Overexpression of wtEGFR or EGFRvIII has been found to increase activation of EGFR proteins⁴. Increased activation is seen in most GBM, but about 10% of GBM do not have an amplification of EGFR proteins despite having the mutant or overexpression of EGFR^{4,8}.

About 54% of GBM overexpress wtEGFR and 31% overexpress wtEGFR and EGFRvIII⁸. Not only are there variants in protein expression, the levels of overexpression are found to vary throughout the tumor^{4,8}. The variation in EGFR expression could range between 10% - 60% in different areas of the tumor^{4,8}. EGFRvIII is known to be largely expressed at the borders of the tumor as wtEGFR is found more centralized. However, localized expression of wtEGFR and EGFRvIII do not affect the mechanism of the proteins but is thought to play a role in tumor longevity and pathogenesis^{4,8}.

EGFRvIII VS WTEGFR MECHANISM

EGFRvIII and wtEGFR have the same signaling domain but differ in downstream signaling. Both forms of EGFR can alter the kinetics of signaling, but the difference in alteration coincides with the oncogenic probability^{1,6}. WtEGFR is less likely to be tumorigenic than EGFRvIII, but this difference is based on receptor internalization rate, activation in downstream kinases, downstream phosphorylation and dephosphorylation, and signal termination^{1,6}.

In non-oncogenic wtEGFR, the ligand is able to bind to the wtEGFR receptor. This binding results in rapid internalization of the receptor. Following the internalization of the receptor, dephosphorylation and degradation occurs^{1,7}. If the receptor are not degraded then the receptor gets recycled^{4,4}. When EGFR proteins are not bonded to the receptor, EGFR phosphorylates tyrosine, which activates a downstream mechanism to induce cell proliferation^{1,6}. WtEGFR becomes oncogenic when the recycling of the receptors is turned off. The signal is terminated when the recycling mechanism tries to compensate for excessive signaling to recycle the receptor. Due to the compensation, less receptors are recycled and are then degraded. The degradation of the receptors results in wtEGFR to be less bonded^{1,7}. This causes EGFR to phosphorylate tyrosine repeatedly, resulting in excessive EGFR signaling that increases cell proliferation.

When the EGFRvIII mutation occurs, the protein cannot bind or poorly binds to the receptor due to changes in the exocellular domain of the protein¹. This causes constitutive phosphorylation of tyrosine by EGFRvIII¹. Since EGFRvIII cannot bind to the receptor, the receptor is internalized more slowly¹. The slow internalization of the receptor prevents more EGFRvIII from being produced. However, EGFRvIII produces a low signal which is thought to help keep tumor borders and the environment regulated and spread infiltrative cells into the neuropil^{1,6}.

When wtEGFR and EGFRvIII are expressed together, these mechanisms work together to quickly proliferate tumor cells along the borders and continue to maintain proliferation within the tumor. Other factors that are important to note is that coexpression of wtEGFR and EGFRvIII has been linked to promote ligand production by autocrine and paracrine. However, these ligands have yet to be identified, but they are thought to affect downstream transcription that promotes malignancy.

MUTATION AND DELETION IN PEDIATRICS

In pediatrics, it was originally thought that EGFR played an insignificant role in HGG development. However, several studies published in the last decade have found that EGFRvIII or EGFR deletion may play a role in HGG development because some evidence has shown there is gene alternation in EGFR^{10,11,12,13}. This suggests that EGFR may be a genetic component to tumor development in HGG.

Research shows that deletions of EGFR are more likely to occur than the mutation in HGG. Dorine and colleagues found that the gene deletion of EGFR causes EGFRvIII to be produced and amplified in activity with coexpression of platelet-derived growth factor receptor alpha. However,

the paper did not talk about how this amplification was occurring but eluded that the deletion of EGFR caused a mutation in another exon that resemble the transcription of EGFRvIII₆. Thus, they concluded that this mechanism may be the same mechanism seen in adult EGFRvIII mutation⁷. In another study conducted by Suri and colleagues, they found that HGG are completely different than GBM because the wtEGR or EGFRvIII that are present in GBM are different variants of EGFR. There are three different variants of EGFR protein expression (p); these are p16, p53, and p27². The EGFR that are altered in GBM are p16 and p27². As in HGG, alteration in p16 and p27 are rare and a majority of the p53 are deleted². Also, they found that deletion in p53 results in transcription of EGFRvIII in HGG due to missense mutation². Thus, they concluded that HGG have completely different cellular make up than GBM.

Majority of the papers that evaluated EGFRvIII were clinical studies who had 1 – 4 children that expressed EGFRvIII as a mutation instead of a byproduct^{6,12}. However, from the few research studies conducted on EGFRvIII, researchers found that HGG have no heterogenous expression of wtEGFR and EGFRvIII when the mutation is present. Surprisingly, even though there is no heterogeneity, the level of EGFRvIII expression is equally as high as EGFRvIII that is expressed in GBM^{5,7,12}.

CONCLUSION

Many studies have looked at GBM in adults and how EGFR affects the pathogenesis, longevity, and recurrence of tumor. Yet, there is little research done for pediatrics due to the rarity of HGG. Even though HGG is rare, there is correlation between prognosis and deleted EGFR gene. This correlation shows that children that have this deletion will be more resistant to treatment and expect to die in < 3 years^{3,5,10}. However, there have been strives to translate EGFRvIII treatment therapies from adults to pediatrics. Many studies show that the HGG cellular makeup is different to GBM due to varying gene factors and protein variation which eludes to translating therapies for GBM which is becoming more difficult than originally thought. Meanwhile, some research has shown that HGG are similar to GBM which eludes that treatment translation will be easier to accomplish. However, no one knows who is correct and if this translation is possible. Regardless of treatment option, the questions of ‘why does HGG occur and how the tumor thrives?’ is still unanswered. More research has to be done in order to look into the microenvironment of HGG and answer the question of ‘are HGG similar of different from GBM?’, ‘why is p53 deleted?’, ‘why isn’t p16 and p27 altered in HGG?’. There is still much more research that has to happen and more therapies that need to be tested, but the little steps that have been made is still an accomplishment.

REFERENCES

1. Heimberger, A. B., Suki, D., Yang, D., Shi, W., & Aldape, K. (2005). The natural history of EGFR and EGFRvIII in glioblastoma patients. *Journal of translational medicine*, 3, 38. doi:10.1186/1479-5876-3-38
2. Warren K. E. (2012). Pediatric high-grade gliomas: survival at what cost?. *Translational pediatrics*, 1(2), 116–117. doi:10.3978/j.issn.2224-4336.2012.09.01
3. Arora, R. S., Alston, R. D., Eden, T. O., Estlin, E. J., Moran, A., & Birch, J. M. (2009). Age-incidence patterns of primary CNS tumors in children, adolescents, and adults in England. *Neuro-oncology*, 11(4), 403–413. doi:10.1215/15228517-2008-097
4. Davis M. E. (2016). Glioblastoma: Overview of Disease and Treatment. *Clinical journal of oncology nursing*, 20(5 Suppl), S2–S8. doi:10.1188/16.CJON.S1.2-8
5. Ansari, M., Nasrolahi, H., Kani, A. A., Mohammadianpanah, M., Ahmadloo, N., Omidvari, S., & Mosalaei, A. (2012). Pediatric glioblastoma multiforme: A single-institution experience. *Indian journal of medical and paediatric oncology : official journal of Indian Society of Medical & Paediatric Oncology*, 33(3), 155–160. doi:10.4103/0971-5851.103142
6. Hatanpaa, K. J., Burma, S., Zhao, D., & Habib, A. A. (2010). Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance. *Neoplasia (New York, N.Y.)*, 12(9), 675-84.

7. Suri, V., Das, P., Pathak, P., Jain, A., Sharma, M. C., Borkar, S. A., ... Sarkar, C. (2009). Pediatric glioblastomas: a histopathological and molecular genetic study. *Neuro-oncology*, *11*(3), 274–280. doi:10.1215/15228517-2008-092
8. Jovcevska, I., Zupanec, N., Urlep, Z., Vranic, A., Bostjan, M., Stokin, C.L., Muyldermans, S., Myers, M.P., Buzdin, A.A., Petrov, I. and Komel, R. (2017) Differentially expressed proteins in glioblastoma multiforms identified with a nanobody-based anti-proteome approach and confirmed by OncoFinder as possible tumor-class predictive biomarker candidates *Oncotarget*. *8*(27):44141-44158 (2017).
9. An, Z., Aksoy, O., Zheng, T., Fan, Q. W., & Weiss, W. A. (2018). Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. *Oncogene*, *37*(12), 1561–1575. doi:10.1038/s41388-017-0045-7
10. Bax, D. A., Gaspar, N., Little, S. E., Marshall, L., Perryman, L., Regairaz, M., . . . Jones, C. (2009, September 15). EGFRvIII Deletion Mutations in Pediatric High-Grade Glioma and Response to Targeted Therapy in Pediatric Glioma Cell Lines. Retrieved from <http://clincancerres.aacrjournals.org/content/15/18/5753.long>
11. El-Ayadi, M., Ansari, M., Sturm, D., Gielen, G. H., Warmuth-Metz, M., Kramm, C. M., & von Bueren, A. O. (2017). High-grade glioma in very young children: a rare and particular patient population. *Oncotarget*, *8*(38), 64564–64578. doi:10.18632/oncotarget.18478
12. Li, G., Mitra, S. S., Monje, M., Henrich, K. N., Bangs, C. D., Nitta, R. T., & Wong, A. J. (2012). Expression of epidermal growth factor variant III (EGFRvIII) in pediatric diffuse intrinsic pontine gliomas. *Journal of neuro-oncology*, *108*(3), 395–402. doi:10.1007/s11060-012-0842-3
13. Long, W., Yi, Y., Chen, S., Cao, Q., Zhao, W., & Liu, Q. (2017). Potential New Therapies for Pediatric Diffuse Intrinsic Pontine Glioma. *Frontiers in pharmacology*, *8*, 495. doi:10.3389/fphar.2017.00495
14. Lara-Velazquez, M., Al-Kharboosh, R., Jeanneret, S., Vazquez-Ramos, C., Mahato, D., Tavanaiepour, D., ... Quinones-Hinojosa, A. (2017). Advances in Brain Tumor Surgery for Glioblastoma in Adults. *Brain sciences*, *7*(12), 166. doi:10.3390/brainsci7120166
15. Schmidt MHH, Furnari FB, Cavenee WK, Böglér O. Epidermal growth factor receptor signaling intensity determines intracellular protein interactions, ubiquitination, and internalization. *Proc Natl Acad Sci U S A*. 2003 May 27;100(11):6505-10. doi: 10.1073/

Perineuronal nets influence on neuroplasticity and fear associations

Nicolas Nansel

INTRODUCTION

Anxiety disorders affect 33.7% of the United States population at some point during their lifetime¹. These include generalized anxiety disorder, major depressive disorder, panic disorders, social anxiety disorder, and phobias. These disorders present impairments to daily life through the presence of persistent or situationally activated excessive pervasive and intrusive worrying, tension, and hypervigilance^{2,3}. The presence of anxiety disorders causes a profound impact on an individual's social and economic productivity, resulting in an estimated \$42-47 billion lost per year in the United States alone². Phobias are situationally active anxiety disorders with the development of most cases being linked to a fearful, inescapable situation in their youth which conditioned the fear response^{4,5}. Current treatments for anxiety disorders include antidepressants and cognitive behavioral therapies. With the presence of a link to events in many individual's youth, conversations have moved to investigate the role of neural plasticity in the development and treatment of potential fear and anxiety disorders.

A key target in neural development research has been the perineuronal net (PNN), an extracellular structure surrounding certain neurons in the brain⁶. In this review paper, I outline the current progress on research regarding connecting fear with the presence or absence of perineuronal nets in the central nervous system. PNNs are an extracellular structure that begin forming during early infant development, with production plateauing around postnatal day 48 in mice⁷. The absence of PNNs allow for freer formation of synaptic connections and creation of associations between previously unrelated stimuli⁸. For this reason, it is thought that the construction of perineuronal nets indicates the closure of various critical periods, such as the visual critical period⁶. While the development of phobias and anxiety disorders is an intricate process during an individual's youth, it is possible that the degradation of PNNs can return a pre-critical period phenotype to those experiencing the disorder, allowing easier manipulation of the associations causing their anxiety. Studies have found this to be the case with autonomic neurologic disorders, such as ocular dominance⁹. However, how these procedures can be adapted for therapies and treatments of complex behavioral disorders is still under investigation.

PERINEURONAL NETS IN REOPENING CRITICAL PERIODS

Perineuronal nets have been discussed as far back as 1898, in which Camillo Golgi introduced perineuronal nets as "a delicate covering, mainly reticular in structure, but also in the form of tiny tiled scales... which surrounds the cell body of all nerve cells..."¹⁰. This was met with criticism, deemed an artifact of staining by Ramón y Cajal¹⁰. While morphological research has continued until the present, it wasn't until 2002 that perineuronal nets were explicitly implicated to influence

neuroplasticity. This was done through the examination of ocular dominance (OD), and how PNN status modified adult rat susceptibility to developing OD⁹.

OD is a deficit marked by the disproportion allocation of cortical neurons to one eye over the other⁹. Experimentally, this is accomplished through monocular deprivation, typically the suturing of one eye closed to prevent adequate stimulation of the sutured eye^{9,11}. This treatment works effectively to generate OD in younger rats as it can be performed prior to the closure of the visual critical period. Adult rats who are treated with monocular deprivation do not experience this disproportionate allocation of cortical neurons to the non-deprived eye as they have already passed the closure of the visual critical period; it is more difficult for these adults to reallocate resources to compensate. However, the enzymatic destruction of PNNs in adult rats by chondroitinase-ABC (ChABC) restores plasticity and allowed for OD to occur in monocularly deprived adults⁹. This suggests that formation of PNNs contribute to the closing of the visual critical period and that their removal may help to restore lost plasticity in adults.

The act of closing critical periods by the production of PNNs can also be seen in the mouse barrel cortex. A study by McRae et al. investigated how whisker trimming, and the resulting stimulus deprivation, influenced the development of PNNs in the corresponding barrel cortex. In young mice, layer V of the deprived barrel cortex had significantly less PNN expression than the non-deprived barrel cortex from the same animal¹². When the whisker trimming is performed on adult mice, this is not found to be the case. Both deprived and non-deprived barrel cortex contain the same PNN density¹². This suggests that PNN development is activity dependent, and that development of PNN prevents the reactivation of critical periods despite the removal of stimulus.

PERINEURONAL NETS INFLUENCE ON FEAR AND FEAR CONDITIONED RESPONSES

How well does the success of PNN degradation in the visual cortex to restore plasticity translate to applications within fear and fear conditioned responses? Studies have been done within the auditory cortex and basolateral amygdala (BLA) to examine the implications of PNN degradation^{13,14}. Pavlovian conditioning is used to create a fear association between a conditioned and unconditioned stimulus, which can last for the entire duration of an adult rats' lifetime¹⁵. The two studies investigated the pairing of a constant-pitch auditory stimulus which co-terminated with a foot shock. In cases of learned fear, percentage of time spent frozen in place in response to a fearful stimulus can be used to determine the strength of a fear memory; the higher percent freezing behavior, the stronger the fear association. A behavioral treatment called extinction is used post-conditioning to attenuate fear associations. By administering ChABC during extinction treatment, it's possible to examine the influence of PNNs on fear response strength and retention.

Auditory fear associations show decreased freezing after extinction treatment, however spontaneous recovery does occur^{6,13}. When ChABC is administered to the auditory cortex prior to fear conditioning and then followed by extinction treatment, the freezing percent is significantly lower 24 and 48 hours later compared to extinction treatment alone¹³. This suggest that PNNs within the auditory cortex are critical for the consolidation of auditory based fear associations. Looking at similar procedures performed within the BLA, ChABC injection prior to conditioning followed by extinction training results in lowered freezing percentage compared to a vehicle control⁶. Additionally, ChABC injection prior to conditioning resulted in the prevention of spontaneous recovery of fear associations after being reintroduced to the unconditioned stimulus, unlike the vehicle which did see a revival in fear associations. Something interesting happens

when ChABC is administered immediately to the BLA after fear conditioning; freeze percentage is lower than the vehicle control after both undergo extinction treatment, but the ChABC group do exhibit spontaneous recovery of fear associations after being subjected to the unconditioned stimulus at a later timepoint⁶. This may be due to the mechanism in which extinction training helps to lower these fear associations. Extinction training is not the erasure of associations, but the acquisition of a new association that counters the old association¹⁴. The old association is still present, just over-written. This means that the old association can be retrieved again when subjected to conditions that stimulate spontaneous recovery. In cases where ChABC is administered prior to extinction training, it is possible that a new mechanism is occurring which doesn't mask the association, but actually attenuates the association so that it can't be retrieved later.

THERAPEUTIC TREATMENTS: HOW DO WE GET THERE?

A key feature of studies examining PNNs implications on fear associations has been the introduction of ChABC before or immediately following fear conditioning to degrade PNNs. While this may be optimal for expanding our knowledge of PNNs, it does little to push the field towards new potential therapeutic options for those suffering from various anxiety disorders characterized by fear-like symptoms. Had these studies include a condition examining ChABC administration several days post fear conditioning, the information could be generalized to patients whose anxiety disorders are rooted in traumatizing events from their childhood^{4,16}. The previously mentioned studies did include treatment conditions in which ChABC was administered immediately after fear conditioning, however, this does not allow a long window for the fear associations to become establish and mimic the conditions of actual patient.

In addition to the lack of research examining ChABC administration post fear conditioning, the quality of results obtained from the administration of ChABC is questionable. ChABC targets chondroitine sulfate-glycosaminoglycan (CS-GAG) side chains which attach to the core protein of CSPGs¹⁷. As CSPGs are not expressed solely in PNNs, but also in the surrounding extracellular matrix (ECM), unintended degradation of the ECM does occur in ChABC-mediated degradation of PNNs¹⁸. This makes it difficult to identify PNN degradation as the sole contributor to any behavior changes after ChABC has been introduced as changes have also occurred in the ECM. Mice which have been genetically modified to knock-out the CSPG aggrecan have been shown to have reduced density of PNNs in the barrel cortex, suggesting aggrecans presence is crucial to PNN integrity¹⁹. Current research is focused on finding a temporally-appropriate method to inhibit aggrecan that would allow for typical PNN development during behavioral training and PNN degradation at timepoints during extinction training. The use of siRNA targeting aggrecan, with the intent of transitioning to shRNA, is currently being investigated as a replacement to ChABC treatments. This could strengthen all research investigation PNNs by creating a more specific targeting method for PNN degradation.

While PNN manipulation is far from being used in human treatment, a currently prescribed drug takes advantage of several mechanisms used to analyze PNN function in fear associations to assist those with anxiety disorders. The selective serotonin reuptake inhibitor fluoxetine is a regularly prescribe antidepressant for those with a variety of anxiety disorders²⁰. The administration of fluoxetine to adult rats helps shift cortical neurons to monocularly deprived eyes in OD trials, like the results seen by PNN degradation²⁰. Additionally, the chronic administration of fluoxetine alongside extinction treatments helps to extinguish fear memories faster than extinction alone; the dual treatment mice also showed resistance to spontaneous recovery after being subjected to the unconditioned stimulus later one²¹.

CONCLUSION

Perineuronal nets have become a focal point of research in the last several decades as methods to manipulate their representative density in the CNS have become available. Investigation into PNN development during stimulus-deprivation has given support to PNNs involvement in the closure of various critical periods, such as in the barrel cortex or with the development of visual deficiencies. Recently, research has shifted towards investigating how PNN status can be modified to influence behaviors. By combining PNN degradation via ChABC with extinction training, attenuation of fear-associations has been found to be equal to conditions that solely undergo extinction training. However, rats that undergo PNN degradation and extinction training appear to be resistant to the spontaneous recovery of previously established fear-associations.

REFERENCES

1. Bandelow B., Michaelis S., "Epidemiology of anxiety disorders in the 21st century", *Dialogues in Clinical Neuroscience*; 17:327-335, 2015.
2. Wittchen HU., "Generalized Anxiety Disorder: Prevalence, Burden, and Cost to Society", *Depression and Anxiety*; 16: 162-71, 2002.
3. Holzel BK., Hoge EA., Greve DN., Gard T., Creswell JD., Brown KW., Barret LF., Schwartz C., Viatl D., Lazar SW., "Neural Mechanism of symptom improvements in generalized anxiety disorder following mindfulness training", *NeuroImage: Clinical*; 2, 448-58, 2013.
4. Merckelback, H., Rutter C., Van Den Hout M., Hoekstra R., "Conditioning Experiences and Phobias", *Behavioral Research and Therapy*; 27: 657-62, 1989.
5. Kim JH., "Reducing fear during childhood to prevent anxiety disorders later: insights from developmental psychobiology", *Policy Insights from the Behavioral and Brain Sciences*; 4:131-138, 2017.
6. Gogolla N., Caroni P., Lüthi A., Herry C., "Perineuronal Nets Protect Fear Memories from Erasure", *Science*; 325: 1258-1261, 2009.
7. Ye Q., Miao QL., "Experience-dependent development of perineuronal nets and chondroitin sulfate proteoglycan receptors in mice visual cortex", *Matrix Biology*; 32: 352- 63, 2013.
8. Carulli D., Rhodes KE., Fawcett J., "Upregulation of aggrecan, link protein 1, and hyaluronan synthases during formation of perineuronal nets in the rat cerebellum", *The Journal of Comparative Neurology*; 501: 83-94, 2007.
9. Pizzorusso T., Medini P., Berardi N., Chiezi S., Fawcett J., Maffei L., "Reactivation of ocular dominance plasticity in the adult visual cortex", *Science*; 298:1248-1251, 2002.
10. Celio MR., Spreafico R., Biasi SD., Vitellaro-Zuccarello L., "Perineuronal nets: past and present", *Trends in Neuroscience*; 21: 510-15, 1998.
11. Le Vay S., Wiesel TN., Hubel DH., "The development of ocular dominance columns in normal and visually deprived monkeys", *Journal of Comparative neurology*; 191, 1980.
12. McRae PA, Rocco MM, Kelly G, Brumberg JC, Matthews RT, "Sensory Deprivation Alters Aggrecan and Perineuronal Net Expression in the Mouse Barrel Cortex", *The Journal of Neuroscience*; 27: 5405-13, 2007.
13. Bannerjee SB., Gutzeit VA., Baman J., Aoued HS., Doshi NK., Lio RC., Ressler KJ., "Perineuronal Nets in the Adult Sensory Cortex Are Necessary for Fear Learning", *Neuron*; 95:169-179, 2017.
14. Quirk GJ, Mueller D., "Neural Mechanisms of Extinction Learning and Retrieval", *Neuropsychopharmacology*; 33: 56-72, 2008.
15. Gale GD, Anagnostara SG, Godsil BP., Mitchell S., Nozawa T., Sage JR., Wiltgen B., Fanselow MS., "Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats", *The Journal of Neuroscience*; 24: 3810-15, 2004.
16. Kim JH., "Reducing fear during childhood to prevent anxiety disorders later: insights from developmental psychobiology", *Policy Insights from the Behavioral and Brain Sciences*; 4:131-138, 2017.
17. Siebert JR, Steencken AC, Osterhout DJ, "Chondroitin sulfate proteoglycans in the nervous system: inhibitors to repair", *Biomed Research Internation*; article ID 845323, 2014.
18. Sorg BA, Berretta S, Blacktop JM, Fawcett JW, Kitagawa H, Kwok JCF, Miquel M, "Casting a wide net: role of perineuronal nets in neural plasticity", *The Journal of Neuroscience*; 36:11459-68, 2016.
19. Rowlands D., Lensjø KK., Dinh T., Yang S., Andrews MR., Hafting T., Fyhn M., Fawcett JW., Dick G., "Aggrecan Directs Extracellular Matrix-Mediated Neuronal Plasticity", *The Journal of Neuroscience*; 38: 10102-113, 2018.
20. Vetencourt JFM., Sale A., Viegi A., Baroncelli L., Pasquale R., O'Leary OF., Castrén E., Maffei L., "The Antidepressant Fluoxetine Restores Plasticity in the Adult Visual Cortex", *Science*; 320: 385-88, 2008.
21. Karpova NN., Pickenhagen A., Lindholm J., Tiraboschi E., Kuleskaya N., Ágústsdóttir A., "Fear Erasure in Mice Requires Synergy Between Antidepressant Drugs and Extinction Training", *Science*; 334: 1731-1734, 2011.

