NEUROANATOMY

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 life[1]. The Mayo Clinic[2] 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 expression[5]. 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 situations[6]. The D3 receptor has also been thought to signal when rewards are to be expected in uncertain circumstances[3]. The 5-HT2b receptors are highly expressed on the VENs but rarely in other areas of the brain[3]. However, the VENs are found in relatively large proportions in the human stomach and intestines[3]. 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 punishment[3]. Together,

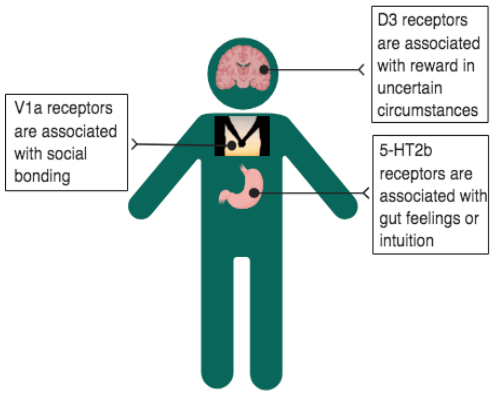

Figure 4. Associations of the three receptors found on von economo neurons of the anterior cingulate cortex.

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 humans[8]. 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.

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NEUROANATOMY

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 in[1]. 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 emotion[2]. 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 memory[5]. 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 consequences[6]. 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 processing[7], 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 disorders[8]. 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.

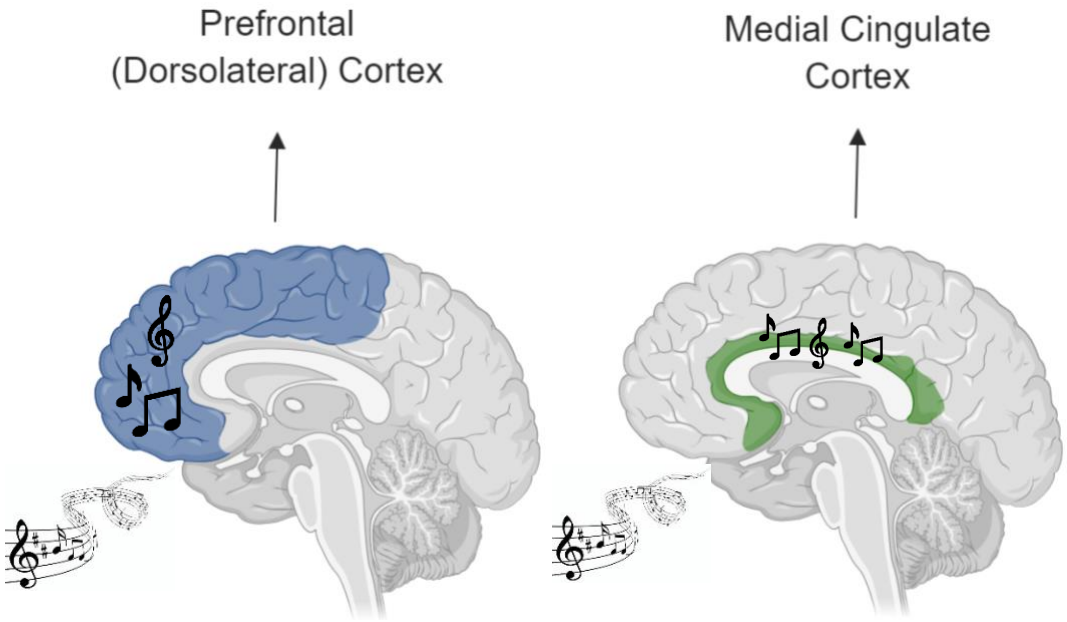

Figure 1

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 function[12].

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NEUROANATOMY

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 neurotropic factor (BDNF) was correlated with the memory restoration in MDD patients being treated with antidepressants[1]. 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 prognosis[2]. 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 memory[3]. 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 normalization[4].

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 treatment[5] 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 depression[6]. 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 stress[7]. 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 outcome[8]. Will BDNF treatment follow the same pattern or will XPJY 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 rodents[9]. 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.

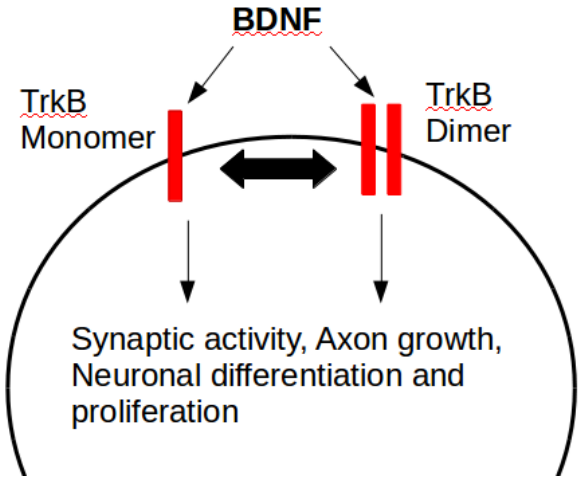

Figure 1. A basic overview of the BDNF-TrkB system. It has been theorized that a large part of the functioning of BDNF is based on the membrane ratio between TrkB monomerization and dimerization. BDNF has a large impact of DNA expression and its effects go well beyond memory and learning, including formation of the CNS by influencing proliferation and differentiation of neurons[12].