Are vital proteins becoming a wrench in the gears?

Lillian Hughes

INTRODUCTION

Parkinson's disease is the second most common neurodegenerative disease, which 50,000 people are diagnosed with each year¹. Parkinson's disease (PD) is a disease that is defined by dysfunction in motor movement as well as the existence of Lewy bodies². While it is not known the exact process by which Lewy bodies are formed, it is known that they are accumulations of many different proteins, such as parkin, SNCA, or PINK-1³. The build-up of these proteins within Lewy bodies correlates with the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc)⁴. In order to diagnose someone with PD post-mortem, the brain sample must contain Lewy bodies and degradation of dopaminergic neurons within the substantia nigra².

Parkinson's disease is an example of pathology within the motor-movement pathway. In the healthy state, the SNpc transfers information through the putamen, then the thalamus which in turn sends the information to the motor cortex². If the information becomes corrupted in the SNpc, there becomes a lack of stimuli continuing to the motor cortex. As more dopaminergic neurons are lost, the degree of stimulation from the SNpc decreases and less activity is triggered in the motor cortex². As the disease progresses, more dopaminergic neurons die, causing more problems with motor cortex functionality.

Currently, research is being done on what causes the death of dopaminergic cells. While it is known that there is protein accumulation within the dopaminergic cells, it is not clear what protein causes the neuropathy. The current hypothesis is that initial protein accumulation can lead to mitochondrial dysfunction, exacerbating further protein accumulation. It is hypothesized that it is not one single pathology that causes the mitochondrial dysfunction, but an accumulation of many different causes. Certain genetic profiles may cause a natural increase in protein that causes a patient to be more susceptible to protein dysregulation². Not only can DNA play a role, but mitochondrial dysfunction and oxidative stress⁴ may also play a role. While there are different causes of protein upregulation, once the proteins are accumulated within the mitochondria they can contribute to further mitochondrial dysfunction⁵. This causes a vicious cycle, where more protein accumulates, causing further mitochondrial dysfunction, and so on. A solid cause of PD has not been found, although information gathered from genetic studies, protein knock-in/knock out studies, and oxidative stress studies point towards a possible cause.

GENETIC DISPOSITION TO PROTEIN ACCUMULATION

While familial cases only make up 3%-5% of PD cases², they do illustrate how dysfunction of one specific gene can cause a case of PD. Familial cases of PD are defined as when PD is passed

through a family due to a genetic mutation, as opposed to sporadic PD, which has no clear cause and does not run in families².

While there are different genetic mutations that can cause PD, one common mutation is in the gene SNCA. SNCA codes for the protein alpha synuclein which is found within Lewy bodies². The higher the number of copies of SNCA the more alpha synuclein produced within the brain². The higher number of copies of SNCA and therefore alpha synuclein concentrations correlates with a higher risk of developing PD².

While it is uncommon that a sporadic case of PD will be caused by a single genetic mutation, a single genetic mutation can cause a disposition to developing PD later in life². Most genetic studies show how an increase of a specific protein can lead to PD. Although genetic studies are good for illustrating what proteins can lead to PD, they are not able to illustrate how lower protein levels can lead to PD.

MUTATIONS OF LEUCINE RICH REPEAT KINASE 2 AND ALPHA SYNUCLEIN

While there is a lack of flexibility in looking at genetic studies, mutation studies allow for researching what occurs during a mutation in a specific gene. For example, a mutation in SNCA that causes familial PD is A53T. The A53T mutation has shown to cause misfolding of Alpha synuclein⁶. The second mutation, G2019S is in the gene coding for LRRK2, which leads to increased kinase activity⁶. Both mutations have been correlated with an increased risk of PD⁶.

Both these mutations were studied by Novello et al. four strains of mice were created, one with leucine rich repeat kinase 2 (LRRK2) wild type with (WT) alpha synuclein production, one with LRRK2 (WT) and the A53T mutation, one with the G2019S and WT alpha synuclein, and one with both G2019S and A53T mutations. The G2019S mutation with the WT alpha synuclein did not cause a significant amount of death of neurons within the substantia nigra, the A53T paired with WT LRRK2 and A53T paired with G2019S caused a significant amount of deaths of neurons. When both mutations occurred together significantly more neurons died than when there was just the A53T mutation. While the G2019S mutation did not cause a mutation on its own it did amplify the effects of the A53T mutation.

KNOCK-IN OF ALPHA SYNUCLEIN INTO *C. ELEGANS*

While Novello et al. study how these mutations occur in an animal that already expresses LRRK2 and alpha synuclein, Cooper et al. study how alpha synuclein reacts in an organism that does not naturally express that protein. *C. elegans* does not naturally express SNCA⁷, which allows a chance to observe synergy between alpha synuclein and other proteins such as PARK2, PINK1 and DJ-1. These worms were genetically modified to express SNCA and then crossed with various models of PD, to see how the addition of alpha synuclein affected PD development.

While adding alpha synuclein into the *C. elegans* nervous system did not cause death of dopaminergic neurons, the strains with alpha synuclein tagged and using red fluorescent protein (asyn:RFP) strain did have increased number of protrusions through the plasma membrane (blebs) on dendrites⁷. Dendritic blebs are associated with toxicity within the neurons⁸. This data implies that introducing alpha synuclein is not enough to cause the death of dopaminergic cells, the neurons do show signs of neurotoxicity. While alpha synuclein may be dangerous to the cell, it may need to be paired with protease dysfunction to cause cell death.

EFFECT OF PROTEINS AND OXIDATIVE STRESS ON MITOCHONDRIA

Not only can over expression of proteins cause PD, but mitochondrial dysfunction can as well [12]. While Cooper et al. and Novello et al. investigate the effects of increase alpha synuclein, Zilocchi et al. investigate the effects of a toxin, MPTP on the mitochondria. MPTP is a toxin that is known to induce parkinsonian symptoms and is commonly used as a PD model². MPTP is quickly converted to MPP⁺ by glial cells, which then can cause oxidative stress within the neuron².

Zilocchi et al. use electron microscopy to view mitochondria in order to determine the concentration of healthy mitochondria after exposure to altered dopamine treatment. Dopamine treatment consisted of increasing intracellular dopamine levels. Both the altered dopamine homeostasis and the MPTP treatment caused a large decrease in healthy mitochondria when compared to the control proportion of healthy mitochondria⁹. The decrease in healthy mitochondria after exposure to an increase of intracellular dopamine or MPTP insinuates that mitochondria may play a role in PD.

In a similar vein, the mitochondrial health during two other PD models rotenone and paraquat [4] are being tested by Wu et al. Rotenone and paraquat are two types of pesticides that cause oxidative stress within the cell². Both rotenone and paraquat caused a large decrease in cristae length [10]. The average cristae length decreased by more than 5 micrometers, to an average length of less than 1 micrometer¹⁰. Since the cristae is where the mitochondrial complex resides, a shrinkage in length is dangerous to normal function.

In contrast, Jang et al. use MPTP to illustrate the change in the concentration of mitochondrial complex proteins. There is a statistical decrease in protein concentration between control samples and samples treated with MPTP¹¹.

Another mutation study done by Zhou et al. also illustrate how mitochondrial dysfunction can be created using a VPS35 mutation to do so. VPS35 D620N is a mutation associated with autosomal dominant PD. The VPS35 D620N is associated with decreased enzymatic activity in complexes I and II¹². PD can be caused by a change in protein production, PD can also be caused due to mitochondrial dysfunction¹².

Parkinson's disease is a widespread neurological disorder, that affects half a million people¹. PD can be caused by various dysfunctions, such as overabundance of alpha synuclein, to heavy oxidative stress. While in extreme cases one of these traits can cause PD, sporadic PD is probably caused by a mixture of these symptoms. A drug that is designed to inhibit only one of these traits will not be able to stop the overall dysfunction. A drug to treat PD must be able to modulate multiple targets, instead of targeting one specific trait.

REFERENCES

1. "NIH Fact Sheets - Parkinson's Disease." *National Institutes of Health*, U.S. Department of Health and Human Services, report.nih.gov/nihfactsheets/ViewFactSheet.aspx?csid=109.
2. Lewis, Patrick A., and Jennifer E. Spillane. *The Molecular and Clinical Pathology of Neurodegenerative Disease*. Academic Press, an Imprint of Elsevier., 2019.
3. Wakabayashi, Koichi, et al. "The Lewy Body in Parkinson's Disease and Related Neurodegenerative Disorders." *Molecular Neurobiology*, vol. 47, no. 2, 2012, pp. 495–508., doi:10.1007/s12035-012-8280-y.
4. Soliman, Amira M., et al. "Dose-Dependent Neuroprotective Effect of Caffeine on a Rotenone-Induced Rat Model of Parkinsonism: A Histological Study." *Neuroscience Letters*, vol. 623, 2016, pp. 63–70., doi:10.1016/j.neulet.2016.04.057.

5. Grünewald, Anne, et al. "New Insights into the Complex Role of Mitochondria in Parkinson's Disease." *Progress in Neurobiology*, 2018, doi:10.1016/j.pneurobio.2018.09.003.
6. Novello, Salvatore, et al. "G2019S LRRK2 Mutation Facilitates α -Synuclein Neuropathology in Aged Mice." *Neurobiology of Disease*, vol. 120, 2018, pp. 21–33., doi:10.1016/j.nbd.2018.08.018.
7. Cooper, Jason F., et al. " α -Synuclein Expression from a Single Copy Transgene Increases Sensitivity to Stress and Accelerates Neuronal Loss in Genetic Models of Parkinson's Disease." *Experimental Neurology*, vol. 310, 2018, pp. 58–69., doi:10.1016/j.expneurol.2018.09.001.
8. Masoudi, Neda, et al. "Tetraspanin (TSP-17) Protects Dopaminergic Neurons against 6-OHDA-Induced Neurodegeneration in *C. Elegans*." *PLoS Genetics*, vol. 10, no. 12, 2014, doi:10.1371/journal.pgen.1004767.
9. Zilocchi, Mara et al. "Mitochondrial alterations in Parkinson's disease human samples and cellular models", *Neurochemistry International*, Volume 118, 2018, Pages 61-72, ISSN 0197-0186, <https://doi.org/10.1016/j.neuint.2018.04.013>.
10. Wu, Siyu et al. "Mutation of hop-1 and pink-1 attenuates vulnerability of neurotoxicity in *C. elegans*: the role of mitochondria-associated membrane proteins in Parkinsonism", *Experimental Neurology*, Volume 309, 2018, Pages 67-78, ISSN 0014-4886, <https://doi.org/10.1016/j.expneurol.2018.07.018>.
11. Jang, Yongchul et al. "Modulation of mitochondrial phenotypes by endurance exercise contributes to neuroprotection against a MPTP-induced animal model of PD", *Life Sciences*, Volume 209, 2018, Pages 455-465, ISSN 0024-3205, <https://doi.org/10.1016/j.lfs.2018.08.045>.
12. Zhou, Leping et al. "Parkinson's disease-associated pathogenic VPS35 mutation causes complex I deficits", *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, Volume 1863, Issue 11, 2017, Pages 2791-2795, ISSN 0925-4439, <https://doi.org/10.1016/j.bbadis.2017.07.032>.

Physical exercise and neuronal plasticity: a review on the relationship between physical activity on enhanced learning and memory formation

Maxwell Neideigh

INTRODUCTION

Physical exercise and the process of learning and storing memories have traditionally been thought to be carried out by independent organ systems in the body. From an evolutionary perspective, however, locomotion and adaptability needed to be in close contact to one another in order to map surroundings and remember locations of danger. When faced with a dangerous situation we not only need the ability to run, but also to have a source of brain plasticity to adapt to new kinds of stress and respond more appropriately in a future encounter. A growing body of research on long-term potentiation (LTP), a form of synaptic plasticity involved in memory encoding, begins to explain the cellular relationship connecting these two processes in the body¹. This paper reviews literature detailing the impact of Physical Exercise on BDNF expression, and how this signaling cascade can benefit processes of learning and memory.

A particular brain protein (brain derived neurotrophic factor) (BDNF) that belongs to the neurotrophin family has been shown to increase in blood serum levels following physical activity². These endogenous peptides secreted from neuronal cells, are associated with facilitating the function, survival, and development of individual cells and neuronal networks that span the entire brain. Neurotrophins demonstrate their neuroprotective effects through the transmembrane receptors they bind to and the signaling cascades they initiate. By means of the PLC pathway for example, binding of BDNF to its receptor Tropomyosin Receptor Kinase B (trkB) leads to increased concentration of intracellular calcium, activating the calcium/calmodulin pathway. This increases the concentration of a cellular transcription factor CREB, increasing transcription of the genes³. Furthermore, BDNF has also been shown to positively modulate hippocampal synapses by binding to trkB.

Previous research indicates that physical activity or neuronal activity significantly enhances *Bdnf* gene expression in the brain and that this increase in BDNF protein has downstream effects activating a number of signaling pathways that result in exercise-dependent enhanced learning and memory formation⁴. In studies involving rodents, a common method to assess whether or not learning has taken place is to implement the Morris water maze. This test involves a pool of water with an adjustable floating platform that can be placed in differing positions around the pool. This challenges rodents to remember, not see where the platform and their eventual safety will be.

It was shown in 2005 by van Praag H and colleagues that physical activity enhanced acquisition of the hidden platform task in the water maze in old mice compared with age-matched sedentary controls. Exercise enhanced acquisition of the hidden platform task in the water maze in 19 month

old mice compared with younger sedentary controls^{5,6}. In order to see the physiological changes associated with exercise, Sleiman and colleagues used a similar method where one half of test subject mice were allowed access to a running wheel but were not forced to run, where the other half were not provided a wheel at all. Physical activity was monitored for 30 days prior to sacrificing the animals and collecting tissue samples. Results from this experiment show 4-weeks of voluntary exercise induces increases in mature brain bdnf levels⁷.

BDNF SIGNALLING IN HD

Unhealthy subjects, like in the case of the Huntington knock-in mouse, can be used as models to study the modulatory effects of BDNF on LTP. Huntington's disease is a dominantly inherited neurodegenerative disease that is characterized by an increased polyglutamine stretch in the translated huntingtin protein that has a devastating effect on neurons. Some of the earliest experiments using HD mouse models found that BDNF levels were diminished in both cortical and striatal neurons, consistent with pathology seen in HD patients. A number of different mechanisms have been proposed to explain the neural deficits seen in HD, including decreased levels of brain-derived neurotrophic factor (BDNF) in the hippocampus. The relationship between BDNF in HD pathogenesis is of particular interest, as BDNF plays a crucial role in synaptic scaling and neuron survival⁸.

POTENTIATION THROUGH THETA BURST STIMULATION (TBS)

Eniko and Kramar show in a 2007 study, that long term plasticity in hippocampal slices from presymptomatic knock-in mice is sub-optimal. They establish that potentiation can be rescued with brain-derived neurotrophic factor (BDNF). In order to test the extent of BDNF as a positive modulator of LTP, the potentiation effect on EPSP's must first be induced by naturalistic theta burst stimulation (TBS). In healthy subjects, binding of BDNF to trkB can induce a variety of intracellular changes including; increases in receptor trafficking, receptor conductance and receptor expression.

A common way to explore the phenomena of LTP is to gradually increase the number of theta bursts administered to induce LTP in the range of 2- 20, where a single burst contains four pulses measuring 100 Hz and the bursts themselves are 200 msec apart. This technique has been shown to cause LTP effects of hippocampal excitation⁹. In the HD knock in model, the typical potentiation efforts using TBS result in an overall reduction in fEPSP amplitude. Observations of the field excitatory postsynaptic potentials of tissue slices from mice carrying the mutant huntingtin gene in the presence of bdnf show a greater fEPSP amplitude, providing evidence for the positive modulation of bdnf on LTP. The binding of BDNF on trkB receptors lowers the EPSP threshold and raises the ceiling required for LTP induction.

CONCLUSION

The purpose of this literature review was to determine the association between exercise and its impact on the processes of learning and memory formation. Higher levels of a specific brain protein BDNF in the hippocampus are seen in response to exercise. Due to bdnf's ability to restore LTP induction to normal levels in HD knock in mice, further research should focus on ways to induce brain bdnf levels, or find other agonists that show high-affinity binding for the trkB receptor. One agonist in particular, dihydroxyflavone shows greater binding affinity to the trkb receptor than BDNF achieving downstream transcriptional regulation much easier. Neurotrophins, specifically

ones that act on trkb receptors, provide evidence supporting the positive impact of exercise on learning and memory. These molecules and their related receptors are capable of alleviating cognitive deficits associated with a number of neurodegenerative diseases. In a future state, these molecules may be used as an “exercise pill,” prescribed to those who are incapable of working out, yet could still benefit from the effects of physical activity.

REFERENCES

1. Enikő A. Kramár, Bin Lin, Ching-Yi Lin, Amy C. Arai, Christine M. Gall and Gary Lynch. *Journal of Neuroscience* 24 June 2004, 24 (22) 5151-5161; DOI:https://doi.org/10.1523/JNEUROSCI.0800-04.2004
2. Ieraci, A. , Mallei, A. , Musazzi, L. and Popoli, M. (2015), Physical exercise and acute restraint stress differentially modulate hippocampal brain-derived neurotrophic factor transcripts and epigenetic mechanisms in mice. *Hippocampus*, 25: 1380-1392. doi:10.1002/hipo.22458
3. Wurzelmann, M., Romeika, J., & Sun, D. (2017). Therapeutic potential of brain-derived neurotrophic factor (BDNF) and a small molecular mimics of BDNF for traumatic brain injury. *Neural regeneration research*, 12(1), 7–12. doi:10.4103/1673-5374.198964.
4. Marosi, K. , Kim, S. W., Moehl, K. , Scheibye-Knudsen, M. , Cheng, A. , Cutler, R. , Camandola, S. and Mattson, M. P. (2016), 3-Hydroxybutyrate regulates energy metabolism and induces BDNF expression in cerebral cortical neurons. *J. Neurochem.*, 139: 769-781. doi:10.1111/jnc.13868
5. van Praag, H., Shubert, T., Zhao, C., & Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(38), 8680–8685. doi:10.1523/JNEUROSCI.1731-05.2005
6. Liu, Patrick Z. Nusslock Robin. 2018, Exercise mediated neurogenesis in the hippocampus via BDNF. *Frontiers in Neuroscience*, 12: 52. doi: 10.3389/fnins.2018.00052
7. Sleiman SF, Henry J, Al-Haddad R, et al. Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body β -hydroxybutyrate. *Elife*. 2016;5:e15092. Published 2016 Jun 2. doi:10.7554/eLife.15092
8. Zuccato, C. *et al.* Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293, 493–498 (2001).
9. Rioult-Pedotti, M. S., Friedman, D., Hess, G. & Donoghue, J. P. Strengthening of horizontal cortical connections following skill learning. *Nat. Neurosci.* 1, 230–234 (1998).
10. Rausch Osthoff, A. K., Juhl, C. B., Knittle, K., Dagfinrud, H., Hurkmans, E., Braun, J., Schoones, J., Vliet Vlieland, T., ... Niedermann, K. (2018). Effects of exercise and physical activity promotion: meta-analysis informing the 2018 EULAR recommendations for physical activity in people with rheumatoid arthritis, spondyloarthritis and hip/knee osteoarthritis. *RMD open*, 4(2), e000713. doi:10.1136/rmdopen-2018-000713
11. Patrick S. Murray and Philip V. Holmes, “An Overview of Brain-Derived Neurotrophic Factor and Implications for Excitotoxic Vulnerability in the Hippocampus,” *International Journal of Peptides*, vol. 2011, Article ID 654085, 12 pages, 2011.

Visual and auditory perceptual load applied to distracted driving

Janee Meengs

INTRODUCTION

There are three types of distracted driving: manual, visual and cognitive¹. The first two are likely familiar since the majority of laws, public service announcements and conversation are centered on keeping drivers' eyes on the road and hands on the wheel. Cognitive distraction is defined as a mental distraction removing the driver's focus from the task of driving. Electronic devices are distinctly dangerous because they require some form of visual, auditory and cognitive processing, which may ultimately lead to increased risk of a motor vehicle accident whether the device is hands-free or not². Our community has understood for some time that the combination of auditory and visual cognition can lead to risky behavior behind the wheel. However, these distractions often present themselves in ways we may not immediately identify as a risk. Some drivers may think they are being safe by not partaking in a long telephone conversation; however, the process of listening to a radio traffic update can decrease visual awareness³ even if the driver's eyes never leave the road.

Support for prohibiting texting while driving and hand-held cell phone use is nearly universal. Yet, as of 2016, the percentage of drivers using headsets had not changed significantly within the last ten years; with use of hand-held devices decreasing by approximately 1.5% and device manipulation increasing at about the same rate⁴. Since the efforts to reduce visual and manual distractions do not seem to be as effective as once hoped, regardless of the ever-increasing restrictions implemented, there must be some way to reduce cognitive load. By restricting the amount of incoming sensory information it could be possible to reduce risk through these means rather than relying on the public to police themselves and each other. Perceptual load theory has previously been applied to driving simulation tasks with mixed results^{3,5,6}. The theory postulates that with increased perceptual load tasks a person's sensory systems will filter task-irrelevant information at an earlier stage than during a low load task, which inhibits deeper processing due to unavailable cognitive resources⁷. This theory sprouted the idea that an auditory stimulus, such as GPS system or phone call, could effectively pull drivers' attention away from the road even if they maintain their gaze on the roadway. However, other studies have suggested that perceptual load theory can only be applied to the visual system and is not cross modal⁶. Therefore, this literature review intends to discover how vision and audition are related and if perceptual load in one modality can hinder another. Exploration will dive further into how these two sensory systems play a role in driving, if laws can be improved and if science can persuade drivers to turn their devices off.

BEGINNINGS OF PERCEPTUAL LOAD THEORY

In 1958, cognitive psychologist Donald Broadbent introduced and tested the theory of early selection of attention. Broadbent's model of attention outlined an initial processing of all incoming stimuli based on physical features such as pitch or orientation. Relevant stimuli are further processed in a second stage for meaning⁸. Broadbent recognized capacity as a limitation of attention, therefore, he proposed that a filter is utilized to choose and further process relevant stimuli.

Contrasting Broadbent's theory, the late selection theory gained support approximately ten years later⁷. Selection on what to attend and its relevance are determined later in processing once the meaning of the stimulus is identified and an appropriate response can be provided⁷. However, many behavioral studies such as the Stroop effect still support early processing. The Stroop effect is the presentation of a word list in various ink colors and asks participants to name the ink color rather than the word itself. In one study, the congruent test where the words matched the color, participants' reaction time was 82 seconds faster than the incongruent test⁹. While this task alone does not imply that unattended stimuli are filtered out, it does show that it is extremely difficult to resist processing of task-irrelevant attributes of the stimulus⁸.

With these conflicting ideas, neuroscientist Nilli Lavie attempted to resolve the dispute by introducing the perceptual load theory. In Lavie's perceptual load theory early processing was only applicable when there was a high degree of cognitive load⁷. In the absence of a high-load task, all stimuli are processed with irrelevant distractors utilizing the unused capacity of the relevant task. The acknowledgement of a limited capacity system with the assumption that processing is automatic so long as there is available capacity yields a compromise implying that attention is not fully automatic while not fully voluntary⁷.

PERCEPTUAL LOAD THEORY AND DISTRACTED DRIVING

To aid in the understanding of perceptual load and its application to driving tasks, many studies have been performed in a driving simulation while participants attended to secondary *stimuli*. A 2017 study monitored driving behavior while university students listened for a change in voice (male to female, female to male) or a specific traffic update on a target road³. The results of this study yielded longer reaction times to roadway hazards and perceptual ignorance of a central stimulus in high load conditions. These data show two things; visual perception is influenced by cognitive load and the capacity of attention is cross modal³. This important factor shows a link between visual and auditory systems, that attention is non-specific and capacity applies to all sensory modalities rather than a specific allowance for each system.

In addition to these findings, fMRI has shown a reduction in brain activity while driving and performing an auditory task. The decrease in activity further shows that auditory and visual systems draw from the same capacity of attention. The participants engaged in a driving task alone and then while listening to general knowledge sentences and verifying their truth or falsehood. Driving behavior deteriorated as the participants *veered over and contacted the road shoulder* more often during the sentence listening task¹⁰. The fMRI revealed a decrease in activation of the bilateral parietal lobes and the superior extrastriate, which are associated with the driving task¹⁰. When the sentence task was added there was increased activation of temporal and prefrontal language areas involved in hearing and language processing⁹. A decrease in overall visual attention is shown through the decrease in spatial processing as well as the reallocation of visual attention¹⁰ to areas associated with auditory processing rather than visual processing.

These studies show behavioral and cognitive declines when an auditory stimulus is applied during a driving task that leads to increased roadway errors and indicate the effect of auditory stimuli on visual processing.

NEURAL LINKAGE OF VISION AND AUDITION

The visual and auditory systems are two distinct systems with respective pathways that lead from the initial stimulus to their respective cortices. Therefore, how can these separate systems work together without blurring the information and its meaning? The principle of labeled lines states that each receptor receives a stimulus and then sends that information through its nerve fibers to the corresponding cortical area¹¹. According to this principle the sharing of information between vision and audition is unlikely and each would have its own capacity of attention. Additionally, some evidence exists that perceptual load cannot be applied to audition because the auditory system acts as an environmental warning system that would require its own capacity to process auditory information regardless of the attended stimulus⁶.

However, this is not reflected in life and especially in the evidence of distracted driving. Here, these systems are closely linked, shown though the blindness due to inattention leading to increased risks while driving. This indicates that in some cases, when perceiving and interacting with our environment vision and audition must be linked in order to accurately predict and respond to an ever-changing world. This is further supported by a study performed in 2010 where a motion stimulus in either vision or audition led to a decrease in brain activity in the non-attended modality. The results were especially pronounced during the auditory task, which showed suppression of activation in the visual cortex¹².

These findings help provide a neural explanation for the driving behavior seen in simulations by depicting a physical reallocation of cognitive resources from the task of driving and onto the distractor. They also show that distractions effectively consume enough cognitive capacity to have an effect on basic driving maneuvers. Understanding how these systems are related to each other could aid in understanding how we can shape laws or inform drivers about possible risks previously thought to be benign.

FUTURE STUDIES AND COGNITIVE DISTRACTION DIRECTED LAWS

Studies that depict a more practical simulation or driving environment would be ideal to understanding how these distractors in a real life setting change behavior. Specifically, studies that allow participants to drive routes they are familiar with or listen to music, news programs or radio talk shows that they would normally indulge in while on their daily commute. Experiments like this would greatly aid in behavioral data but because of their nature likely would not allow for fMRI imaging. Law shaping at this time has greatly emphasized the use of hands-free devices, without understanding that these devices only eliminate two of the three types of distracted driving. It is possible that because of the increasing focus on ridding drivers of manual and visual distractors technology has come too far to ever truly abolish cognitive distractors like phones and navigation systems. For now, implementing laws that ban radio, navigation and hands-free phone use would be a giant step backwards when considering the efforts society has taken to equip newer cars with this technology and the difficulty involved in enforcing those laws. However, further understanding cognitive distractions could help the public make informed decisions about using those devices.

REFERENCES

1. Esurance Insurance Company. (2019). 3 Types of Distracted Driving. Retrieved March 17, 2019 from <https://www.esurance.com/info/car/3-types-of-distracted-driving>.
2. Chase, J.D.C. (2014). U.S. State and Federal Laws Targeting Distracted Driving. *Annals of Advances in Automotive Medicine* 58: 84-98.
3. Murphy, G. and Green, C.M. (2017). The Elephant in the Road: Auditory Perceptual Load Affects Driver Perception and Awareness. *Applied Cognitive Psychology* 31: 258-263
4. National Highway Traffic Safety Administration (2017). Electronic Device Use in 2016. *U.S. Department of Transportation* [Online]. <https://crashstats.nhtsa.dot.gov/Api/Public/ViewPublication/812426>
5. Tellinghuisen, D.J. and Nowak, E.J. (2003) The inability to ignore auditory distractors as a function of visual task perceptual load. *Perception & Psychophysics* 65: 817-828
6. Murphy, S., Fraenkel, N. and Dalton, P. (2013). Perceptual load does not modulate auditory distractor processing. *Cognition* 129: 345-355
7. Lavie, N. (1995). Perceptual Load as a Necessary Condition for Selective Attention. *Journal of Experimental Psychology: Human Perception and Performance* 21: 451-468
8. Lachter, J., Forster, K.I. and Ruthruff, E. (2004). Forty-Five Years After Broadbent (1958): Still No Identification Without Attention. *Psychological Review* 111: 880-913
9. Ghimire, N., Paudel, B.H., Khadka, R. and Singh, P.N. (2014). Reaction Time in Stroop Test in Nepalese Medical Students. *Journal of Clinical and Diagnostic Research*. 8:BC14-BC16
10. Just, M.A., Keller, T.A. and Cynkar, J. (2008). A Decrease in Brain Activation Associated with Driving while Listening to Someone Speak *Brain Research* 1205: 70-80
11. Pereira Jr., J.C. and Alves, R.C. (2011). The Labelled-Lines Principle of the Somatosensory Physiology Might Explain the Phantom Limb Phenomenon. *Medical Hypotheses* 77: 853-856
12. Lewis, J.W., Beauchamp, M.S. and DeYoe, E.A. (2000). A Comparison of Visual and Auditory Motion Processing in Human Cerebral Cortex. *Cerebral Cortex* 10: 873-888

Cytokine storm signaling and potential therapies

Liubov Beregova

INTRODUCTION

Inflammation is an essential part of the body's defense mechanism, without which a resolution of cellular damage would not be possible. The immune system recognizes and removes harmful bacteria and viruses and begins the healing process to return an organism to its homeostatic state. However, emerging viral threats have continued to challenge medical and public health systems and incur economic costs to both individuals and countries. Influenza virus is the leading cause of those threats and responsible for 250,000–500,000 deaths each year. This virus can cause extensive morbidity and mortality in a high-risk group such as the elderly or people with compromised immune systems¹. Normally, when a person is infected with the flu virus, the immune system swings into action, using different types of immune white blood cells, such as macrophages, B-cells, and T-cells, to attack the invader². To coordinate communication between those cells a set of proteins called cytokines are released which activate a target cell and regulate the production of more cytokines to help deliver a knockout blow to the infection. While a strong immune system is a key to keeping an infection in check, ironically, an excessive immune reaction in response to viruses present the greatest danger to a healthy person. Some pathogens, such as influenza virus, trigger life-threatening conditions called “cytokine storms,” which can ultimately lead to death³. A cytokine storm is an overproduction of immune cells and their activating compounds – cytokines⁴. Essentially, the immune system is unable to adjust to changes triggered by certain strains of flu virus, which causes a severe immune overreaction in young adults since they tend to have stronger health. This review will focus on understanding and analyzing potential cytokine storm signaling using the influenza virus as an example. Additionally, this review will investigate the current candidates for the treatment of cytokine storms and compare them to more traditional treatments that target the pathogen rather than the host response. Comparing treatments will help determine which therapies are the most promising to prevent cytokine storms and prove useful in future clinical settings.

WHAT ARE CYTOKINES: DIFFERENT SUBTYPES OF CYTOKINES AND THEIR SIGNALING

To understand the pathology of cytokine storm, it is important to appreciate an essential part of the human body regulation – cytokine signaling. Most cytokines are cell-secreted proteins which are necessary for intracellular signaling where they play the role of local regulators that alter and activate immune cells such as macrophages, dendritic cell, lymphocytes etc. Macrophages and dendritic cells attack foreign invaders and send cytokine signals to activate nearby dormant lymphocytes, specialized to recognize certain antigens. Cytokines bind to receptors on target cells and activate a cascade of intracellular signals which help keep the body in homeostasis. When cytokines bind, a rapid tyrosine phosphorylation of the cytokine receptor occurs since cytokine receptors transmit their signal through a distinct family of tyrosine kinases (JAKs)^{5,6}. This process

leads to activation of the signaling molecules (STAT) which trigger transcriptional regulation of target genes. Disruption of the JAK-STAT signaling may cause a variety of diseases, such as skin conditions, cancers, and disorders affecting the immune system like cytokine storms.

Cytokines are a diverse group of small proteins that are secreted by cells for intercellular signaling and communication to activate immune responses. There are many types of cytokines that contribute to maintaining internal equilibrium including four major types: chemokines, interferons (IFNs), interleukins (ILs), and tumor necrosis factor (TNF). The first type, chemokines, function as mediators of directional migration of immune cells to the site of inflammation by interacting with G protein-linked transmembrane chemokine receptors of target cells⁷. The second type of cytokines are interferons named after their ability to “interfere” with viral replication within host cells. Similarly to ILs, IFNs mediate a continual conversation between cells about growth and defenses. The initiated response inside the cell heavily depends on the type of IFNs which either modify immune function or play a role in defense⁸. The term “interleukin” is used to describe a group of cytokines with complex immunomodulatory functions – including cell proliferation, maturation, migration, and adhesion. ILs, produced by lymphocytes, exert both inflammatory and anti-inflammatory actions to modulate growth, differentiation, and activation of immune cells. That subset of cytokines binds with high-affinity to receptors located on the surface of cells initiating a response⁹ to aid B-cells in producing antibodies and attract white blood cells to the site of infection. About thirty years ago, researchers discovered an additional type of cytokine termed tumor necrosis factor (TNF) which exert anti-tumoral effects on mouse models¹⁰. Depending on the TNF receptor type, TNF can either induce or inhibit apoptosis. This knowledge about the functions of different cytokine molecules will help explain the pathology behind cytokine storm signaling.

TOO MUCH OF A GOOD THING: CYTOKINE STORM SIGNALING

In 1918, the influenza pandemic or Spanish flu was responsible for about 50 million deaths worldwide which is the greatest loss of life to any known medical condition. For 75 years, scientists had a difficult time answering this basic question: ‘Why was the Spanish flu so fatal?’ In 1993, the term “cytokine storm” first appeared in the discussion of graft-versus-host disease (GvHD), a medical complication of a recipient who received a transplantation of a tissue from a genetically different donor¹¹. In the early 2000s, more papers began to use the term “cytokine storm” to describe infectious diseases such as the influenza virus¹². However, scientists still do not understand the complex nature of the immune response that triggers a cytokine storm.

Modern research mimics the 1918 influenza virus pandemic (H1N1) using experimental animal models to understand the pathology behind cytokine storm. Patients with severe influenza viral infections tend to have elevated levels of pro-inflammatory cytokines including interferons, tumor necrosis factors, interleukins, and chemokines¹³. Previous studies suggest that ILs are expressed in the early stages of infection in the H5N1-infected (Bird flu) mice. Previous studies made on IL1R KO mice suggests that transcriptional response is strongly associated with an increase in TNF gene expression, revealing the interplay between ILs and TNS molecules. For unknown reasons, ILs deactivate apoptotic TNF receptors leading to an upregulation of the TNF anti-apoptotic receptor which result in a buildup of cytokine molecules and triggers an onset of cytokine storm¹⁴. It is known that as the IFNs signaling pathway is activated, several proteins with antiviral or immunomodulatory properties are produced. However, in IFNsR KO mice, infected with the 1918 virus, a cytokine signaling pathway redundancy is also observed as there is strong upregulation of IFN-stimulated gene expression. Furthermore, several research groups looked at the role of some chemokine receptors, such as CCR5 and CCR2, in severe influenza infections. The CCR5 knockout mice displayed an excessive inflammatory response and increased mortality,

while *CCR2*^{-/-} mice had reduced inflammatory response and mortality but developed a significantly elevated viral load¹⁵. Figure 1 demonstrates the overall review of cytokine storm in the lung following severe influenza infection. Collectively, the knowledge about different pathological effects of cytokine molecules present key targets for therapeutic intervention and diagnostics. Yet, not all factors of the pathogenesis of severe influenza mechanism are unknown.

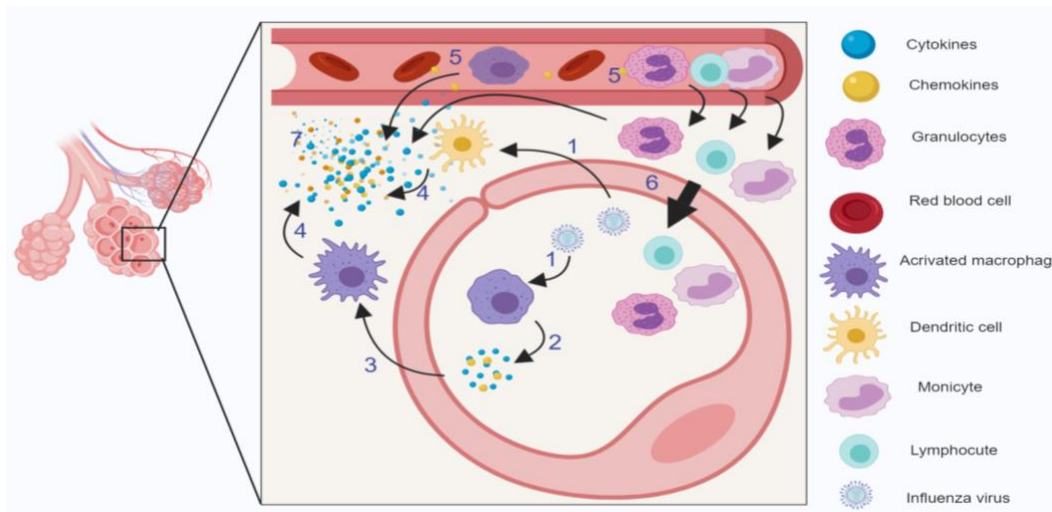


Figure 1. Cytokine storm in the lung following severe influenza infection. (1) Viruses infect lung epithelial cells, alveolar macrophages, and dendritic cells to produce progeny viruses and release cytokines/chemokines (2). (3) Cytokine/chemokine-activated macrophages and virally infected dendritic cells lead to a more extensive immune response and the initiation of cytokine storm (4). (5) Released chemokines attract more inflammatory cells to migrate from blood vessels into the site of inflammation (6), and these cells release additional chemokines/cytokines to amplify cytokine storm (7).

IMMUNOMODULATION AS A THERAPEUTIC APPROACH

The immune system is unable to adjust to changes triggered by certain strains of influenza virus, which causes severe immune overreaction. Since our body cannot adjust to new microenvironments at the same rate as microbes, pharmaceutical companies developed drugs such as antibiotics and antivirals in order to support the body's defenses¹⁶. However, many pathogens have evolved resistance to these medications designed to kill them. Therefore, immunomodulation gained more attention as a viable therapeutic approach to enhance the efficacy of antimicrobials. Specifically, a combination of some of them, such as Sphingosine-1-phosphate receptor 1 agonists (S1P1R), Peroxisome proliferator-activated receptors (PPAR), and COX-2 inhibitors show the most promising results.

In recent years, five specific S1P receptors were found to regulate the immunopathological signaling pathway; however, only one receptor (S1P1) exhibits cytokine-storm-blunting activity by suppressing innate cellular cytokine/chemokine responses. The location of the S1P1 receptor is mainly on the pulmonary endothelial cell. In murine models infected with 2009 H1N1 influenza, a S1P1 receptor agonist is reported to reduce death outcome by 80% compared to 50% protection offered by the antiviral inhibitor^{4,17}. This leads to the idea of a combined therapy that can achieve the most optimal protection of 96% according to the paper published by Oldstone and his colleagues in 2013.

Peroxisome proliferator-activated receptor (PPARs), including PPAR- α , PPAR- β , and PPAR- γ , are important for regulation of inflammation. Among those three types, PPAR- γ agonist is the most promising candidate to suppress cytokine storm due to its ability to increase the survival of influenza-infected mice as well as downregulate the inflammatory response to viral pneumonia¹⁸. Moreover, the benefits of PPAR- γ agonist treatment were found to be higher than PPAR- α ¹⁹. Even that PPAR- α agonist exerts the ability to inhibit TNF, IL-6, and IFN- γ , and the study by Zheng reported that PPAR- α administered 48-h post-infection had no effects on the mortality of H5N1 avian influenza-infected mice²⁰.

Selective COX inhibitors are known in clinical settings due to their antipyretic, analgesic, and anti-inflammatory properties. Differently from the previous two immunomodulatory therapies, COX inhibitors do not significantly modulate disease severity in influenza-induced murine model; however, in combination with neuraminidase inhibitors, COX-2 inhibitors improve the survival of infected mice²¹. A triple combination therapy of zanamivir, celecoxib (COX-2 inhibitors) and mesalazine significantly decreased the mortality and cytokines/chemokines levels of infected mice.

CONCLUSION

The episodic outbreaks of avian influenza virus in Asia and parts of Africa pose a major threat to public health. As a result, many severe influenza-infected patients died from serious complications caused by cytokine storm. This review highlighted the pathology of cytokine storm signaling, in particular, how different cytokine-subtypes influence the outcomes of influenza virus infection. Although, immunomodulatory strategies and novel approaches in targeting the host response during cytokine storm that shows the most promising results have been previously described. Knowing that these agents work on different intracellular pathways, combination of them might be an ideal therapeutic approach to obtain a better outcome. Based on the presented experimental results, S1PR, PPAR agonists, and COX-2 inhibitors immunomodulation treatments deserve further study in randomized clinical trials.

By investigating the host signaling pathways connected with pathogenesis, it may be helpful to manipulate these pathways for the benefit of a vaccine or development of better therapeutic approaches. For future investigations, it would be essential to underly genetic variants influencing host responses that contribute toward a cytokine storm during infection. It will help to explain why some individuals, but not others, seem relatively resistant to cytokine storm.

REFERENCES

1. Kash, J. C., Tumpey, T. M., Proll, S. C., Carter, V., Perwitasari, O., Thomas, M. J., Basler, C. F., Palese, P., Taubenberger, J. K., Garcia-Sastre, A., Swayne, D. E., Katze, M. G. (2006). Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. *Nature*. 443(7111):578-81. doi:10.1038/nature05181
2. Maloy, J. K., Salaun, L., Cahill, R., Dougan, G., Saunders, J. N., Powrie, F. (2002). CD4+CD25+ T_R cells suppress innate immune pathology through cytokine-dependent mechanisms. *JEM*. 197(1):111. 10.1084/jem.20021345
3. Teijaro, J. R., Walsh, K. B., Rice, S., Rosen, H., Oldstone, M. B. (2014). Mapping the innate signaling cascade essential for cytokine storm during influenza virus infection. *PNAS*. 111 (10) 3799-3804. <https://doi.org/10.1073/pnas.1400593111>
4. Teijaro, J. R., Walsh, K. B., Cahalan, S., Fremgen, D. M., Roberts, E., Scott, F., et al. (2011). Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. *Cell*. 146:980–991. 10.1016/j.cell.2011.08.015

5. Wilks, A. F., Harpur, G. A., Kurban, R. R., Ralph, J. S., Zürcher, G., Ziemiecki, Z. (1991). Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. *Mol Cell Biol.* 11:2057–2065. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC359893/>
6. Lerner, A. C., David, M., Feldman, G. M., Igarashi, K., Hackett, H. R., Webb, S. D., Sweitzer, M. S., Petricoin, F. E. 3rd., Finbloom, S. D. (1993). Tyrosine phosphorylation of DNA binding proteins by multiple cytokines. *Science.* 261(5129):1730-1733 10.1126/science.8378773
7. Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends in Immunology.* 25(12):677-686 <https://doi.org/10.1016/j.it.2004.09.015>
8. Borden, C. E. (2015). Interferons. *The Molecular Basis of Cancer* 4(52):739-752 <https://doi.org/10.1016/B978-1-4557-4066-6.00053-6>
9. Gately, K. M., Renzetti, M. L., Magram, J., Stern, S. A., Adorini, L., Gubler, U., Presky, H. D. (1998). The Interleukin-12/Interleukin-12-Receptor System: Role in normal and pathologic immune responses. *Annual Review of Immunology.* 16:495-521 <https://doi.org/10.1146/annurev.immunol.16.1.495>
10. Brunda, J. M., Bellantoni, D., Sulich, V. (1987). In vivo anti-tumor activity of combinations of interferon alpha and interleukin-2 in a murine model. Correlation of efficacy with the induction of cytotoxic cells resembling natural killer cells. *International Journal of Cancer.* 40(3):365-371 <https://doi.org/10.1002/ijc.2910400314>
11. Ferrara, J. L., Abhyankar, S., Gilliland, D. G. (1993). Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. *Proc.* 25:1216–1217 [https://doi.org/10.1016/0952-7915\(93\)90139-J](https://doi.org/10.1016/0952-7915(93)90139-J)
12. Yokota, S. (2003). Influenza-associated encephalopathy—pathophysiology and disease mechanisms. *Nippon Rinsho.* 61:1953–1958 <https://europepmc.org/abstract/med/14619437>
13. Beigel, J. H., Farrar, J., Han, A. M., Hayden, F. G., Hyer, R., Jong, de M. D. (2005). Avian influenza A (H5N1) infection in humans. *N Eng J Med.* 353:1374–1385 10.1056/NEJMra052211
14. Muramoto, Y., Shoemaker, J. E., Le, M. Q., Itoh, Y., Tamura, D., Sakai-Tagawa, Y., et al. (2014). Disease severity is associated with differential gene expression at the early and late phases of infection in nonhuman primates infected with different H5N1 highly pathogenic avian influenza viruses. *J Virol.* 88: 8981–8997. 10.1128/JVI.00907-14
15. Dawson, T. C., Beck, M. A., Kuziel, W. A., Henderson, F., Maeda, N. (2000). Contrasting effects of CCR5 and CCR2 deficiency in the pulmonary inflammatory response to influenza A virus. *Am J Pathol.* 156:1951–1959 [https://doi.org/10.1016/S0002-9440\(10\)65068-7](https://doi.org/10.1016/S0002-9440(10)65068-7)
16. Walsh B. K., Teijaro, R. J., Wilker, R. P., Jatzek, A., Fremgen, M. D., Das, C. S., Watanabe, T., Hatta, M., Shinya, K., Suresh, M., Kawaoaka, Y., Rosen, H., Oldstone B. A. M. (2011). Suppression of cytokine storm with a sphingosine analog provides protection against pathogenic influenza virus. *PNAS.* 108(29):12018–12023. <https://doi.org/10.1073/pnas.1107024108>
17. Oldstone, M. B., Teijaro, J. R., Walsh, K. B., Rosen, H. (2013). Dissecting influenza virus pathogenesis uncovers a novel chemical approach to combat the infection. *Virology.* 435:92–101. 10.1016/j.virol.2012.09.039
18. Bassaganya-Riera, J., Song, R., Roberts, P. C., Hontecillas, R. (2010). PPAR-gamma activation as an anti-inflammatory therapy for respiratory virus infections. *Viral Immunol.* 23: 343–352. <https://doi.org/10.1089/vim.2010.0016>
19. Darwish, I., Mubareka, S., Liles, W.C., (2011). Immunomodulatory therapy for severe influenza. *Expert Rev Anti Infect Ther.* 9:807–822. 10.1586/eri.11.56
20. Zheng, B. J., Chan, K. W., Lin, Y. P., Zhao, G. Y., Chan, C., Zhang, H. J. (2008). Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. *Proc Natl Acad Sci USA* 2008; 105: 8091–8096 10.1073/pnas.0711942105
21. Carey, M. A., Bradbury, J. A., Reboloso, Y. D., Graves, J. P., Zeldin, D. C., Germolec, D. R. (2010). Pharmacologic inhibition of COX-1 and COX-2 in influenza A viral infection in mice. *PLoS One.* 5:11610 10.1371/journal.pone.0011610

Approaches to capturing nonlinear dynamics in auditory encoding

Jacob Pennington

INTRODUCTION

Computational modeling is a useful approach for understanding how the brain represents information about sounds. At a broad level, the evoked activity of an auditory neuron can be represented as a function of a time-varying stimulus plus some amount of error due to recording quality, noisy responses, or some downfall of the particular model used¹. A common framework that utilizes this approach is the linear-nonlinear spectro-temporal receptive field (LN STRF) model. This model casts a neuron's sound-evoked activity at each moment in time as the linear weighted sum of the immediately preceding sound spectrogram, followed by nonlinear rectification¹. The LN STRF has been a useful model in part because research has shown that it provides a representation of a neuron's characteristics that is independent of the stimulus presented². In other words, after an STRF has been computed for a neuron, one can use it to predict that neuron's response to a novel stimulus.

Unfortunately, auditory neurons have a frustrating tendency to change their responses to identical sounds based on *sensory context*: the other sounds that came just before the sound of interest³. With these observations in mind, the LN STRF must be an incomplete model since it cannot account for context-dependent encoding or other nonlinear aspects of auditory processing⁴. Several studies have attempted to bridge this gap in modeling capacity by extending the LN STRF to incorporate experimentally-observed biological mechanisms like synaptic depression and contrast-dependent gain control^{3,5}. This review will begin with an overview of the history of the LN STRF model as well as a description of how the model can be implemented in practice. Subsequent sections will discuss two of the proposed methods for extending the LN STRF model. The concluding section will discuss the possibility of efficiently combining multiple these approaches in order to build a single model that captures all of the separate nonlinearities without an unreasonable increase in computational complexity^{6,7}.

THE SPECTRO-TEMPORAL RECEPTIVE FIELD (STRF) MODEL

The core of the STRF model is a matrix of weights, each of which corresponds to the degree of correlation between a neural response and the sound pressure level of a particular frequency band at some time preceding the response^{1,2}. This weighting matrix is then used as the kernel for a convolution or filtering operation applied to the stimulus spectrogram: a representation of a sound in which it is broken up into its component frequencies¹. The result is a prediction of a neuron's instantaneous firing rate, which can then be compared to that neuron's recorded response averaged over many repetitions.

The impetus for the STRF model came from observations of the average stimulus characteristics that preceded action potentials in the auditory midbrain^{2,8}. Some details of STRF implementations are likewise biologically motivated. When constructing a stimulus spectrogram, for example, the frequency bins are often log-spaced to reflect the tonotopic organization in the cochlea¹.

An important improvement to the STRF is the inclusion of an output nonlinearity, which at minimum constrains the firing rate predicted by the STRF to be positive to maintain biological plausibility. Many of the functions used also emulate the effect of thresholding and/or saturating a neuron's response by scaling the predicted firing rate along a sigmoidal curve between a minimum and maximum value¹. When including this output nonlinearity, the model as a whole is often referred to as the linear-nonlinear spectro-temporal receptive field (LN STRF) model¹. This model has been used to describe neural responses in several auditory brain regions and animal models including the primary auditory cortex, anterior auditory field, and auditory thalamus in mice, cats and ferrets^{9,10,11,12}.

EXTENSIONS TO THE LN STRF MODEL

Despite the LN STRF model's success it fails to account for a significant portion of auditory neurons' sound-evoked activity, especially in response to natural sounds^{4,13}. The major shortcoming of the LN STRF model is that it assumes a neuron's response to a particular stimulus to be a static property: no matter how or when a stimulus is presented, the neuron will always respond the same way to the same stimulus. However, real neurons are plastic: repeated stimulation or a noisy environment can make an auditory neuron respond to identical sounds in dramatically different way^{3,5}. Several models have been proposed to explain this plastic behavior, two of which aim to describe short-term plasticity and contrast-dependent gain control.

The short-term plasticity model attempts to emulate synaptic depression or facilitation by scaling each channel of the stimulus spectrogram as though it were passing through a virtual synapses. Several studies from the model's authors have shown a significant increase in model performance when the LN STRF is adapted to account for short-term plasticity^{4,5,7}. One study also found that neurons whose responses were best explained by a model that included depressive synapses enabled the reconstruction of natural sounds at longer latencies, implying that synaptic depression plays an important computational role in auditory cortex⁴.

The model for contrast-dependent gain control does not attempt to recreate a specific mechanism since one has not been definitively identified¹⁴. Instead, this model aims to incorporate a functional observation: that auditory neurons can increase or decrease their sensitivity to stimulation based on the amount of contrast present in recently heard sounds¹⁴. As with the short-term plasticity model, the authors of the contrast-dependent gain control model have demonstrated that it significantly improves the performance of the LN STRF model³. The authors have also shown that the gain control mechanism contributed to auditory neurons' ability to faithfully represent relevant sounds despite noisy environments, a feature that was also described for the short-term plasticity mechanism^{12,15}.

EFFICIENT APPROACHES TO A UNIFIED NONLINEAR MODEL

The models described above certainly represent advances in modeling the computations performed in the auditory cortex. However, there is still plenty of room for improvement. Even after adjusting for recording noise, the models' authors report that on average between forty and sixty percent of the variance present in the neural responses examined was left unexplained^{3,4}.

Still, these models provide important insight into the types of computations that contribute to complex sensory representations in auditory cortex, and they do so while adding minimal computational complexity. This begs the question: since both models are closely related to the LN STRF model and both independently improve performance, could they be combined to improve predictive power even further?

In the case of auditory encoding models, computational complexity can be measured as the number of free parameters that need to be fit for a given model. Low complexity is an important characteristic for any successful model to have since more parameters require more data to fit, and simply increasing the number of parameters quickly yields diminishing returns in terms of model performance⁷. Lower complexity also means less time and money devoted to the process of fitting the models. Accordingly, while simply cramming two unmodified models together may be a reasonable starting point for a new model, an attempt should be made to eliminate redundant parameters.

An important example of parameter reduction was achieved for the LN STRF model, in which it was demonstrated that the weight matrix at the core of the STRF could be split up into much simpler components with minimal loss of predictive power⁷. For example, a weight matrix consisting of 18 spectral channels and 15 temporal bins would normally consist of $18 \times 15 = 270$ parameters. The authors found that a decent approximation to this matrix was to treat the spectral and temporal weights separately for a total of $18 + 15 = 33$ parameters that are sufficient to recover the full matrix. Further, these separate components could then be described by simple functions with even fewer parameters, a method referred to as parameterization⁷. Combining both strategies, the authors showed that they could not only reduce the parameter count for the LN STRF model from 277 to 29, but actually improve prediction accuracy in the process⁷. The same study showed a similar reduction using the short-term plasticity model, and demonstrated that this model benefitted from the simplification even more than the LN STRF model alone⁷.

Another study used a completely different strategy to improve upon the LN STRF, in which the authors recast the encoding model as a combination of several simpler nonlinear layers⁶. The authors of the study found that their multi-layer approach not only performed better than the standard LN STRF, but explained neural responses to some stimuli better than single-layer nonlinear models like short-term plasticity⁶. The results of this study offer further evidence that there are likely several kinds of nonlinear computations that contribute to central processing in the auditory cortex⁶.

Taken together, these results imply that one may be able to unify the advantages of each approach in a single model. Short-term plasticity and contrast-dependent gain control models have helped explain some of the plastic nonlinearities present in auditory responses, but other mechanisms of plasticity mediated by g-proteins or fluctuations in arousal could also be modeled. All of these mechanisms could ideally be efficiently parameterized and treated like distinct pathways, reminiscent of separate ascending circuits. The output of each layer could then be integrated into a final prediction in a manner similar to how cortical neurons summate ascending inputs. Whether successful or not, some attempt to combine the advantages of both novel and existing nonlinear models is needed if the massive gap in explained variance for auditory encoding models is ever to be bridged. Every step toward better explaining auditory neurons' responses is a step toward understanding the auditory system in general, which in turn could inform the design of better cochlear implants or the development of novel audio-neural prostheses.

REFERENCES

1. David, S. (2018). Incorporating behavioral and sensory context into spectro-temporal models of auditory encoding. *Hearing Research*, 360: 107-123. doi: 10.1016/j.heares.2017.12.021.
2. Aertsen, A. and Johannesma, P. (1981). The Spectro-Temporal Receptive Field: A Functional Characteristic of Auditory Neurons. *Biological Cybernetics*, 42: 133-143.
3. Rabinowitz, N., Willmore, B., Schnupp, J., and King, A. (2012). Spectrotemporal Contrast Kernels for Neurons in Primary Auditory Cortex. *The Journal of Neuroscience*, 32(33):11271-11284. doi: 10.1523/JNEUROSCI.1715-12.2012.
4. David, S. and Shamma, S. (2013). Integration over Multiple Timescales in Primary Auditory Cortex. *The Journal of Neuroscience*, 33(49): 19154-19166. doi: 10.1523/JNEUROSCI.2270-13.2013.
5. David, S., Mesgarani, N., Fritz, J., and Shamma, S. (2009). Rapid Synaptic Depression Explains Nonlinear Modulation of Spectro-Temporal Tuning in Primary Auditory Cortex by Natural Stimuli. *The Journal of Neuroscience*, 29(11): 3374-3386. doi: 10.1523/JNEUROSCI.5249-08.2009.
6. Deneux, T., Kempf, A., Daret, A., Ponsot, E., and Bathellier, B. (2016). Temporal asymmetries in auditory coding and perception reflect multi-layered nonlinearities. *Nature Communications*. doi: 10.1038/ncomms12682.
7. Thorson, I., Liénard, J., and David, S. (2015). The Essential Complexity of Auditory Receptive Fields. *PLOS Computational Biology*, 11(12): e1004628. doi: 10.1371/journal.pcbi.1004628.
8. Aertsen, A. and Johannesma, P. (1981). A Comparison of the Spectro-Temporal Sensitivity of Auditory Neurons to Tonal and Natural Stimuli. *Biological Cybernetics*, 42: 145-156.
9. Gourevitch, B., Norena, A., Shaw, G., and Eggermont, J. (2009). Spectrotemporal Receptive Fields in Anesthetized Cat Primary Auditory Cortex Are Context Dependent. *Cerebral Cortex*, 19: 1448-1461. doi: 10.1093/cercor/bhn184.
10. Linden, J., Liu, R., Sahani, M., Schreiner, C., Merzenich, M. (2003). Spectrotemporal Structure of Receptive Fields in Areas AI and AAF of Mouse Auditory Cortex. *Journal of Neurophysiology*, 90: 2660-2675. doi: 10.1152/jn.00751.2002.
11. Miller, L., Escabi, M., Read, H., and Schreiner, C. (2002). Spectrotemporal Receptive Fields in the Lemniscal Auditory Thalamus and Cortex. *Journal of Neurophysiology*, 87: 516-527. doi: 10.1152/jn.00395.2001.
12. Rabinowitz, N., Willmore, B., King, A., Schnupp, J. (2013). Constructing Noise-Invariant Representations of Sound in the Auditory Pathway. *PLOS Biology*, 11(11): e1001710. doi:10.1371/journal.pbio.1001710.
13. Laudanski, J., Edeline, J., and Huetz, C. (2012). Differences Between Spectro-Temporal Receptive Fields Derived from Artificial and Natural Stimuli in the Auditory Cortex. *PLOS ONE*, 7(11): e50539. doi: 10.1371/journal.pone.0050539.
14. Rabinowitz, N., Willmore, B., Schnupp, J., and King, A. (2011). Contrast Gain Control in Auditory Cortex. *Neuron*, 70(6): 1178-1191. doi: 10.1016/j.neuron.2011.04.030.
15. Mesgarani, N., David, S., Fritz, J., and Shamma, S. (2014). Mechanisms of noise robust representation of speech in primary auditory cortex. *PNAS*, 111(18): 6792-6797. doi: 10.1073/pnas.1318017111.

Perineuronal net modulation of parvalbumin interneurons and the excitatory/inhibitory balance

Jonathan Anguiano

INTRODUCTION

The cerebral cortex is composed of many distinct cell types each operating in their own intricate manner to perform a specific function within a circuit. When investigating the cortical circuitry by which cell types communicate and influence behavior, it is useful to simplify the circuit in terms of an excitatory/inhibitory balance. Contributing to this excitatory/inhibitory balance are glutamatergic principal neurons and γ -aminobutyric acid (GABA)-ergic interneurons. Within the cortex, glutamatergic neurons and GABAergic interneurons account for approximately 80-90% and 10-20% of the neuronal population, respectively¹. GABAergic interneurons are indeed a minority, however, their ability to modulate neuronal circuitry is not to be understated. GABAergic interneurons provide potent inhibition of glutamatergic neurons, with a single interneuron capable of inhibiting >50% of principle neurons in a roughly 100 μ m region². GABAergic interneurons may also provide inhibitory input onto other GABAergic interneurons¹. One GABAergic interneuron, the fast spiking parvalbumin-positive (PV⁺) interneuron, has been the focus of many studies due to the ease by which it may be distinguished and targeted for manipulation¹. PV⁺ interneurons regulate the output of prefrontal cortex pyramidal neurons³. Interestingly, roughly 60-80% of PV⁺ cells in the cortex are surround by perineuronal nets (PNNs)⁴. PNNs are an extracellular matrix structure wrapping around the somas and proximal dendrites of neurons in the brain and spinal cord. Research on PNNs has revealed them to be important regulators of neuronal plasticity and involved in learning and memory^{5,6}. Additionally, PNNs may act to regulate the activity of PV⁺ interneurons⁵. Understanding the ability of PNNs to modulate PV⁺ interneuron function is important as many disorders, such as schizophrenia, are characterized by diminished PV⁺ interneuron activity³. Thus, this review synthesizes research on PV⁺ interneurons, PNNs and the interplay between the two in maintaining the excitatory/inhibitory balance in the cortex.

PV⁺ INTERNEURON MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL PROPERTIES FACILITATE POTENT INHIBITION

While PV⁺ interneurons are the most abundant interneuron in the neocortex⁷, their prevalence in terms of the total neuronal population is notably low. The minority stake of PV⁺ interneurons in the cortex requires them to be efficient and reliable modulators of neuronal activity. The morphological properties of PV⁺ interneurons allow for efficient processing and transfer of information to target cells. PV⁺ interneuron dendrites are of considerable length and spread, allowing them to incorporate input from a variety of sources¹. Additional evidence for the ability of PV⁺ interneurons to integrate information comes from a high density of synaptic input, with an average of 16,294 synapses found on hippocampal CA1 PV⁺ interneurons⁸. In consideration of relative dendritic

length, the synaptic input onto CA1 PV⁺ interneurons rivals that of CA1 pyramidal neurons, which contain an average of 32,000 synapses⁹. PV⁺ interneurons are generally classified into two distinct subgroups, basket cells and chandelier cells, both of which innervate areas near action potential initiation sites in the target cell. Basket cell axons target the somata of principal neurons while chandelier cells target the axon initial segment of principal neurons¹. This axon morphology equips PV⁺ interneurons with the ability to strongly inhibit target cells. The reliable, quick, and temporally precise electrophysiology of PV⁺ interneurons further enables potent inhibition⁷. For example, the high density of Kv3-type potassium channels found in PV⁺ interneuron dendrites ensures tight coupling of excitatory post synaptic potentials to action potential firing in the interneuron¹⁰. Action potentials are initiated in the axon initial segment for PV⁺ interneurons¹¹ and propagate with rare failure and relatively high speeds¹. Further contributing to PV⁺ interneuron function are the PNNs that surround the majority of PV⁺ interneurons and serve as a structural and biochemical support system.

COMPOSITION AND FUNCTIONAL PROPERTIES OF PERINEURONAL NETS

PNNs were first described by Camillo Golgi in the 1890s as a reticular structure enveloping cells and their branches¹². The structure and function of PNNs has been greatly expanded upon since this original description. The basic components of PNNs are hyaluronan, proteoglycans, and link proteins. Hyaluronan acts as the structural backbone, allowing for the attachment of proteoglycans¹³. The majority of proteoglycans composing PNNs are chondroitin sulfate proteoglycans (CSPGs) including aggrecan, brevican, versican, and neurocan⁵. CSPGs and the hyaluronan backbone are joined via hyaluronan and proteoglycan link proteins while CSPGs are linked by tenascin-R⁵. In general, PNNs regulate plasticity by acting as physical barrier, regulating new synaptic contacts through binding of specific molecules such as chemorepulsive molecules, and limiting the lateral mobility of synaptic proteins⁵.

IMPACT OF PERINEURONAL NET AND PV⁺ INTERNEURON INTERACTION ON CORTICAL EXCITATORY/INHIBITORY BALANCE

The cortex houses intricate networks of neurons through which sensory information is processed for behavioral output. Processing information of such magnitude requires great computational power. GABAergic interneurons endow the cortex with this necessary computational flexibility via inhibition of excitatory signaling³. This regulation by inhibitory interneurons is important as there exists a balance of excitatory and inhibitory responses to a given neuronal event¹⁴. For example, rodent models of epilepsy are marked by loss of interneurons and inhibitory synapses¹⁵. In PV⁺ interneurons, there is a shift in the excitatory/inhibitory balance that is dependent upon experience. Specifically, Donato et al. (2016) found that environmental enrichment promoted low excitatory-to-inhibitory synaptic density ratios while fear conditioning promoted high excitatory-to-inhibitory synaptic density ratios on PV⁺ interneurons¹⁶. Could these changes in PV⁺ interneurons be the result of interactions with PNNs? It is apparent that changes in PV⁺ interneurons are often accompanied by changes in PNNs. Balmer et al. (2009) reported a correlation between the percentage of PV⁺ interneurons surrounded by PNNs and the stage of song learning in zebra finches. Deprivation of song learning decreased both PV and PNN staining intensity¹⁷, indicating a correlation between PV and PNNs in learning. A codependence of PV and PNNs is supported by research on cocaine-induced modulation of PV and PNNs, in which changes in PV intensity generally follow changes in PNN intensity¹⁸. Additionally, cocaine exposure modulated the excitatory/inhibitory balance via increases in inhibitory synapses onto PV⁺ interneurons, which

may have corresponded to observed decreases in PNN staining¹⁸. This is consistent with the ability of PNNs to restrict new synapse formation¹⁹.

The ability of PNNs and PV+ interneurons to impact the excitatory/inhibitory balance has been further elucidated by research using the PNN degrading enzyme, chondroitinase ABC (Ch-ABC). Treating medial prefrontal cortex tissue with Ch-ABC reduces miniature-inhibitory postsynaptic currents (mIPSCs) onto pyramidal neurons⁴. Because PNNs are highly colocalized with PV+ interneurons⁴, the decrease in mIPSCs on pyramidal neurons may be due to changes in the function of PV+ interneurons. In concordance with this hypothesis, recent work has shown that cortical PV+ interneurons exhibit decreased excitability in response to Ch-ABC treatment²⁰. Balmer (2016) further proposed that PNNs act to enhance synaptic inhibition. Interestingly, deletion of brevican, a structural component of PNNs, increased excitability of PV+ interneurons in the hippocampus²¹. Differences in these findings may be due to a brevican-specific roll in modulating PV+ function as PV+ interneurons expressing brevican are less excitable than PV+ interneurons not expressing brevican²¹. The opposing findings may also arise from the different brain regions studied (cortex versus hippocampus).

PERINEURONAL NETS AND PV+ INTERNEURONS: FUTURE DIRECTIONS

The morphological and electrophysiological properties of PV+ interneurons enable the integration of information so that potent inhibition may be applied to target cells. PNNs possess the structural and functional properties necessary to modulate the PV+ interneurons they surround and impact the flow of information throughout the cortex. It is indeed evident that PNNs and PV+ interneurons interact to shape the excitatory/inhibitory balance. Modulation of PNNs changes the inhibitory and excitatory synaptic inputs as well as the intrinsic electrophysiological properties of PV+ interneurons. Much of the research on PNNs and PV+ interneurons centers around external manipulation of healthy animals. However, it would be worthwhile to study PNN and PV+ interactions in various animal disease/disorder models so that the PNN and PV+ system may be targeted for treatment. For example, in a rodent model of addiction, Ch-ABC treated animals displayed decreased cocaine-seeking behavior⁴. Furthermore, Alzheimer's disease, schizophrenia, and autism share a commonality in that they are all associated with abnormalities in PNNs and PV+ interneurons^{1,13}. Future research should seek to understand how modulation of PNNs or PV+ interneurons, and therefore the excitatory/inhibitory balance, may reverse or attenuate characteristics of the aforementioned diseases/disorders.

REFERENCES

1. Hu, H., Gan, J., & Jonas, P. (2014). Fast-spiking, parvalbumin GABAergic interneurons: From cellular design to microcircuit function. *Science* 345:1255263-1255263. doi:10.1126/science.1255263
2. Isaacson, J. S., & Scanziani, M. (2011). How inhibition shapes cortical activity. *Neuron* 72:231-43.
3. Ferguson, B. R., & Gao, W. J. (2018). PV Interneurons: Critical Regulators of E/I Balance for Prefrontal Cortex-Dependent Behavior and Psychiatric Disorders. *Frontiers in neural circuits* 12:37. doi:10.3389/fncir.2018.00037
4. Slaker, M., Churchill, L., Todd, R. P., Blacktop, J. M., Zuloaga, D. G., Raber, J., . . . Sorg, B. A. (2015). Removal of Perineuronal Nets in the Medial Prefrontal Cortex Impairs the Acquisition and Reconsolidation of a Cocaine-Induced Conditioned Place Preference Memory. *Journal of Neuroscience* 35:4190-4202. doi:10.1523/jneurosci.3592-14.2015
5. van't Spijker, H. M., & Kwok, J. (2017). A Sweet Talk: The Molecular Systems of Perineuronal Nets in Controlling Neuronal Communication. *Frontiers in integrative neuroscience*, 11, 33. doi:10.3389/fnint.2017.00033
6. Romberg, C., Yang, S., Melani, R., Andrews, M. R., Horner, A. E., Spillantini, M. G., Bussey, T. J., Fawcett, J. W., Pizzorusso, T., . . . Saksida, L. M. (2013). Depletion of perineuronal nets enhances recognition memory and long-term depression in the perirhinal cortex. *The Journal of Neuroscience* 33:7057-65.
7. Tremblay, R., Lee, S., & Rudy, B. (2016). GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. *Neuron*,91(2), 260-292. doi:10.1016/j.neuron.2016.06.033

8. Gulyás, A. I., Megias, M., Emri, Z., & Freund, T. F. (1999). Total Number and Ratio of Excitatory and Inhibitory Synapses Converging onto Single Interneurons of Different Types in the CA1 Area of the Rat Hippocampus. *The Journal of Neuroscience*, *19*(22), 10082-10097. doi:10.1523/jneurosci.19-22-10082.1999
9. Megias, M., Emri, Z., Freund, T., & Gulyás, A. (2001). Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience*, *102*(3), 527-540. doi:10.1016/s0306-4522(00)00496-6
10. Hu, H., Martina, M., & Jonas, P. (2010). Dendritic Mechanisms Underlying Rapid Synaptic Activation of Fast-Spiking Hippocampal Interneurons. *Science*, *327*(5961), 52-58. doi:10.1126/science.1177876
11. Li, T., Tian, C., Scalmani, P., Frassoni, C., Mantegazza, M., Wang, Y., . . . Shu, Y. (2014). Action Potential Initiation in Neocortical Inhibitory Interneurons. *PLoS Biology*, *12*(9). doi:10.1371/journal.pbio.1001944
12. Spreafico, R., Biasi, S. D., & Vitellaro-Zuccarello, L. (1999). The Perineuronal Net: A Weapon for a Challenge. *Journal of the History of the Neurosciences*, *8*(2), 179-185. doi:10.1076/jhin.8.2.179.1834
13. Wen, T. H., Binder, D. K., Ethell, I. M., & Razak, K. A. (2018). The Perineuronal 'Safety' Net? Perineuronal Net Abnormalities in Neurological Disorders. *Frontiers in Molecular Neuroscience*, *11*. doi:10.3389/fnmol.2018.00270
14. Okun, M., & Lampl, I. (2009). Balance of excitation and inhibition. *Scholarpedia*, *4*(8):7467
15. Kumar, S. S., & Buckmaster, P. S. (2006). Hyperexcitability, Interneurons, and Loss of GABAergic Synapses in Entorhinal Cortex in a Model of Temporal Lobe Epilepsy. *Journal of Neuroscience*, *26*(17), 4613-4623. doi:10.1523/jneurosci.0064-06.2006
16. Donato F, Rompani SB, Caroni P (2013) Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* 504:272–276.
17. Balmer, T. S., Carels, V. M., Frisch, J. L., & Nick, T. A. (2009). Modulation of perineuronal nets and parvalbumin with developmental song learning. *The Journal of Neuroscience* 29:12878-85.
18. Slaker, M. L., Jorgensen, E. T., Hegarty, D. M., Liu, X., Kong, Y., Zhang, F., . . . Sorg, B. A. (2018). Cocaine Exposure Modulates Perineuronal Nets and Synaptic Excitability of Fast-Spiking Interneurons in the Medial Prefrontal Cortex. *ENEURO* 5. doi:10.1523/eneuro.0221-18.2018
19. de Winter, F., Kwok, J. C., Fawcett, J. W., Vo, T. T., Carulli, D., & Verhaagen, J. (2016). The Chemorepulsive Protein Semaphorin 3A and Perineuronal Net-Mediated Plasticity. *Neural plasticity*, *2016*, 3679545. doi:10.1155/2016/3679545
20. Balmer, T. S. (2016). Perineuronal Nets Enhance the Excitability of Fast-Spiking Neurons. *ENEURO*, *3*(4). doi:10.1523/eneuro.0112-16.2016
21. Favuzzi E, Marques-Smith A, Deogracias R, Winterflood CM, Sánchez-Aguilera A, Mantoan L, Maeso P, Fernandes C, Ewers H, Rico B (2017) Activity-dependent gating of parvalbumin interneuron function by the perineuronal net protein brevicin. *Neuron* 95:639–655.e10.

A review on one mechanism of aminoglycoside-induced ototoxicity and mitochondria specific treatments

Jordan Donaldson

INTRODUCTION

Sensorineural hearing loss is defined as a decreased ability to hear due to ototoxic drugs, aging, genetic mutations or excessive noise exposure¹. Specifically, ototoxic drugs are medications such as aminoglycosides (AG), which damage mechanosensory hair cells of the inner ear resulting in hearing deficits or vestibular dysfunction due to the inability to regenerate hair cells in mammals². Therefore, ototoxic damage from AG is irreversible. Ototoxic AG are bactericidal agents commonly used against aerobic gram-negative bacteria associated with diseases such as cystic fibrosis, tuberculosis, bacteremia, and endocarditis^{3,4}. Because benefits of treatment outweigh risks of AG treatment for these severe illnesses, people are often left to suffer from sensorineural hearing loss without any treatments to reverse these effects. AG-induced hearing loss affects 20-30% of people treated with this type of antibiotic and equates to nearly \$350,000 in socioeconomic burdens over the person's lifetime^{4,5,6}.

Fortunately, there have been notable findings from a developing body of new research evaluating mechanisms of AG-induced hearing loss. However, there are still many details not yet discovered about AG-induced ototoxicity. Researchers have hypothesized that intracellular accumulation of cytotoxic levels of calcium and reactive oxygen species (ROS) after AG exposure eventually leads to death of hair cells⁷. ROS are natural biologically harmful products derived from molecular oxygen during production of ATP within cells⁸. Attempts at attenuating cytotoxic levels of ROS with nonspecific ROS scavengers have been somewhat successful and currently there are clinical trials underway with mixed results⁹. Research continues to uncover additional mechanistic details of AG-induced hearing loss in hopes of developing a more effective treatment for a wider array of AG.

Research has uncovered that in bacteria, AG disrupt the citric acid cycle and electron transport chain in mitochondria causing cytotoxic levels of ROS¹⁰. Disruption of mitochondrial metabolism leads to necrosis and cell death, making AG an effective antimicrobial treatment. In mammals, AG generally act by inhibiting protein translation, binding to the endoplasmic reticulum (ER), disrupting mitochondrial metabolism, and signaling apoptotic pathways^{11,12}. This is biologically consistent with a large body of evidence showing mitochondria produce large amounts of ROS during metabolic activity to produce ATP for cells⁸. Therefore, it is hypothesized that the primary location for ROS accumulation after AG exposure is within mitochondria of mammals⁹. Further investigations have shown that there is a dose-dependent relationship between rapid calcium flux from ER to mitochondria after AG administration¹³. This finding led to the discovery that elevated

mitochondrial Ca_2^+ is necessary for cytotoxic levels of ROS accumulation during neomycin-induced ototoxicity¹². Studies with mitochondrial-specific antioxidants provide further evidence that preventing oxidative changes in mitochondria protect hair cells from AG damage^{12,14,15,16}. This paper will review current findings on mechanisms of AG-induced hearing loss in more detail. Additionally, I hypothesize new treatments which specifically target cytotoxic mitochondrial ROS accumulation will exhibit increased protection against AG in comparison to general ROS scavengers.

ENTRY OF AG THROUGH THE MET CHANNELS OF HAIR CELLS

Hair cells consists of a basal cell body with apical stereocilia which contain non-selective mechanotransduction channels (MET) on stereocilia tips¹⁷ (Figure 1). Each stereocilia tip is physically connected to each other with tip links, proteins that synchronize stereocilia deflection by direct tethering¹⁷. AG primarily enter through non-selective MET channels¹⁸ (Figure 1). Under physiological conditions, MET channels influx potassium causing a chemical depolarization and signal propagation along afferent nerves. Once AG enter the apical stereocilia of hair cells, they diffuse to the basal portion of hair cells¹⁹ (Figure 1). AG entry causes disruption of cellular processes such as ribosomal dysfunction, ER dysfunction, binding to apoptotic signal proteins such as CLIMP-63 and toxic mitochondrial ROS generation^{20,21}. Of specific focus to this review are ER and mitochondrial dysfunctions which have been found to cause ROS accumulation and apoptosis through calcium-dependent transduction.

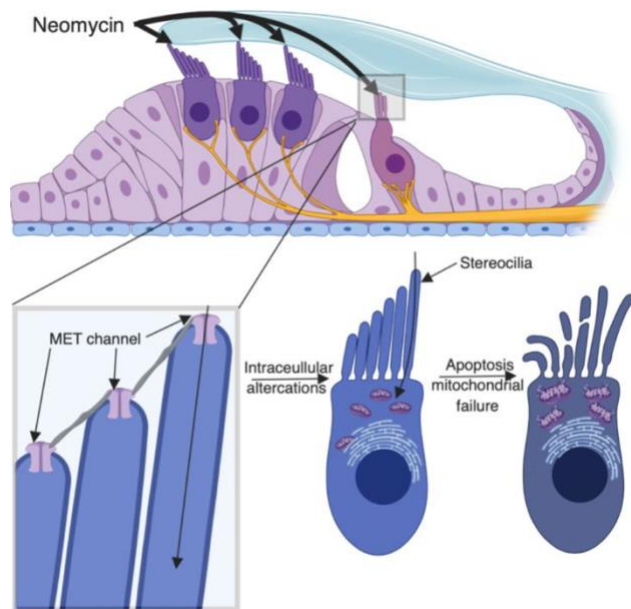


Figure 1. Neomycin entry into hair cells of *Homo sapiens* through MET channel. Neomycin entry induces intracellular dysfunction and subsequent apoptosis. MET = mechanotransduction channel.

EFFECTS OF INTRACELLULAR NEOMYCIN ON MITOCHONDRIAL LEVELS OF CALCIUM

Researchers reported several intracellular hallmarks after AG entry to hair cells. Accumulation of cytotoxic levels of ROS have been observed just prior to cell death²². Attempted attenuation of AG induced hearing loss with antioxidants has produced less than optimal results for a wide range of antibiotics^{14,15,16}. Further characterization of cellular events after AG entry has revealed mobilization of calcium from ER to mitochondria which is facilitated through activation of inositol trisphosphate receptors (IP3R) by IP3^{11,12,22} (Figure 2). In proximal tubules of nephrons, aminoglycosides have an agonistic effect on IP3 receptors (IP3R)²³. Therefore, I hypothesize that AG also act as an agonist on IP3R in hair cells. Previous studies have identified physically close approximation of ER to mitochondria¹³. Approximation of ER and mitochondria have been recognized for roles in bioenergetic signaling, cytotoxic events, and apoptotic signaling¹¹.

Researchers have found that differentiation between apoptotic or bioenergetic signals is due to dose-dependent releases of calcium. Large releases of calcium from ER have been shown to induce apoptotic pathways (Figure 2), whereas low levels of calcium mobility have been observed to increase metabolism within mitochondria²⁴. Calcium release is largely regulated by the phosphorylation of IP3R by protein kinase C (PKC) and calmodulin-dependent protein kinase II (CaMKII) which is activated by large cytosolic calcium levels^{25,26} (Figure 2). Under physiological conditions, PKC/CaMKII regulation of calcium release would prevent cytotoxic calcium accumulation. However, upon AG entry, agonistic effects of AG act too rapidly for regulation by PKC and CaMKII on IP3R (Figure 2). Cytotoxic calcium efflux from ER increases both cytosolic calcium and subsequent mitochondrial calcium levels²² (Figure 2). Therefore, understanding how accumulation of calcium can induce apoptotic pathways may lead to better development for treatments preventing AG-induced ototoxicity.

APOPTOSIS DUE TO EXCESSIVE MITOCHONDRIAL CALCIUM ACCUMULATION: AN IRREVERSIBLE FATE

Upon calcium efflux from ER, calcium travels to voltage-dependent anion channels (VDAC) on the outer mitochondrial membrane (OMM) (Figure 2). VDAC is one of the most abundant OMM proteins and is a high-conductance weakly selective anion channel which prefers cation flux at lower conductances²⁷. Thus, the selectivity of VDAC implies that when it is in a “closed” state, calcium and other cations are able to freely flow across VDAC^{11,27}. This hypothesis of variable anion/cation selectivity was further supported in a study which overexpressed VDAC, resulting in increased calcium uptake across the OMM²⁸. Upon large ER calcium efflux, VDAC creates a bottleneck of calcium micro-domains allowing for large amounts of calcium to pass the OMM²⁷ (Figure 2).

Under physiological conditions, another OMM protein, Na⁺/3Ca²⁺ antiporter (commonly reported as the sodium/calcium exchanger) acts to restore the concentration of calcium within mitochondria through exchanges of one sodium cation for every three calcium ions^{29,30} (Figure 2). However, calcium ATPases are unable to adjust intracellular calcium levels upon rapid and sustained calcium influx (Figure 2). This leads to mitochondrial calcium overload³⁰. Beyond entry of calcium through the OMM, transduction across the inner mitochondrial membrane (IMM) is less characterized¹¹. Fortunately, Rizzuto and colleagues discovered an additional transmembrane protein on the IMM which may further explain AG-induced toxicity.

Studies have identified a mitochondrial inner membrane uniporter (MCU), MCU preferentially transduces calcium into mitochondrial matrices where it can act by increasing mitochondrial metabolism under physiological conditions¹¹ (Figure 2; Supplementary Figure B). Large quantities of calcium inhibit transduction^{11,31}. However, lower levels of calcium can increase transport probability of MCU through phosphorylation by CaMKII^{11,31}. One study reported that uptake induction occurs with a time constant of 6 seconds, whereas inactivation occurs at 17 seconds³². They suggested that biphasic regulation allows for oscillations of calcium flux but prevents cytotoxic calcium accumulation in mitochondrial matrices when there are prolonged periods of calcium in the cytosol³² (Figure 2). Given that MCU are regulated and prevent excessive accumulation of calcium in the matrices, further studies investigated if other calcium transducers existed on the IMM, as this may be a protein responsible for rapid calcium accumulation.

Ruthenium Red 360 (RuR360) is a fluorescent, non-selective IMM channel blocker^{31,33} (Figure 2). RuR360 was used to both identify and inhibit MCU^{31,33} (Figure 2). Robert Esterburg and colleagues (2014) have shown that indeed RuR360 is otoprotective after exposure to AG.

However, RuR360 is not suitable as a clinically effective treatment because it inhibits total calcium flux into to mitochondrial matrices in all cells which causes necrosis due to an inability to produce ATP^{11,31,33}. Continued investigation of the IMM by Genevieve Sparagna defined another mode of calcium entry to the IMM described as rapid mode of uptake (RaM). RaM was found to allow very rapid pulsing of calcium into the mitochondrial matrix. Although there are not more recent studies further characterizing RaM, studies have reported that RaM functions physiologically similar to MCU. This leaves the question of whether RaM is a different functional state of MCU not yet identified or a separate protein entity¹¹. Regardless, Rizzuto hypothesized that rapid flux of calcium may create microdomains of calcium within the matrix where processes such as the tricarboxylic acid cycle (TCA) cycle can be upregulated¹¹ (Supplementary Figure B).

McCormack and his colleagues extensively studied how calcium acts as a modulator of the TCA cycle and reported that calcium affects pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and isocitrate dehydrogenase, collectively known as the calcium sensitive mitochondrial dehydrogenases (CSMDHs)³⁴ (Supplementary Figure B). These findings intrigued Laurence and his colleagues to investigate how calcium affects ATP production. They reported that CSMDHs increased the electrons available to generate a proton gradient via complexes I and III in oxidative phosphorylation (OxPhos) resulting in an increase in ATP production³⁵ (Supplementary Figure A). Unfortunately, with excessive levels of calcium, this pathway is on overdrive and rapidly accumulates ROS from complexes I and III (Supplementary Figure A). Together, calcium-induced disruption of the membrane potential results in the classically observed organelle swelling^{12,22} and ROS accumulation leading to recruitment of apoptotic factors such as Bax. Bax has been shown to activate the permeability transition pore (PTP) which classically effluxes cytochrome C (cyto C) into the cytosol³⁶ (Figure 2). Accumulation of cytosolic cyto C activates the cyto C-dependent caspase signaling pathway³⁷ (Figure 2). Caspases are cysteine-dependent/independent proteases which participate in the cascade of events signaling apoptosis³⁷. Activation of caspases specifically leads to apoptosis through recruitment of P53 mediated apoptosis³⁸ (Figure 2). P53 is another apoptotic signaling molecule associated with AG-induced ototoxicity³⁹. If the lack of ATP does not yet cause cell necrosis, activation of these pathways ensures apoptosis, the inevitable cell fate after AG-induced ototoxicity.

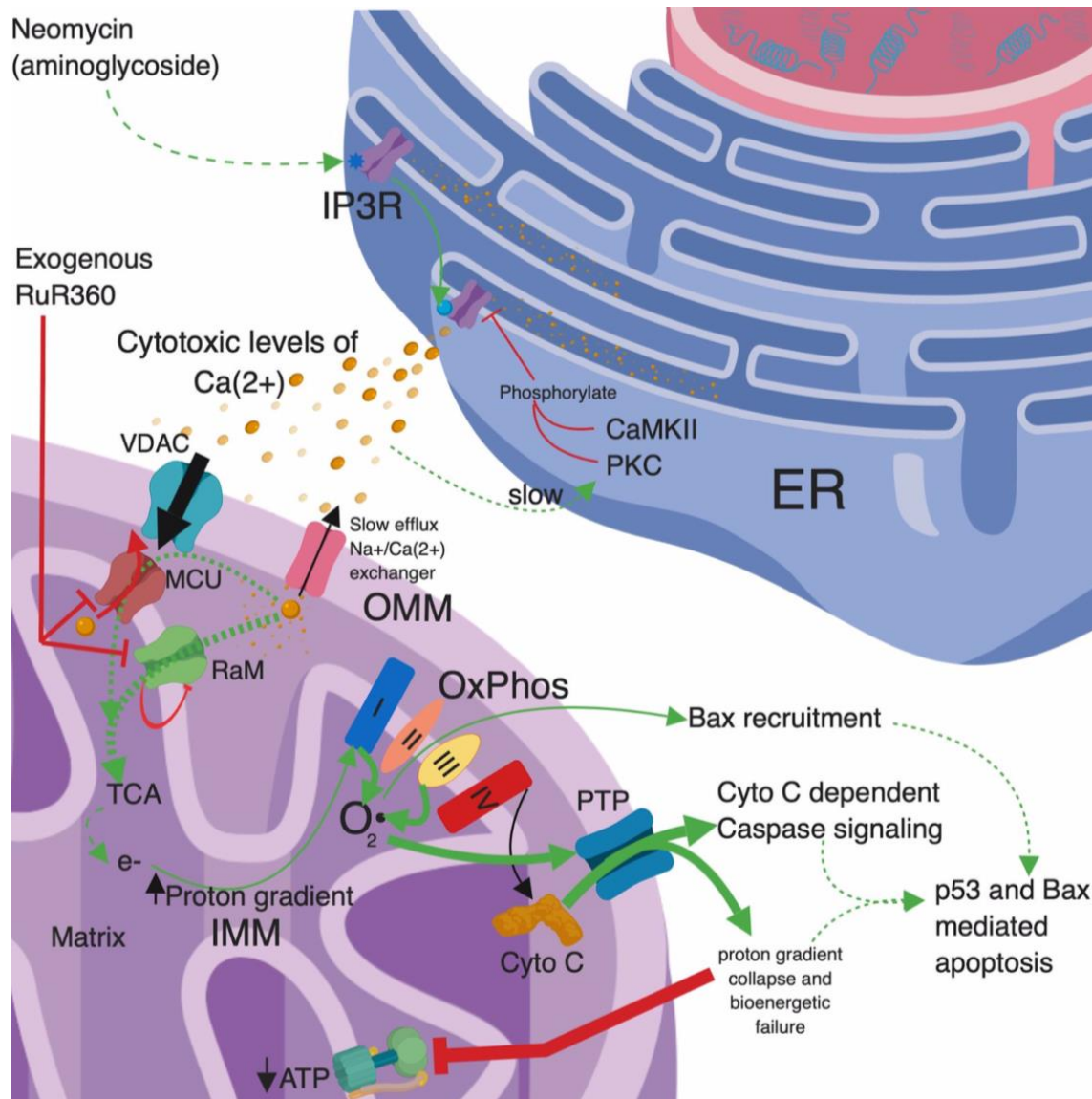


Figure 2. Current proposed mechanism of neomycin-induced toxicity on mitochondrial function. Neomycin likely acts as an agonist on the IP₃R causing a prolonged release of cytototoxic calcium levels onto the OMM. Calcium is readily transduced into the intramitochondrial space through VDAC. The Na⁺/Ca²⁺ exchanger slowly restores homeostatic ion levels across the membrane. Calcium is further transduced through the MCU with biphasic oscillatory regulations by calcium and CaMKII (not shown). Calcium is possibly transduced across the less characterized RaM in rapid, short bursting intervals. Exogenous RuR360 inhibits the actions of MCU and RaM. Cytotoxic calcium accumulation results in mitochondrial swelling just prior to cell death. Microdomains in the mitochondrial matrix enhance electron production in the TCA cycle. Shuttling of electrons to the IMM occurs where a proton gradient is generated, increasing the rate of ATP production and consequently, ROS accumulation within the matrix. Excessive ROS accumulation recruits Bax, an apoptotic factor, leading to apoptosis. Cytotoxic levels of calcium in the matrix and severe ROS accumulation signal the PTP to release Cyto C into the cytosol. Regular oscillation of calcium under physiological conditions does not activate PTP. The apoptotic Cyto C dependent caspase signaling is activated, leading to p53 dependent apoptosis. Additionally, upon PTP activation, the proton gradient is collapsed causing ATP to no longer be produced. The lack of ATP additionally causes cell necrosis. Refer to supplementary figures 1 and 2 for abbreviations and additional mechanistic details of oxidative phosphorylation (OxPhos) and how calcium affects the TCA cycle.

DEVELOPING TARGETED MITOCHONDRIAL TREATMENTS: THE FUTURE OF OTOTOXIC BIOMEDICAL RESEARCH

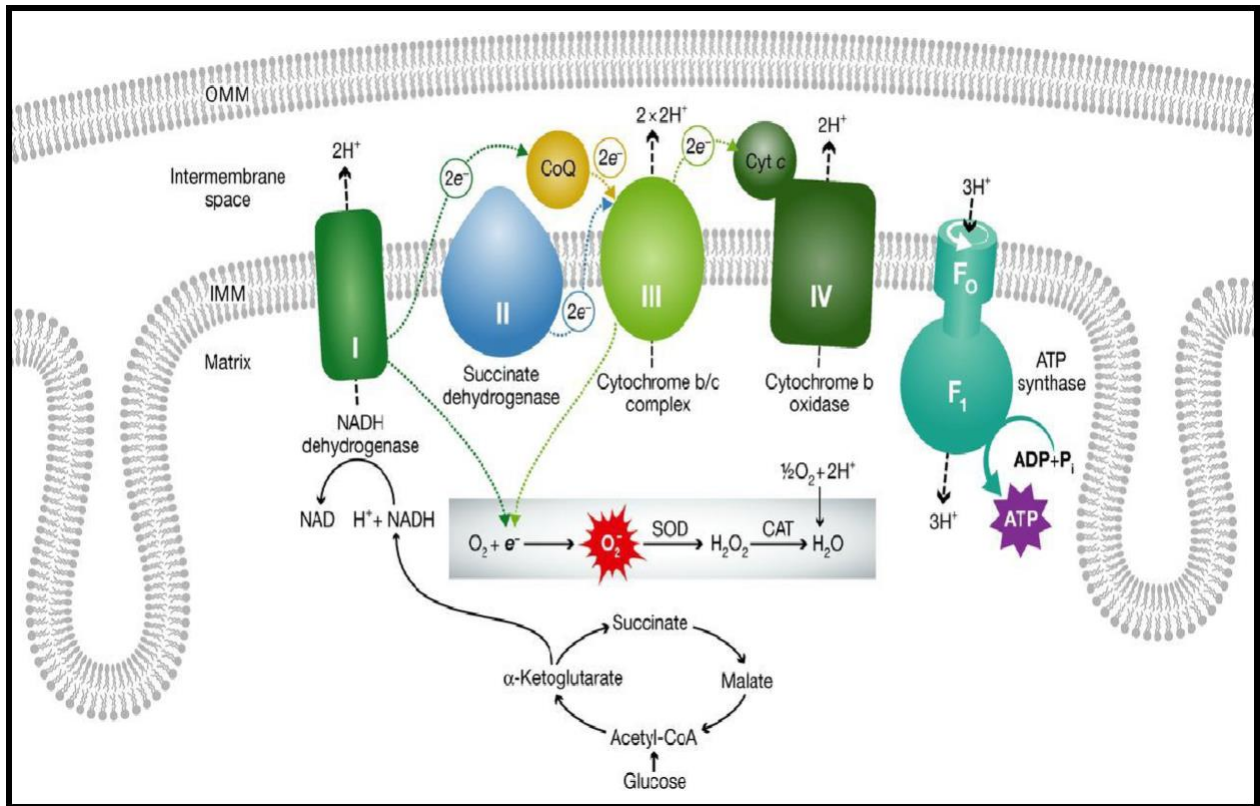
Characterization of AG-induced mitochondrial disruption allows a new avenue for treatments. The mechanism of AG-induced ototoxicity explained in Figure 2 exposes many new targets including mitochondrial-specific ROS scavengers. One of the largest hurdles in developing therapies for AG-induced ototoxicity is creating a treatment which targets hair cells specifically without harmful offsite effects. Mitochondria are essential to cell survival, therefore, biomedical researchers must develop a treatment which does not cause long term depression of mitochondrial activity and acutely blocks AG ototoxic effects. Additionally, understanding other mechanisms of AG-induced ototoxicity may allow for development of comprehensive treatments which completely attenuate ototoxic effects of AG. Studies which used general cytosolic ROS scavengers, although somewhat successful, have not been able to effectively eliminate the ototoxic effects of AG induced hearing loss, likely due to the inability to deliver the ROS scavengers to the mitochondria (Jauslin et al., 2003; Esterberg 2016). With the development of mitochondrial-specific treatments, more effective attenuation of AG induced hearing loss may finally be realized.

REFERENCES

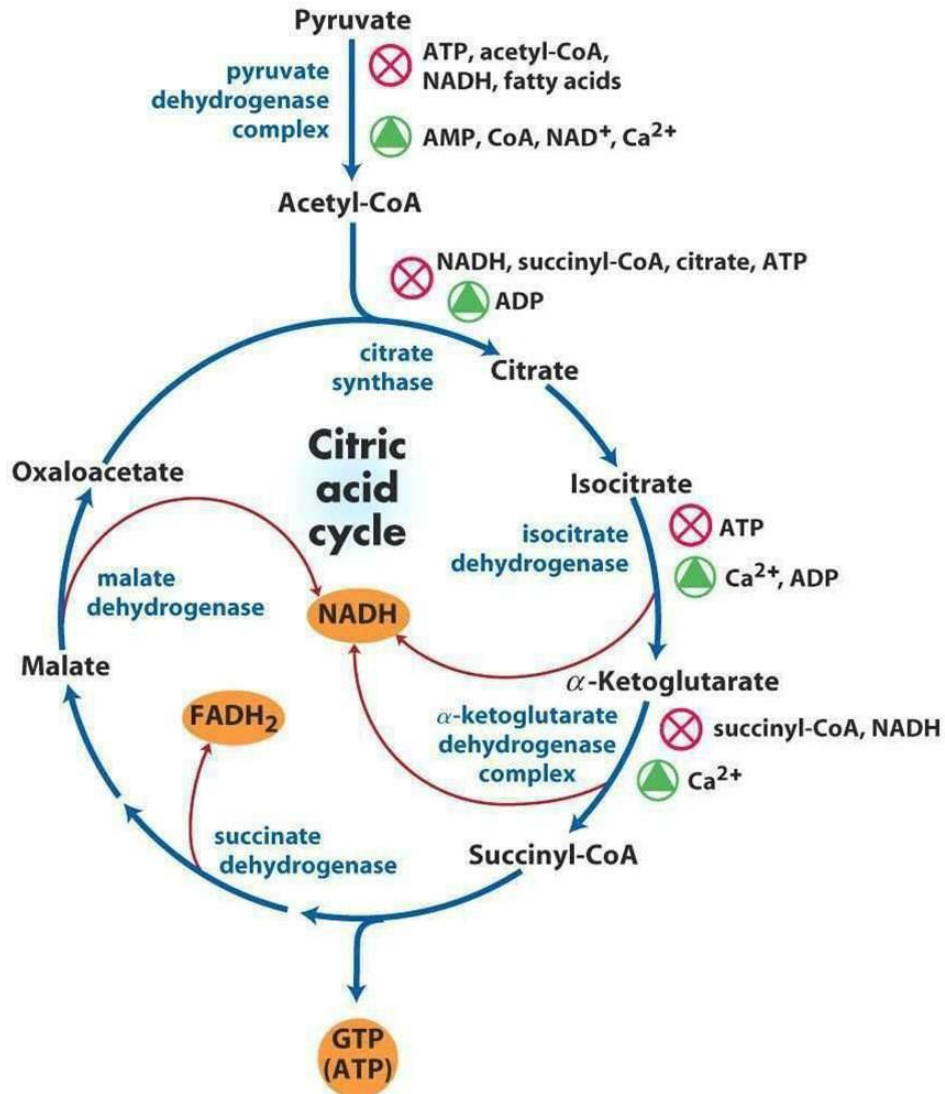
1. ASHA. "Sensorineural Hearing Loss." *American Speech-Language-Hearing Association*, ASHA, Web. 19, March, 2019.
2. VMC. "Ototoxicity Information." *MyVMC*, 26 May 2018. Web. 19 March, 2019.
3. UIS S. GONZALEZ III, PHARM.D., and JEANNE P. SPENCER, M.D., Conemaugh Memorial Medical Center, Johnstown, Pennsylvania *Am Fam Physician*. 1998 Nov 15;58(8):1811-1820.
4. Jiang, Meiyang et al. "AG-Induced Cochleotoxicity: A Review" *Frontiers in cellular neuroscience* vol. 11 308. 9 Oct. 2017, doi:10.3389/fncel.2017.00830
5. Moore R. D., Smith C. R., Lietman P. S. (1984). "Risk factors for the development of auditory toxicity in patients receiving aminoglycosides." *J. Infect. Dis.* 149, 23–30. 10.1093/infdis/149.1.23
6. Mohr, P. E., Feldman, J. J., Dunbar, J. L., McConkey-Robbins, A., Niparko, J. K., Rittenhouse, R. K., et al. (2000). "The societal costs of severe to profound hearing loss in the United States." *Int. J. Technol. Assess. Health Care* 16, 1120–1135. doi: 10.1017/s0266462300103162
7. Choung YH, Taura A, Pak K, Choi SJ, Masuda M, Ryan AF. "Generation of highly-reactive oxygen species is closely related to hair cell damage in rat organ of Corti treated with gentamicin." *Neuroscience*. 2009;161(1):214–226.
8. Dorn, Gerald W. "Mitochondrial Dynamism and Heart Disease: Changing Shape and Shaping Change." *EMBO Molecular Medicine*, EMBO Press, 1 July 2015, embomolmed.embopress.org/content/7/7/865.
9. Stawicki, Tamara M et al. "Using the zebrafish lateral line to uncover novel mechanisms of action and prevention in drug-induced hair cell death" *Frontiers in cellular neuroscience* vol. 9 46. 18 Feb. 2015.
10. Kohanski MA, Dwyer DJ, Wierzbowski J, Cottarel G, Collins JJ. "Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death." *Cell*. 2008;135(4):679–690.
11. Rizzuto Rosario, Saverio Marchi, Massimo Bonora, Paola Aguiari, Angela Bononi, Diego De Stefani, Carlotta Giorgi, Sara Leo, Alessandro Rimessi, Roberta Siviero, Erika Zecchini, Paolo Pinton, "Ca²⁺ transfer from the ER to mitochondria: When, how and why," *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, Volume 1787, Issue 11, 2009, Pages 1342-1351
12. Esterberg, Robert et al. "Mitochondrial calcium uptake underlies ROS generation during AG-induced hair cell death" *Journal of clinical investigation* vol. 126.9 (2016): 3556-66.
13. Rizzuto, Rosario, et al. "Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca²⁺ responses." *Science* 280.5370 (1998): 1763-1766.
14. Garetz, S. L., Rhee, D. J., & Schacht, J. (1994). "Sulfhydryl compounds and antioxidants inhibit cytotoxicity to outer hair cells of a gentamicin metabolite in vitro." *Hearing Research*, 77(1-2), 75–80. doi:10.1016/0378-5955(94)90254-2
15. Conlon, B. J., Aran, J.-M., Erre, J.-P., & Smith, D. W. (1999). "Attenuation of aminoglycoside-induced cochlear damage with the metabolic antioxidant α -lipoic acid." *Hearing Research*, 128(1-2), 40–44.
16. Jiang, H., Sha, S.-H., & Schacht, J. (2005). "NF- κ B pathway protects cochlear hair cells from aminoglycoside-induced ototoxicity." *Journal of Neuroscience Research*, 79(5), 644–651. doi:10.1002/jnr.20392

17. Pickles, J. O., Comis, S. D., & Osborne, M. P. (1984). Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction. *Hearing Research*, 15(2), 103–112. doi:10.1016/0378-5955(84)90041-8
18. Alharazneh A, Luk L, Huth M, Monfared A, Steyger PS, Cheng AG, et al. (2011) "Functional Hair Cell Mechanotransducer Channels Are Required for Aminoglycoside Ototoxicity." *PLoS ONE* 6(7): e22347.
19. Hashino, E., Shero, M., & Salvi, R. J. (1997). "Lysosomal targeting and accumulation of aminoglycoside antibiotics in sensory hair cells." *Brain Research*, 777(1-2), 75–85.
20. Wu, W.-J., Sha, S.-H., & Schacht, J. (2002). "Recent Advances in Understanding Aminoglycoside Ototoxicity and Its Prevention." *Audiology and Neurotology*, 7(3), 171–174.
21. Matsui, J. I., Gale, J. E., & Warchol, M. E. (2004). "Critical signaling events during the AG-induced death of sensory hair cells in vitro." *Journal of Neurobiology*, 61(2), 250–266.
22. Esterberg, R., Hailey, D. W., Rubel, E. W., & Raible, D. W. (2014). "ER-Mitochondrial Calcium Flow Underlies Vulnerability of Mechanosensory Hair Cells to Damage." *Journal of Neuroscience*, 34(29), 9703–9719.
23. Kaloyanides, George J., and Leslie S. Ramsammy. "Aminoglycoside Antibiotics Inhibit the Phosphatidylinositol Cascade in Renal Proximal Tubular Cells: Possible Role in Toxicity." *Nephrotoxicity*. Springer, Boston, MA, 1989. 193-200.
24. Berridge, M. J. (2002). "The endoplasmic reticulum: a multifunctional signaling organelle." *Cell Calcium*, 32(5-6), 235–249.
25. Bagni, Claudia, et al. "Chemical stimulation of synaptosomes modulates α -Ca²⁺/calmodulin-dependent protein kinase II mRNA association to polysomes." *Journal of Neuroscience* 20.10 (2000): RC76-RC76.
26. Vermassen, Elke, et al. "Regulation of the phosphorylation of the inositol 1, 4, 5-trisphosphate receptor by protein kinase C." *Biochemical and biophysical research communications* 319.3 (2004): 888-893.
27. Colombini, Marco. "VDAC: the channel at the interface between mitochondria and the cytosol." *Molecular and cellular biochemistry* 256.1-2 (2004): 107-115.
28. Rapizzi, Elena, et al. "Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca²⁺ microdomains to mitochondria." *J Cell Biol* 159.4 (2002): 613-624.
29. Szalai, Gábor, et al. "Calcium signal transmission between ryanodine receptors and mitochondria." *Journal of Biological Chemistry* 275.20 (2000): 15305-15313.
30. Baysal, K., et al. "Na⁺-dependent Ca²⁺ efflux mechanism of heart mitochondria is not a passive Ca²⁺/2Na⁺-exchanger." *American Journal of Physiology-Cell Physiology* 266.3 (1994): C800-C808.
31. Montero, Mayte, et al. "Direct activation of the mitochondrial calcium uniporter by natural plant flavonoids." *Biochemical Journal* 384.1 (2004): 19-24.
32. Moreau, Ben, Charmaine Nelson, and Anant B. Parekh. "Biphasic regulation of mitochondrial Ca²⁺ uptake by cytosolic Ca²⁺ concentration." *Current biology* 16.16 (2006): 1672-1677.
33. Litsky, Monica L., and Douglas R. Pfeiffer. "Regulation of the mitochondrial Ca²⁺ uniporter by external adenine nucleotides: the uniporter behaves like a gated channel which is regulated by nucleotides and divalent cations." *Biochemistry* 36.23 (1997): 7071-7080.
34. McCormack, J. G., Halestrap, A. P., & Denton, R. M. (1990). Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiological Reviews*, 70(2), 391–425. doi:10.1152/physrev.1990.70.2.391
35. Jouaville, Laurence S., et al. "Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming." *Proceedings of the National Academy of Sciences* 96.24 (1999): 13807-13812.
36. Chami, Mounia, et al. "Bcl-2 and Bax exert opposing effects on Ca²⁺ signaling, which do not depend on their putative pore-forming region." *Journal of Biological Chemistry* 279.52 (2004): 54581-54589.
37. Kuida, K., Haydar, T. F., Kuan, C.-Y., Gu, Y., Taya, C., Karasuyama, H., ... Flavell, R. A. (1998). "Reduced Apoptosis and Cytochrome c-Mediated Caspase Activation in Mice Lacking Caspase 9." *Cell*, 94(3), 325–337.
38. Feng Gao, C., Ren, S., Zhang, L., Nakajima, T., Ichinose, S., Hara, T., ... Tsuchida, N. (2001). "Caspase-Dependent Cytosolic Release of Cytochrome c and Membrane Translocation of Bax in p53-Induced Apoptosis." *Experimental Cell Research*, 265(1), 145–151. doi:10.1006/excr.2001.5171
39. Coffin, Allison B., et al. "Bax, Bcl2, and p53 Differentially Regulate Neomycin- and Gentamicin-Induced Hair Cell Death in the Zebrafish Lateral Line." *Journal of the Association for Research in Otolaryngology*, vol. 14, no. 5, 2013, pp. 645–659.
40. Nathan, Abhi. "30.1 (Uworld) Flashcards." *Memorang*, Web. 2019. April 6, 2019.
41. Smith RA, Porteous CM, Gane AM, Murphy MP. "Delivery of bioactive molecules to mitochondria in vivo." *Proc Natl Acad Sci U S A*. 2003; 100 (9):5407–5412.

SUPPLEMENTAL INFORMATION



Supplementary Figure A: Biochemical pathway of oxidative phosphorylation. Electron transfer to the intermembrane space from the mitochondria generates a proton gradient in complexes I and III. The generation of a proton gradient generates reactive oxygen species (ROS) in the matrix where superoxide dismutase (SOD) reduces the ROS to hydrogen peroxide (H_2O_2). Generation of the proton gradient across the IMM allows for ATP production (Dorn et al., 2015). ADP = adenosine diphosphate. Pi = inorganic phosphate. ATP = adenosine triphosphate. NADH = nicotinamide adenine dinucleotide. CoQ = coenzyme Q. Cyto C = Cytochrome C.



Supplementary Figure B: Effects of calcium on the TCA (citric acid cycle). Researchers have identified increases in the activation of pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase and alpha-ketoglutarate complex upon increases in calcium in the matrix^{34,40}. ADP = adenosine diphosphate. ATP = adenosine triphosphate. NADH = nicotinamide adenine dinucleotide. FADH₂ = Flavin adenine dinucleotide. CoA = Coenzyme A.

Martial arts related neurological damage: a review

Anthony Ziegenfuss

INTRODUCTION

Martial arts are a form of physical activity that generally is practiced with the focus of gaining strength, discipline, and greater capability in fights. Recently the sport of mixed martial arts (MMA) has developed. MMA consists of scheduled fights between two combatants where a win is accomplished through two main methods: a knock-out (KO) or a submission. A submission involves joint manipulation or choking until the receiving fighter either submits or is unconscious. To achieve a KO a fighter must be hit, generally in the head, until they lose consciousness. A subsection of a KO is a technical knock-out (TKO), this can be deceptively damaging as it involves a fighter capitulating due to being conscious but unable to defend themselves. The results of the head injuries obtained in various matches can be deceptively subtle and lead to fighters accumulating neurological damage over time.

This review aims to investigate the available data on martial arts, MMA, and brain injuries. Fighters accept danger when they enter the ring to fight, but many may not know the lasting impact of the head trauma they receive. A 2015 study out of the British Journal of Sports Medicine found a correlation between fighting experience and lower brain structure volumes.¹ This points out a possible connection between fighting and structural deviations in the brain. These hits to the head do not always come from strikes by fists or limbs. Sports with grappling such as wrestling, and judo can also induce damage. A 2018 study investigated the occurrence of concussions in high-school athletes. They found out of 958 athletes that engage in wrestling and martial arts, 204 concussions were recorded.² The incidence of concussions in high-school athletes presents a very real danger to adolescents and their developing brains.

THE DANGER OF REPEATED HEAD INJURY

Across all ages of participants, there is a danger of repeated head-injury which may result in permanent brain damage. However, the duration of a fighter's career may be an important factor. There is a trend within both MMA fighters and boxers to have more severe brain injuries as they have more fights in their career⁴. The understated danger of the strikes fighters receive to the head is not the concussive force of an individual strike, but repeated sub-concussive strikes. Repeated sub-concussive strikes to the head correlate to reduced volume of brain structures such as the caudate, and thalamus^{1,6}. The thalamus plays a significant role in the transmission of signals into the cortex and a reduction in volume could have a profound effect. The risk was greater in boxers when compared to both MMA fighters and controls¹.

Repetitive head trauma can lead to a condition: chronic traumatic encephalopathy (CTE)^{6,7}. Athletes that experience sub-concussive impacts such as boxers or American football players are

frequently at risk to receive these impacts. It has been hypothesized that there is a difference in the type of damage received by each athlete. Boxers, especially those with concussive injuries, mostly experience rotational damages⁸. Football players are thought to experience mostly straight blows from the front to back⁷. This is believed to especially damage areas with mixed cell types such as blood vessels directly next to neurons and astrocytes⁷. The chance for cell death due to these impacts could contribute to the reduction in volume observed in fighters such as boxers.

Fighters receiving repeated strikes to the head are more likely to have an increased cavum septum pellucidum (CSP) as well¹. A CSP is a space that forms as the thin membrane that is the septum pellucidum separates. The septum pellucidum itself is formed with this space during the normal development of a fetus¹¹. The membrane fuses together for most people before they reach sixth months of age¹¹. CSP does not cause of many symptoms itself but is present in several conditions including traumatic CTE⁷. The presence of a CSP when paired with a history of fighting or hard sport activity could be used as a late marker to further confirm conditions such as CTE.

DETECTION AND DIAGNOSING

The injuries obtained in combat sports generally are obvious in that there can be bruises or bleeding. However, there is great difficulty in diagnosing brain related trauma. Athletes may not present symptoms of concussions and be allowed to return to fighting. This was the case of a 16-year-old judoka that received medical treatment but was not advised to wait long enough before returning to practice. They fell unconscious on the mat and later passed away due to an acute subdural hematoma⁵. The danger presented calls for earlier detection. There has been some focus in identifying serum samples and saliva miRNA to assess mild traumatic brain injury (mTBI) in MMA fighters. The main finding was that levels of 47 miRNA in saliva and serum were changed over time after an individual's exposure to a fight³. The promise of a saliva method of brain injury detection is that an miRNA sample in the future could be analyzed for a fighter to give a better idea of the damage they received in a fight.

ADOLESCENT EXPOSURE

Throughout America there is a culture that encourages athleticism in schools starting at a very young age. Exposure to the risks discussed above at such critical points in adolescent development could amplify the long-term effects felt. The reduced processing speed and cell death discussed above may hamper the potential of each student¹. There are regulations in place to limit the damage to adolescents, such as the combative sports license in Washington state which requires applicants be 18 years old⁹. Having the regulations in place to curb damage to developing minds is crucial. Exposure to impacts that would cause concussions in popular culture also may contribute to a lack of understanding of the danger presented within the general public.

Comic book movies have grown in popularity over time, they show heroes battling without exploring the long-term head trauma they receive. Batman has been part of American culture as an expert martial arts vigilante fighting crime. However, there are moments throughout the films that demonstrate Batman receiving impacts from various villains that should have been concussive or deadly. There were 176 head injuries in all of Batman's movie appearance that should have been a concussion and 5 that would have been fatal¹⁰. This may create a bias in the general public to not know when a strike to the head may result in a concussion. Batman is portrayed as a strong, but not supernatural human, with the same physical limitations as the viewers when it comes to his mortality.

CONCLUSION

There needs to be further understanding of the long-term effects of participating in combat sports such as MMA and boxing. The risk of brain injury to adolescents as they participate in these sports, and how that effects their future development should be further investigated. Having doctors on staff at events as well using cutting-edge biomarker analysis to identify brain injuries. The biomarkers could be used to stop athletes from unknowingly causing further damage. Additionally, the risks of participation should be made clear to all those interested. Disseminating the information to high school age athletes that are beginning wrestling, or those looking to participate in amateur MMA before allowing them to do so may be a positive method of risk management.

REFERENCES

1. Bernick C, Banks SJ, Shin W, et al (2015). Repeated head trauma is associated with smaller thalamic volumes and slower processing speed: the Professional Fighters' Brain Health Study. *Br J Sports Med*, 49:1007-1011.
2. Tsushima WT, Ahn HJ, Siu AM, Yoshinaga K, Choi SY, Murata NM, (2018). Effects of repetitive subconcussive head trauma on the neuropsychological test performance of high school athletes: A comparison of high, moderate, and low contact sports, *Applied Neuropsychology: Child*, DOI: 10.1080/21622965.2018.1427095
3. LaRocca D, Barns S, Hicks SD, et al. (2019). Comparison of serum and saliva miRNAs for identification and characterization of mTBI in adult mixed martial arts fighters. *PLOS ONE*. <https://doi.org/10.1371/journal.pone.0207785>
4. Lee JK, Wu J, Banks S, et al. (2017) Prevalence of Traumatic Findings on Routine MRI in a Large Cohort of Professional Fighters. *American journal of Neuroradiology*, 38, 1303-1310. <https://doi.org/10.3174/ajnr.A5175>
5. Nambu S, Noji M, (2014) Case of Fatal Head Trauma Experienced During Japanese Judo. *Current Sports Medicine Reports*, 13, 11-15. doi: 10.1249/JSR.0000000000000024
6. Banks S, Mayer B, Obuchowski N, et al. (2014). Impulsiveness in Professional Fighters. *Journal of Neuropsychiatry and Clinical Neuroscience*, 26. <https://doi.org/10.1176/appi.neuropsych.12070185>
7. Montenigro, P. H., Bernick, C. and Cantu, R. C. (2015) Clinical Features of Repetitive Traumatic Brain Injury and Chronic Traumatic Encephalopathy. *Brain Pathology*, 25: 304-317. doi:10.1111/bpa.12250
8. Filley CM, Arciniegas DB, Brenner LA, et al. (2019) Chronic Traumatic Encephalopathy: A Clinical Perspective. *The Journal of Neuropsychiatry and Clinical Neurosciences*. 31:2, 170-172
9. Amateur mixed martial arts participant. (2019). Retrieved May 2, 2019, from <https://www.dol.wa.gov/business/athletics/amateurMMA.html>
10. Zehr EP, Wright B, (2016). Can Concussion Constrain the Caped Crusader? *Br J Sports Med*, 50, 1481-1484. <http://dx.doi.org/10.1136/bjsports-2016-096792>
11. Dremmen MHG, Bouhuis RH, Blanken LME, et al. (2019) Cavum Septum Pellucidum in the General Pediatric Population and Its Relation to Surrounding Brain Structure Volumes, Cognitive Function, and Emotional or Behavioral Problems. *American Journal of Neuroradiology*. DOI: <https://doi.org/10.3174/ajnr.A5939>

Racial and ethnic differences in postpartum depression and cortisol function: a literature review

Cheyenne Lewis

INTRODUCTION

Postpartum depression (PPD) is a condition following childbirth during which the mother experiences depressive symptoms such as restlessness, hopelessness and severe irritability. It is one of the most prevalent perinatal conditions with as many as 50% of women in certain populations being diagnosed each year¹. Unlike depression experienced during other periods in a woman's life, PPD is comorbid with anxiety related to childcare. A mother may have trouble bonding with the child and forming emotional attachment, leading to doubts about the ability to provide proper support. Although these feelings are often experienced by new mothers, the severity of the symptoms is what sets these two cases apart. Therefore, it is important to distinguish between PPD and a case of "baby blues". Baby blues syndrome, while sharing symptoms with PPD, usually develops a few days after delivery and lasts anywhere from 2-6 weeks. This phenomenon is experienced by 50-80% of new mothers and the symptoms typically go away on their own². For mothers with PPD, symptoms can last years if left untreated, and it not only affects the mother's ability to function, but it is also detrimental to the social, cognitive and emotional development of the child³. For these reasons, early diagnosis and treatment is vital to the health of both the mother and her baby.

The etiology of PPD, despite being largely unknown, has been linked to genetic predisposition, trauma and history of mental illness. Together, these factors can lead to the disruption of maternal-fetal endocrine dynamics, particularly within the hypothalamic-pituitary-adrenocortical (HPA) axis⁴. Cortisol, a steroid hormone produced by the adrenal cortex in response to stress, has been debated as a direct mediator of PPD for many years^{5,6}. Although there is only some evidence to suggest that cortisol alone plays a significant role in the development of PPD, both stress and the HPA axis are closely linked to onset of major depressive symptoms. Among the hundreds of thousands of women affected annually, the burden of PPD symptoms is especially high on women of color who are increasingly vulnerable to physiological changes following cumulative stress. This race-related stress is exacerbated by the existing disparity in maternal outcomes especially within the United States. The United States has the highest rates of maternal mortality among high-income countries with Black women dying at three to four times the rate of white women from pregnancy-related complications⁷. To explore the relationship between race-related stress and the development of PPD, this literature review will focus on alternations in cortisol function during perinatal and postpartum periods and how dysfunction of the HPA-axis relates to physiological changes in cases of PPD. Cortisol function will also be explored as a therapeutic target to alleviate symptoms, especially in women of color.

NEUROBIOLOGY OF PPD

The postpartum period is associated with plasticity changes with total brain volume decreasing during pregnancy and returning to normal after 6 months postpartum⁴. Currently, very few studies have investigated the neural correlates of PPD in depth, but it is suspected that the distinct functional, metabolic and structural changes that occur naturally during pregnancy play a major role in both the onset and duration of several postpartum conditions⁸. Findings from clinical studies using functional magnetic resonance imaging (fMRI) indicate that there is a distinct network of brain systems involved in postpartum affective disorders such as postpartum depression, anxiety and psychosis. In instances of PPD, there are significant changes in neural activity in brain regions important for self-regulation, empathy and emotion as well as decreases in corticocortical and corticolimbic connectivity⁹. These changes are often lateralized and exist in the absence of cues during the brain's resting state. Upon presentation of emotional infant-related cues, distinct activation patterns occur within the maternal caregiving network – a network of cortical and subcortical limbic regions important for regulating maternal motivation and behavior. Depressed mothers experience hypoactive resting-state activity within the maternal caregiving network when compared to healthy controls, and when exposed to either negative or positive infant-related cues, the pattern of activity changes^{10,11}. For example, a collection of studies found that when faced with positive infant-related cues, there was decreased activation in the anterior cingulate cortex, insula, inferior frontal gyrus, and orbital frontal cortex with increased activity in the right amygdala^{12,13,14}. It is hypothesized that significant alterations in these neural networks can negatively impact a mother's empathy, stress, motivation and executive functioning as well as their desire to interact with their child¹⁵.

The use of animal models, particularly rat models, to understand the biology underlying postpartum disorders is still underdeveloped as animal models are typically used to mimic one contributing biological or psychosocial factor at a time. Applying stressors, administering exogenous glucocorticoids, or separation of the mother from pups are often ways to induce PPD-like behaviors in rats. From these models, recent findings suggest that there are modifications in synaptic plasticity in certain areas. Studies focusing on the hippocampus, a brain area heavily associated with depression, found alterations in neurogenesis and dendritic plasticity following bouts of stress during the postpartum period, suggesting that the HPA-axis may play a major role in the development of PPD^{16,17,18}.

CORTISOL & RACE-RELATED STRESS

Cortisol, a steroid hormone, is produced by the adrenal cortex in response to stress and is regulated by the HPA-axis. Its primary functions include regulating metabolism and playing a role in negative feedback in inflammatory processes as well as aiding in memory formation⁶. Following stressful stimuli, corticotrophin releasing hormone (CRH) is produced in the paraventricular nucleus of the hypothalamus and released into the pituitary gland. CRH then stimulates the release of adrenocorticotrophic hormone (ACTH) in the anterior pituitary which signals the adrenal cortex to release cortisol. During pregnancy, maternal cortisol release promotes stimulation of CRH in the placenta resulting in an exponential increase in maternal plasma cortisol levels¹⁹. This process alters the mechanisms of the HPA-axis, and it is suspected that high levels of cortisol secretion may lead to hypercortisolemia²⁰. Hypercortisolemia can increase a woman's risk of developing PPD in the context of environmental stressors as well as produce structural and functional changes within the limbic system. Excessive amounts of glucocorticoids are associated with decreases in hippocampal volume and can result in damage of glucocorticoid receptors²¹. As a result, the hippocampus becomes metabolically stressed²². Furthermore, high levels of

cortisol can lead to a significant reduction in the volume of the anterior cingulate cortex, one of the major brain areas involved in the maternal caregiving network²³.

One of the major psychosocial risk factors for PPD is cumulative stress as it may disrupt maternal-fetal endocrine dynamics especially within the HPA-axis. Despite popular belief, chronic stress does not consistently lead to increased cortisol levels. Studies suggest that those who experience chronic stress have lower baseline cortisol and a flattening of the typical diurnal cortisol slope²⁴. The diurnal rhythm of cortisol typically peaks after waking and gradually decreases throughout the day, and in instances of chronic stress, cortisol levels become irregular. This association between cortisol levels and stress exposure was noted in women of color especially among Black and Hispanic women²⁴. Black women often cope with socioeconomic stress and poor birth outcomes with Black mothers dying at 3-4 times the rate of white women from pregnancy related complications^{7,25}. Additionally, by the end of 2014, Hispanic women were twice as likely as white women to die during childbirth, with numbers only increasing each year²⁶. Although socioeconomic status (SES) is not race-dependent, there is a correlation between the two. Particularly during pregnancy, women of low SES are more exposed to environmental stressors such as financial hardship, exposure to community violence, racism or discrimination, and interpersonal violence and are 11 times more likely to develop PPD²⁷. This suggests that for women of color, stress management is important as part of ongoing treatment.

SOCIAL SUPPORT AND MATERNAL CARE

Another major psychosocial risk factor for PPD is lack of social support and poor interpersonal relationships. Although social support is largely subjective, there is still consensus on some of the resources that it provides including emotional, informational, and instrumental support²⁸. Not only is receiving this support influential on health outcomes, but perceived availability is equally as important for the mother's feeling of well-being. Ultimately, supportive relationships during pregnancy can enhance mood to help women perceive pregnancy-related changes as less stressful. These effects may be more pronounced among women who experience high levels of stress and can persist well into the postpartum period. As a form of treatment, psychosocial intervention has been shown to be effective in improving symptoms of PPD in order to reduce external stress factors²⁹. PPD has been associated with a wide range of negative outcomes for the mother and the child including lack of maternal emotional and behavioral sensitivity to the infant, poor bonding, atypical neurodevelopment and later emotional and behavioral problems for the children when older³.

FUTURE DIRECTIONS

For women who suffer from postpartum conditions such as depression, anxiety and psychosis, there are a number of barriers that prevent them from seeking help. Over the years, social stigma about mental health and insufficient knowledge among healthcare providers regarding mental health have hindered the treatment processes for these women. This trend is especially seen in women of color as stigma and support varies depending on cultural expectations, beliefs and resource availability. The disorders are heterogeneous in that etiology is diverse, and differences in results among various studies is likely a result of variations in selected populations and criteria used for diagnosis. For these reasons, finding precise rates of postpartum disorders is difficult to establish. For future studies, accounting for variations in race, ethnicity, demographics, SES and levels of stress will be important in determining proper treatment options for each case of PPD. To do this, researchers should have a better understanding of the ways risk factors like socioeconomic stress and social support play a role in the development of PPD. Using this

knowledge will help build associations between risk factors and biomarkers such as cortisol which can then be used as both predictive and therapeutic tools. This insight will be important in determining whether the mother or expecting mother should utilize treatments such as behavioral therapy or anti-depressants. If proper social support, whether emotionally, informationally, or financially, will be useful in diminishing symptoms, then those services should be available.

CONCLUSION

Every mother is at risk for developing PPD regardless of socioeconomic status, race, education, or geographical location. It is a real problem that often goes unreported with many instances resulting in negative outcomes for both the mother and the child. Therefore, finding better ways to diagnose and treat these women is of utmost importance. The neurological and endocrine changes that result from PPD may not be permanent and are reversible if symptoms are caught early on, indicating that personalized therapy can be effective both during pregnancy and postpartum to monitor the health of mother and child while also providing social support. Women of color are at greater risk for developing PPD while also having higher risks of death due to pregnancy-related complications so providing proper resources and support are essential for both the livelihood of the mother and the child.

REFERENCES

1. Howell E.A., Mora P.A., Horowitz C.R. & Leventhal H. (2005) Racial and Ethnic Differences in Factors Associated With Early Postpartum Depressive Symptoms, *Obstetrics & Gynecology*, 105(6): 1142-1450
2. Koshchavtsev A.G, Mul'tanovskaya V.N. & Lorer V.V. (2008) Baby Blues Syndrome as an Adaptation Disorder in the Early Stages of Formation of the Mother–Child System, *Neuroscience and Behavioral Physiology*, 38(4): 439-442
3. Field T. (2010) Postpartum depression effects on early interactions, parenting, and safety practices: A review, *Infant Behavior & Development*, 33: 1-6
4. Brummelte S. & Galea L.A.M. (2016) Postpartum depression: Etiology, treatment and consequences for maternal care, *Hormones and Behavior*, 77: 153-166
5. Bloch M., Daly R.C. & Rubinow D.R. (2003) Endocrine Factors in the Etiology of Postpartum Depression, *Comprehensive Psychiatry*, 44(3): 234-246
6. Liuzza J.W., Gallaher M.J. & Powers R.W. (2015) Urinary cortisol and depression in early pregnancy: role of adiposity and race, *BMC Pregnancy and Childbirth*, 15(30)
7. Essien U.R., Molina R.L. & Lasser K.E. (2018) Strengthening the postpartum transition of care to address racial disparities in maternal health, *Journal of the National Medical Association*
8. Pawluski J.L., Lonstein J.S. & Fleming A.S (2017) The Neurobiology of Postpartum Anxiety and Depression, *Trends in Neurosciences*, 40(2): 106-120
9. Silverman M.E., Loudon H., Safier M., Protopopescu X., Leiter G., Liu X. & Martin Goldstein (2014) Neural Dysfunction in Postpartum Depression: An fMRI Pilot Study, *CNS Spectrums*, 12(11): 853-862
10. Wang X-j., Wang J., Liu Z-h., Ming Y. & Zhang S-w. (2011) Increased Posterior Cingulate, Medial Frontal and Decreased Temporal Regional Homogeneity in Depressed Mothers. A Resting-State Functional Magnetic Resonance Study, *Procedia Environmental Sciences*, 8: 737-743
11. Wonch K.E., Medeiros C.B., Barrett J.A., Dudin A., Cunningham W.A., Hall G.B., Steiner M. & Fleming A.S. (2016) Postpartum depression and brain response to infants: Differential amygdala response and connectivity, *Social Neuroscience*, 11(6): 600-617
12. Laurent H.K. & Ablow J.C. (2012) A cry in the dark: depressed mothers show reduced neural activation to their own infant's cry, *Social Cognitive and Affective Neuroscience*, 7(2): 125-134
13. Moses-Kolko E.L., Perlman S.B., Wisner K.L., James J., Saul T. & Phillips M.L. (2010) Abnormally Reduced Dorsomedial Prefrontal Cortical Activity and Effective Connectivity With Amygdala in Response to Negative Emotional Faces in Postpartum Depression, *The American Journal of Psychiatry*, 167(11): 1373-1380
14. Barrett J., Wonch K.E., Gonzalez A., Ali N., Steiner M., Hall G.B. & Fleming A.S. (2011) Maternal affect and quality of parenting experiences are related to amygdala response to infant faces, *Social Neuroscience*, 7(3): 252-268
15. Gress-Smith J.L., Luecken L.J., Lemery-Chalfant K. & Howe R. (2012) Postpartum Depression Prevalence and Impact on Infant Health, Weight, and Sleep in Low-Income and Ethnic Minority Women and Infants, *Maternal and Child Health Journal*, 16(4): 887-893

16. Pawluski J.L., van den Hove D.L.A., Rayen I., Prickaerts J. & Steinbusch H.W.M. (2011) Stress and the pregnant female: Impact on hippocampal cell proliferation, but not affective-like behaviors, *Hormones and Behavior*, 59(4): 572-580
17. Kinsley C.H., Trainer R., Stafisso-Sandoz G., Quadros P., Marcus L.K., Hearon C., Meyer E.A., Hester N., Morgan M., Kozub F.J., Lambert K.G. (2006) Motherhood and the hormones of pregnancy modify concentrations of hippocampal neuronal dendritic spines, *Hormonal Behavior*, 49: 131–142.
18. Darnaudery M., Perez-Martin M, Del Favero F., Gomez-Roldan C., Garcia-Segura L.M, Maccari S. (2007) Early motherhood in rats is associated with a modification of hippocampal function, *Psychoneuroendocrinology*, 32(7): 803-812
19. Kammerer M., Taylor A. & Glover V. (2006) The HPA axis and perinatal depression: a hypothesis. *Archives Womens Mental Health*. 9(4):187-96.
20. Seth S., Lewis A. & Galbally M. (2016) Perinatal maternal depression and cortisol function in pregnancy and the postpartum period: a systematic literature review, *BMC Pregnancy and Childbirth*, 16(124)
21. Lupien S., Leon M., Santi S., Convit A., Tarshish C., Nair N.P.V., Thakur M., McEwen B., Haugher R., & Meaney M. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits, *Nature Neuroscience*, 1: 69-73
22. Liu S., Wang Y., Xu K., Ping F., Wang R., Li F. & Cheng X. (2016) Brain glucose metabolism is associated with hormone level in Cushing's disease: A voxel-based study using FDG-PET, *NeuroImage: Clinical*, 12: 415-419
23. Cerqueira J.J., Cataniab C., Sotiropoulos I., Schubert M., Kalischc R., Almeida O.F.X., Auerc D.P. & Sousaa N. (2005) Corticosteroid status influences the volume of the rat cingulate cortex – a magnetic resonance imaging study, *Journal of Psychiatric Research*, 39(5): 451-460
24. Suglia S.F., Staudenmayer J., Cohen S., Enlow M.B., Rich-Edwards J.W. & Wright R.J. (2010) Cumulative Stress and Cortisol Disruption among Black and Hispanic Pregnant Women in an Urban Cohort, *Psychological Trauma*, 2(4): 326-334
25. Zayas L.H., Cunningham M., McKee M.D. & Jankowski K.R.B. (2002) Depression and Negative Life Events Among Pregnant African-American and Hispanic Women, *Women's Health Issues*, 12(1): 16-22
26. Booker W., Gyamfi-Bannerman C., Sheen J., Wright J., Siddiq Z., D'Alton M. & Friedman A. (2018) Maternal Outcomes by Race for Women Aged 40 Years or Older, *Obstetrics & Gynecology*, 132(2): 404-413
27. Goyal D., Gay C. & Lee K.A. (2010) How much does Low Socioeconomic Status Increase the Risk of Prenatal and Postpartum Depressive Symptoms in First Time Mothers?, *Womens Health Issues*, 20(2): 96-104
28. Collins N.L, Dunkel-Schetter C., Lobel M. & Scrimshaw S.C. (1993) Social Support in Pregnancy: Psychosocial Correlates of Birth Outcomes and Postpartum Depression, *Journal of Personality and Social Psychology*, 65(6): 1243-1258
29. Wisner K.L., Parry B.L. & Piontek C.M. (2002) Postpartum Depression, *New England Journal of Medicine*, 347(3): 194-199
30. U.S. Department of Health and Human Services, Child Health USA 2013

The role of the gut microbiome on depression

Daniel Salazar

INTRODUCTION

Depression is a mental disorder which affects 16 million people around the US and an estimated 322 million worldwide¹. The majority of those affected are young adults aged 18-25¹. Depression is a complex mental disorder which results from a combination of different triggers such as stress, genetic predispositions, and environmental factors¹. Roughly 20% of adolescents with major depression are treated with antidepressants such as selective serotonin reuptake inhibitors². Unfortunately, traditional antidepressants can have adverse effects on adolescents potentially increasing the risk of self-harm and suicide³. Other side effects of antidepressants are fatigue, dizziness, and weight gain which can indirectly exacerbate depression (Stahl et al., 1998). Therefore, new therapies must be developed to treat mental illness with minimal side effects. A growing field of research has characterized the influence of the gut microbiome as a target for potential treatments of mental illness (Dinan et al., 2017; Martin et al., 2017; Wang 2016). Interestingly, 70-90% of patients who suffer from psychiatric disorders also suffer from irritable bowel syndrome (IBS) (Kawoos et al., 2017). Furthermore, a 2018 study discovered that fecal transplantation of healthy bacteria decreased depression symptoms in patients with major depressive disorder (Kurokawa et al., 2018). This evidence demonstrates an intimate relationship between the gut microbiome and mental health.

The enteric nervous system (ENS) is found in the gastrointestinal tract. The ENS sends signals directly to the brain via the vagus nerve (CN X)⁴. Additionally, the gut microbiome is also able to indirectly communicate with the brain. For example, inflammation in the gut signals for the degradation of tryptophan (TRP) to one of its metabolites, kynurenic acid (KYNA) (Cheng et al., 2018). TRP is the precursor to 5-HT (5-HT) and thus metabolism of TRP leads to decreased net levels of 5-HT in the body (Israelyan et al., 2019). Therefore, one hallmark of depression is decreased levels of 5-HT. Moreover, KYNA can modulate brain function because the brain has binding sites for these neurochemicals produced by the gut microbiome (Holzer et al 2014). This review will explore how the gut microbiome influences mental health which will allow for exploration of gut-brain axis targeted therapies.

THE ROLE OF THE GUT MICROBIOME ON NEUROCHEMICALS LINKED WITH DEPRESSION

The gut microbiome is composed of over 100 species of bacteria which make up 90% of the cells found in the human body (Wang 2016). Bacteria in the GI tract modulate neurochemicals such as brain derived neurotrophic factor (BDNF)⁶. BDNF is a neuropeptide important in promoting neuroplasticity⁶. A 2011 study found that germ-free (GF) mice showed decreased levels of BDNF⁷. Importantly, these GF mice exhibited anxiety and depression-like behaviors⁷. Similarly, decreased

levels of BDNF have also been observed in depressed human patients⁶. Thus, manipulation of BDNF in the brain by the gut microbiome has shown to contribute to depression.

Another neurochemical modulated by the gut microbiome is 5-HT. In fact, some species of bacteria such as *Bifidobacterium* are able to produce 5-HT (Mittal et al., 2017). Unsurprisingly, a reported 90% of 5-HT is produced in the GI tract⁸. Interestingly, a recent study by Mandic and colleagues discovered that *Clostridium ramosum* is capable of increasing expression of peripheral 5-HT-producing enterochromaffin cells⁹. Thus, the gut microbiome is able to directly influence levels of 5-HT. One hallmark of depression is low levels of 5-HT¹⁰. 5-HT is a ubiquitously expressed neurotransmitter in the brain and periphery important in regulating mood, appetite, and sleep. Additionally, peripheral 5-HT is vital in brain development. Knockout studies of tryptophan hydroxylase (tph), the enzyme which converts TRP to 5-HT, revealed that peripheral placental maternal 5-HT plays a vital role in murine developing brains more so than embryonic 5-HT levels¹¹. Cote found that homozygous mothers lacking the tph gene impacted pup brain development more so than the genotype of the pups themselves. Peripheral 5-HT therefore plays a vital role in neural processes. Gut dysbiosis can lead to decreased levels of BDNF and 5-HT proving the importance of the gut microbiome in the etiology of depression.

THE GUT MICROBIOME STRESS RESPONSE LEADS TO TRP METABOLISM

One of the biggest risk factors for developing depression is chronic stress¹². It is well established that the gut microbiome is one mediator of immune and inflammatory response mechanisms in the body^{13,14}. In response to stress, enteroendocrine cells signal for the release of cytokines such as tumor necrosis factor (TNF) alpha¹³. Fascinatingly, bacteria found in the GI tract such as *Fusobacterium nucleatum* mediate levels of TNF gene expression in humans¹⁵. TNF can induce fever, cell apoptosis, and the release of other proinflammatory cytokines¹³. TNF also increases expression of indoleamine (2,3)-dioxygenase, an enzyme which converts TRP to kynurenine¹⁶. Thus, chronic inflammation in the gut can trigger the degradation of TRP via the kynurenine pathway¹⁷.

Kynurenine is able to influence the brain since this compound is able to cross the blood brain barrier¹⁸. Kynurenine is then converted to KYNA by kynurenine aminotransferases found in astrocytes¹⁸. At normal levels, KYNA acts as an endogenous ROS scavenger and competitive antagonist at the glycine binding site of N-Methyl-D-aspartic acid receptors (NMDARs). Fascinatingly, NMDAR antagonists such as ketamine act as fast-acting antidepressants and thus KYNA may be acting to attenuate depression symptoms¹⁹. In fact, patients with depression in remission and currently depressed both showed disproportionately low levels of KYNA compared to healthy individuals²⁰. Furthermore, dysregulated NMDARs have been linked to decreased levels of BDNF and dysfunctional serotonergic signaling²¹. Therefore, KYNA regulation by gut microbes influences the etiology of depression.

CONCLUSION

A growing body of evidence has shown that the gut microbiome is able to influence neurochemicals and pathophysiology of mental disorders. Future directions include investigating treatments for mental disorders which target the gut microbiome. Such treatments include anti-inflammatory drugs and compounds which improve the life of gut microbes such as prebiotics and probiotics²². A challenge in developing gut microbiome specific treatments for mental disorders is

that the environment of the gut microbiome is highly individualized. Thus, future studies will focus on possibly genotyping the bacteria in the gut to develop patient specific treatments²³. The gut microbiome provides a powerful clinical tool for treatment of mental disorders.

REFERENCES

1. Major Depression. (2017, November). Retrieved from <https://www.nimh.nih.gov/health/statistics/major-depression.shtml>
2. Rushton, J., Bruckman, D., & Kelleher, K. (2002, June). Primary care referral of children with psychosocial problems. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/12038893>
3. Didham, R. C., McConnell, D. W., Blair, H. J., & Reith, D. M. (2005, November). Suicide and self-harm following prescription of SSRIs and other antidepressants: Confounding by indication. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1884935/>
4. Breit, S., Kupferberg, A., Rogler, G., & Hasler, G. (2018). Vagus Nerve as Modulator of the Brain-Gut Axis in Psychiatric and Inflammatory Disorders. *Frontiers in psychiatry*, 9, 44. doi:10.3389/fpsy.2018.00044
5. Holzer, P., & Farzi, A. (2014). Neuropeptides and the microbiota-gut-brain axis. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/24997035>
6. Caviedes, A., Lafourcade, C., Soto, C., & Wyneken, U. (2017). BDNF/NF- κ B Signaling in the Neurobiology of Depression. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28078988>
7. Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., . . . Pettersson, S. (2011, February 15). Normal gut microbiota modulates brain development and behavior. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3041077/>
8. Keszhelyi, D., Troost, F. J., & Masclee, A. A. (2009, December). Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/19650771>
9. Mandić, A. D., Woting, A., Jaenicke, T., Sander, A., Sabrowski, W., & Rolle-Kampczyk, U. (2019, February 04). Clostridium ramosum regulates enterochromaffin cell development and serotonin release. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/30718836>
10. Kraus, C., Castrén, E., Kasper, S., & Lanzenberger, R. (2017, June). Serotonin and neuroplasticity - Links between molecular, functional and structural pathophysiology in depression. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28342763>
11. Cote F, Fligny C, Bayard E, Launay JM, Gershon MD, Mallet J, Vodjdani G. Maternal serotonin is crucial for murine embryonic development. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:329–334. [PubMed: 17182745]
12. Kendler K.S., Karkowski L.M., Prescott C.A. Causal relationship between stressful life events and the onset of major depression. *Am. J. Psychiatry*. 1999;156:837–841. doi: 10.1176/ajp.156.6.837
13. Schirmer, M., Smeekens, S. P., Vlamakis, H., Jaeger, M., Oosting, M., Franzosa, E. A., Xavier, R. J. (2016, November 03). Linking the Human Gut Microbiome to Inflammatory Cytokine Production Capacity. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/27814509>
14. Shi, N., Li, N., Duan, X., & Niu, H. (2017, April 27). Interaction between the gut microbiome and mucosal immune system. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28465831>
15. Ye, X., Wang, R., Bhattacharya, R., Boulbes, D. R., Fan, F., Xia, L., . . . Ellis, L. M. (2017, July). Fusobacterium Nucleatum Subspecies Animalis Influences Proinflammatory Cytokine Expression and Monocyte Activation in Human Colorectal Tumors. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28483840>
16. Weiss, G., Schroecksadel, K., Mattle, V., Winkler, C., Konwalinka, G., & Fuchs, D. (2004, February). Possible role of cytokine-induced tryptophan degradation in anaemia of inflammation. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/14962250>
17. Waclawiková, B., & El Aidy, S. (2018, June 25). Role of Microbiota and Tryptophan Metabolites in the Remote Effect of Intestinal Inflammation on Brain and Depression. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6160932/>
18. Herédi, J., Berkó, A. M., Jankovics, F., Iwamori, T., Iwamori, N., Ono, E., . . . Gellért, L. (2017, May). Astrocytic and neuronal localization of kynurenine aminotransferase-2 in the adult mouse brain. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/27568378>
19. Niciu, M. J., Ionescu, D. F., Richards, E. M., & Zarate, C. A. (2014, August). Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4048804/>
20. Savitz, J., Drevets, W. C., Wurfel, B. E., Ford, B. N., Bellgowan, P. S., Victor, T. A., . . . Dantzer, R. (2015, May). Reduction of kynurenic acid to quinolinic acid ratio in both the depressed and remitted phases of major depressive disorder. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/25686798>
21. Ghasemi, M., Phillips, C., Fahimi, A., McNERney, M. W., & Salehi, A. (2017, September). Mechanisms of action and clinical efficacy of NMDA receptor modulators in mood disorders. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28711661>

22. Wilkins, T., & Sequoia, J. (2017, August 01). Probiotics for Gastrointestinal Conditions: A Summary of the Evidence. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28762696>
23. Vrakas, S., Mountzouris, K. C., Michalopoulos, G., Karamanolis, G., Papatheodoridis, G., Tzathas, C., & Gazouli, M. (2017, January 18). Intestinal Bacteria Composition and Translocation of Bacteria in Inflammatory Bowel Disease. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28099495>
24. Schwarcz, R., & Stone, T. W. (2017, January). The kynurenine pathway and the brain: Challenges, controversies and promises. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/27511838>

Analyzing the cellular basis behind permanent central nervous system damage

Jason Driver

INTRODUCTION

In 2007 the World Health Organization (WHO) estimated the prevalence of those suffering from neurological disorders to be greater than 1 in 7, with more than 1 billion cases of neurological damage and 6.8 million deaths each year attributed to neurological damage¹. The central nervous system (CNS) complicates clinical recovery due to its lack of significant natural regeneration; when damage occurs it is generally permanent². The defining factor contributing to the permanent nature of CNS damage is related to the extracellular matrix (ECM) found in the CNS. The ECM is filled with growth inhibiting factors which prevents neuron growth and causes glial scarring to occur at the site of injury; the most prevalent of these growth inhibiting factors are chondroitin sulfate proteoglycans (CSPGs)³. The CSPGs found in the ECM interact with various proteins such as neural cell adhesion molecule (NCAM), neural glial cell adhesion molecule (NgCAM), and growth-inhibitory receptors expressed on the surfaces of axons^{4,10}. These interactions result in an overall inhibition of neural growth and the promotion of glial scars forming^{4,5,10}. These factors largely contribute to CNS damage being permanent if external measures are not taken.

A current area of research focuses on counteracting the growth inhibition of the CNS in order to allow for damage repair, challenging the notion that damage is permanent. Chondroitinase ABC application is a promising approach to counteracting the previously mentioned CSPGs. Chondroitinase ABC catalyzes the degradation of the chondroitin sulfate and dermatan sulfate side chains of proteoglycans, an effective way of depleting the CSPG concentration and removing a large portion of the inhibitory element found in the CNS⁶. Research into both the mechanisms making CNS damage unique and improving treatment methods is extremely important for the 1 billion suffering from CNS damage. This literature review will discuss the unique physiology of CNS damage and highlight current research being done inhibiting CSPGs in order to regain neurological health.

CSPGS AND THE UNIQUE PHYSIOLOGY OF CENTRAL NERVOUS SYSTEM DAMAGE

The resistance of the adult CNS to regeneration is rooted in its physiology, specifically its ECM. The ECM inhibits regeneration through three classes of molecules; myelin-associated growth inhibitors, chemorepulsive guidance molecules, and highly sulfated proteoglycans with the greatest contributing factor being CSPGs⁴. CSPGs are proteoglycans consisting of a protein core and a sugar chondroitin sulfate side chain; the sugar side chain sulfonation pattern directly influences the CSPG binding properties¹¹. Based on binding affinity, sulfonation pattern, and

binding location, CSPGs will regulate a neuron differently, making them highly diverse molecules¹⁰. CSPGs work through binding to members of at least two distinct classes of growth-inhibitory receptors expressed on the surfaces of axons, activating specific growth-inhibitory pathways^{4,10}.

Along with preventing regeneration, CSPGs discourage CNS damage recovery; glial scar formation is promoted preventing neural connection⁴. CSPG expressing astrocytes release the primary precursors to form a glial scar at the distal end of a damaged axon^{7,8}. At the site of an injury, astrocytes are found to rapidly upregulate the production and release of CSPGs at the injury site, causing an overexpression of CSPGs helping to contribute to the scar formation⁴. When mice models were provided both glial scar degrading factors and growth promoting factors, the axon readily regrew; when provided only growth promoting factors the axon lacks the ability to break through the glial scar and remains non-functional⁸.

CSPGs have been found to inhibit NCAM and NgCAM in the body; two molecules vital towards neuronal and glial extension during neurogenesis^{14,15}. NCAM is an immunoglobulin-like neuronal surface glycoprotein which mediates neuronal growth and adhesion guidance through binding to other cell adhesion proteins¹⁹. NgCAMs works similarly to NCAM stimulating the movement of both neural and glial cells within the CNS²⁰. The core proteins of CSPGs have high affinities for NCAMs and NgCAMs, neutralizing these growth factors through occupying what would be the site for connection²¹.

COMPARING AXON REGENERATION IN THE PERIPHERAL AND CENTRAL NERVOUS SYSTEMS

The peripheral nervous system (PNS), unlike the CNS, does regenerate post-damage and is a comparative model to better understand why the CNS does not readily regenerate¹⁰. The glia found in the PNS functionally change the ECM to be one that promotes the repair of axonal injury and prevents scarring from occurring¹⁰. Through a handful of PNS specific cell adhesion and guidance molecules neural reinnervation is supported²⁶. To prepare for recovery, Wallerian degeneration occurs; a mechanism in which the distal portion of an injured axon is cleaved and degraded to clear room for neural recovery³⁰. This process occurs in both the PNS and CNS, though in the PNS no glial scar forms, allowing for the axon to extend from the site of injury and reform a neural connection^{2,9,30}. On average, the PNS has a damaged neuron prepared for reinnervation in roughly one week. At this point Wallerian degeneration is normally finished and has cleared the way for the new axon to travel for neural recovery²⁹.

The hypothesis that glia play an important regulatory role is supported through regeneration occurring despite CSPGs and other growth inhibitory factors still being present in the PNS²⁴. When comparing the genetics of the CNS and PNS healthy glia, it was found PNS glia had genes such as GPX3, an extracellular peroxidase gene that allowed neurons to overcome inhibition and EIF2B5 a contributor to neurite growth factors²⁴. The CNS lacked many growth-promoting and counter-inhibitory genes found within the PNS²⁴. Schwann cells have the ability to secrete heparin sulfate proteoglycans (HSPGs), unlike CSPGs these molecules support cell signaling and motility in the PNS^{26,27,28}. Combined with growth promoting factors, the PNS environment supports neuronal regrowth; this genetic and physiological information can be used to better treat CNS damage towards recovery^{24,25}.

CSPGs: A FUTURE FOCUS FOR CNS DAMAGE TREATMENT

CSPGs have been shown to be the largest component of growth inhibiting factors found in the CNS^{3,5}, contributing greatly to glial scar formation and the permanent nature of CNS damage^{2,4,7}. Due to this contribution, CSPGs are a prime candidate to target for CNS recovery research. Chondroitinase ABC (ChABC) is a bacterial enzyme used to degrade the sugar sulfate side chain of CSPGs, which makes the ECM much more tolerant to axonal regrowth¹³. Direct ChABC injections to the site of injury and ChABC saturated gelfoams have been used with limited promotion of CNS regeneration observed^{16,17,18}. Rat spinal cord injuries have been counteracted using injections of ChABC with a covering of gelfoam and dental acrylic; when this method was combined with physical therapy to allow for functional movement³¹.

ChABC has also been combined with other CNS treatments in rodent trials with promising results^{22,23}. When combining a PNS nerve graft with ChABC treatment, the ChABC is administered to cleave inhibitory CSPGs in the scar matrix while growth-permissive peripheral nerve grafts allow for the reinnervation of the damaged neuronal tissue²². Melatonin has been shown to have anti-inflammatory, antioxidant, and neuroprotective effects; combining melatonin and ChABC can promote CNS regeneration²³. This result was achieved by reducing oxidative damage and inflammatory reaction in the injury area and inhibiting glial scar formation and allowing for neural regrowth²³.

Overall, there are many possible directions to take this information, and many provide promising clinical applications. CSPGs make up a large portion of the ECM in the nervous system, and as such play a large role in regulating the CNS's function. When combined with other treatment methods, research into regulating CSPGs or other neuronal matrix proteins in the body has the possibility of significantly aiding in the treatment of the high proportion of individuals suffering or who will suffer from CNS damage.

ChABC provides a promising clinical approach to trauma-based injuries such as mild traumatic brain injuries and spinal-cord crushing. When the damage is based in the loss of axonal connections and not the death of the cell itself, degrading the inhibitory molecules within the CNS allows the connection to extend. Currently, there are dozens of studies spanning decades focused on rodent models using ChABC therapeutically. The next step for this research is to use a simple model such as spinal cord lesions in primate models in order to get one step closer to clinical applications in humans.

With 1 billion cases of neurological damage, progress towards reinnervating axons in areas of damage is of great clinical importance¹. A defining reason that has caused the amount of cases to become so large is the lack of natural regeneration found in the CNS; if axonal connections are lost, they do not reconnect². This lack of regeneration is largely attributed to the extracellular matrix of the CNS, specifically CSPGs inhibiting axon extension³. It has been seen in previous research that enzymes such as ChABC successfully degrade CSPGs to help alleviate the inhibitory effects¹⁵. This method combined with other neural supportive procedures such as PNS grafts have been shown to be successful in restoring neural function in rodent models²². These tested treatment methods provide opportunities to do further research into primate models in hopes to provide progress towards human clinical use.

REFERENCES

1. Neurological disorders affect millions globally: WHO report. (2007, February 27). Retrieved March 18, 2019, from <https://www.who.int/mediacentre/news/releases/2007/pr04/en/>
2. Purves D, Augustine GJ, Fitzpatrick D, et al., editors. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Recovery from Neural Injury. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK10856/>
3. Miller, G. M., & Hsieh-Wilson, L. C. (2015). Sugar-dependent modulation of neuronal development, regeneration, and plasticity by chondroitin sulfate proteoglycans. *Experimental neurology*, 274(Pt B), 115-25.
4. Sharma, K., Selzer, M. E., & Li, S. (2012). Scar-mediated inhibition and CSPG receptors in the CNS. *Experimental neurology*, 237(2), 370-8.
5. Tan, C. L., Kwok, J. C., Patani, R., Ffrench-Constant, C., Chandran, S., & Fawcett, J. W. (2011). Integrin activation promotes axon growth on inhibitory chondroitin sulfate proteoglycans by enhancing integrin signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(17), 6289-95.
6. Corvetto, L., & Rossi, F. (2005). Degradation of chondroitin sulfate proteoglycans induces sprouting of intact purkinje axons in the cerebellum of the adult rat. *Journal of Neuroscience*, 25(31), 7150-7158.
7. McKeon, R. J., Jurynek, M. J., & Buck, C. R. (1999). The chondroitin sulfate proteoglycans neurocan and phosphacan are expressed by reactive astrocytes in the chronic CNS glial scar. *Journal of Neuroscience*, 19(24), 10778-10788.
8. Anderson, M. A., Burda, J. E., Ren, Y., Ao, Y., O'Shea, T. M., Kawaguchi, R., & Sofroniew, M. V. (2016). Astrocyte scar formation aids central nervous system axon regeneration. *Nature*, 532(7598), 195.
9. Höke, A., Redett, R., Hameed, H., Jari, R., Zhou, C., Li, Z. B., ... & Brushart, T. M. (2006). Schwann cells express motor and sensory phenotypes that regulate axon regeneration. *Journal of Neuroscience*, 26(38), 9646-9655.
10. Lutz, A. B., & Barres, B. A. (2014). Contrasting the glial response to axon injury in the central and peripheral nervous systems. *Developmental cell*, 28(1), 7-17.
11. Properzi, F., Asher, R. A., & Fawcett, J. W. (2003). Chondroitin sulphate proteoglycans in the central nervous system: changes and synthesis after injury.
12. Lemons, M. L., Sandy, J. D., Anderson, D. K., & Howland, D. R. (2003). Intact aggrecan and chondroitin sulfate-depleted aggrecan core glycoprotein inhibit axon growth in the adult rat spinal cord. *Experimental neurology*, 184(2), 981-990.
13. Crespo, D., Asher, R. A., Lin, R., Rhodes, K. E., & Fawcett, J. W. (2007). How does chondroitinase promote functional recovery in the damaged CNS?. *Experimental neurology*, 206(2), 159-171.
14. Prag, S., Lepekhin, E. A., Kolkova, K., Hartmann-Petersen, R., Kawa, A., Walmod, P. S., & Pedersen, N. (2002). NCAM regulates cell motility. *Journal of cell science*, 115(2), 283-292.
15. Hu, J., Curinga, G. M., & Smith, G. M. (2015). Chondroitinase Gene Therapy for Spinal Cord Injury. In *Extracellular Matrix*(pp. 139-149). Humana Press, New York, NY.
16. Lemons, M. L., Howland, D. R., & Anderson, D. K. (1999). Chondroitin sulfate proteoglycan immunoreactivity increases following spinal cord injury and transplantation. *Experimental neurology*, 160(1), 51-65.
17. Yick, L. W., Cheung, P. T., So, K. F., & Wu, W. (2003). Axonal regeneration of Clarke's neurons beyond the spinal cord injury scar after treatment with chondroitinase ABC. *Experimental neurology*, 182(1), 160-168.
18. Yick, L. W., Wu, W., So, K. F., Yip, H. K., & Shum, D. K. Y. (2000). Chondroitinase ABC promotes axonal regeneration of Clarke's neurons after spinal cord injury. *Neuroreport*, 11(5), 1063-1067.
19. Weledji, E. P., & Assob, J. C. (2014). The ubiquitous neural cell adhesion molecule (N-CAM). *Annals of medicine and surgery (2012)*, 3(3), 77-81. doi:10.1016/j.amsu.2014.06.014
20. Grumet, M., & Edelman, G. M. (1988). Neuron-glia cell adhesion molecule interacts with neurons and astroglia via different binding mechanisms. *The Journal of cell biology*, 106(2), 487-503.
21. Dow, K. E., & Wang, W. (1998). Cell biology of astrocyte proteoglycans. *Cellular and Molecular Life Sciences CMLS*, 54(6), 567-581.
22. Tom, V. J., Sandrow-Feinberg, H. R., Miller, K., Santi, L., Connors, T., Lemay, M. A., & Houlié, J. D. (2009). Combining peripheral nerve grafts and chondroitinase promotes functional axonal regeneration in the chronically injured spinal cord. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(47), 14881-14890. doi:10.1523/JNEUROSCI.3641-09.2009
23. Guo, W. L., Qi, Z. P., Yu, L., Sun, T. W., Qu, W. R., Liu, Q. Q., ... Li, R. (2019). Melatonin combined with chondroitin sulfate ABC promotes nerve regeneration after root-avulsion brachial plexus injury. *Neural regeneration research*, 14(2), 328-338. doi:10.4103/1673-5374.244796
24. Buchser, W. J., Smith, R. P., Pardinis, J. R., Haddox, C. L., Hutson, T., Moon, L., ... Lemmon, V. P. (2012). Peripheral nervous system genes expressed in central neurons induce growth on inhibitory substrates. *PLoS one*, 7(6), e38101. doi:10.1371/journal.pone.0038101
25. Markus, A., Patel, T. D., & Snider, W. D. (2002). Neurotrophic factors and axonal growth. *Current opinion in neurobiology*, 12(5), 523-531.
26. Navarro, X., Vivó, M., & Valero-Cabré, A. (2007). Neural plasticity after peripheral nerve injury and regeneration. *Progress in neurobiology*, 82(4), 163-201.

27. Sarrazin, S., Lamanna, W. C., & Esko, J. D. (2011). Heparan sulfate proteoglycans. *Cold Spring Harbor perspectives in biology*, 3(7), a004952. doi:10.1101/cshperspect.a004952
28. Lawrence, R., Yabe, T., Hajmohammadi, S., Rhodes, J., McNeely, M., Liu, J., ... Shworak, N. W. (2007). The principal neuronal gD-type 3-O-sulfotransferases and their products in central and peripheral nervous system tissues. *Matrix biology : journal of the International Society for Matrix Biology*, 26(6), 442–455. doi:10.1016/j.matbio.2007.03.002
29. Menorca, R. M., Fussell, T. S., & Elfar, J. C. (2013). Nerve physiology: mechanisms of injury and recovery. *Hand clinics*, 29(3), 317–330. doi:10.1016/j.hcl.2013.04.002
30. Gaudet, A. D., Popovich, P. G., & Ramer, M. S. (2011). Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *Journal of neuroinflammation*, 8, 110. doi:10.1186/1742-2094-8-110
31. Manohar, A., Foffani, G., Ganzer, P. D., Bethea, J. R., & Moxon, K. A. (2017). Cortex-dependent recovery of unassisted hindlimb locomotion after complete spinal cord injury in adult rats. *eLife*, 6, e23532. doi:10.7554/eLife.23532