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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 programed 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

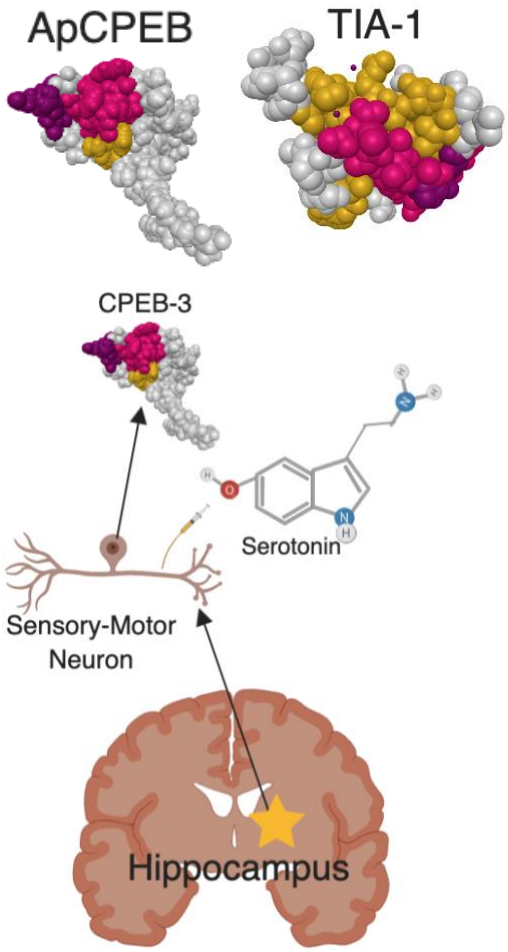

Figure 5. The persistence of synaptic plasticity and memory storage requires CPEB-3-mediated protein synthesis in the hippocampus. Serotonin pulse injections for synaptic memory-consolidation.

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 were 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 were 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 post 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-1for 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 programed 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.

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NEUROPHYSIOLOGY

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 body[1]. 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 functions[5].

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 body[6]. CB1 receptors are predominately found in the brain as neuronal plasma membrane receptors[7]. They are also responsible for binding delta-9-Tetrahydrocannnabinol (THC), the main psychoactive compound of cannabis[8]. 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 anandamide[11]. 2-AG and anandamide are both molecules produced within the body that may bind to CB1 receptor[12]. Gaining great attention is new research finding improved cognitive function in old mice receiving chronic low doses of THC[13]. 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).

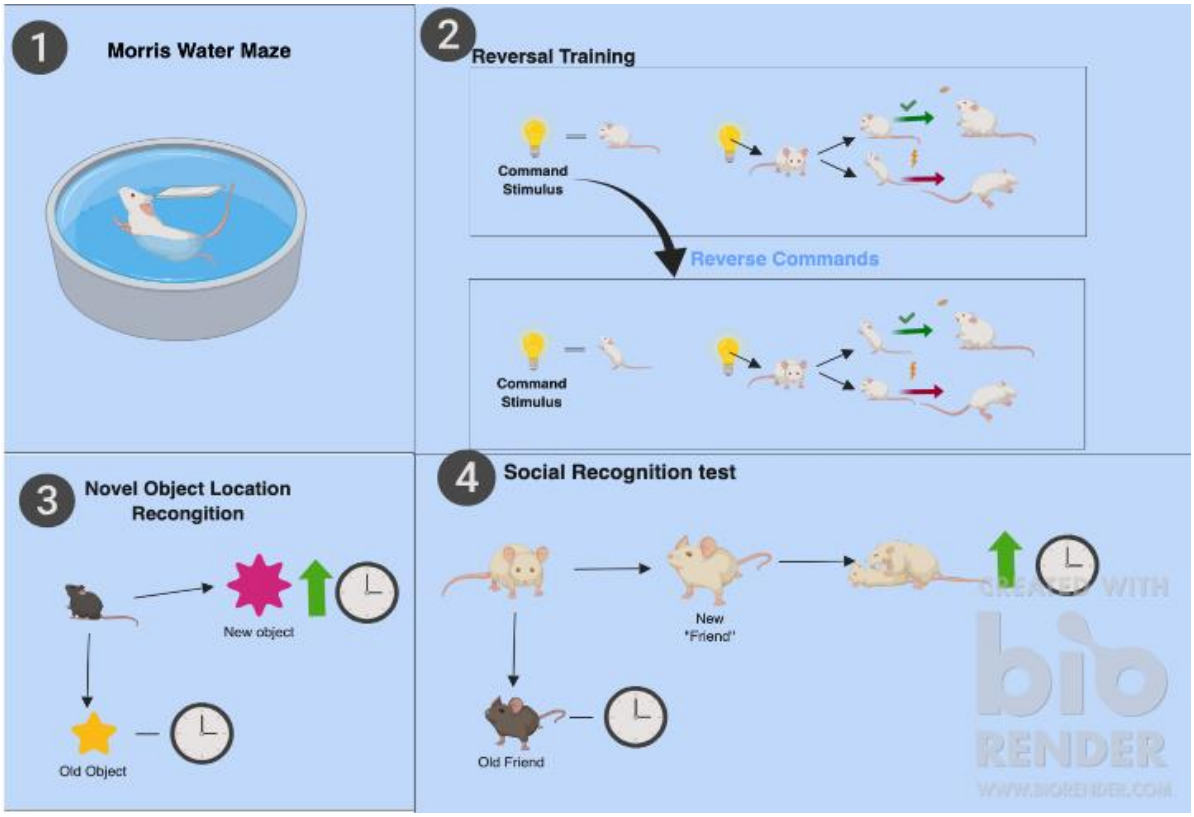

Figure 6. *1) Morris Water Maze to test spatial learning and memory 2) Reversal Training to test for learning flexibility 3) Novel Object Location Recognition and 4) Social Recognition test to test long-term memory. (Bilkei-Gorzo et al. 2017)*

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.

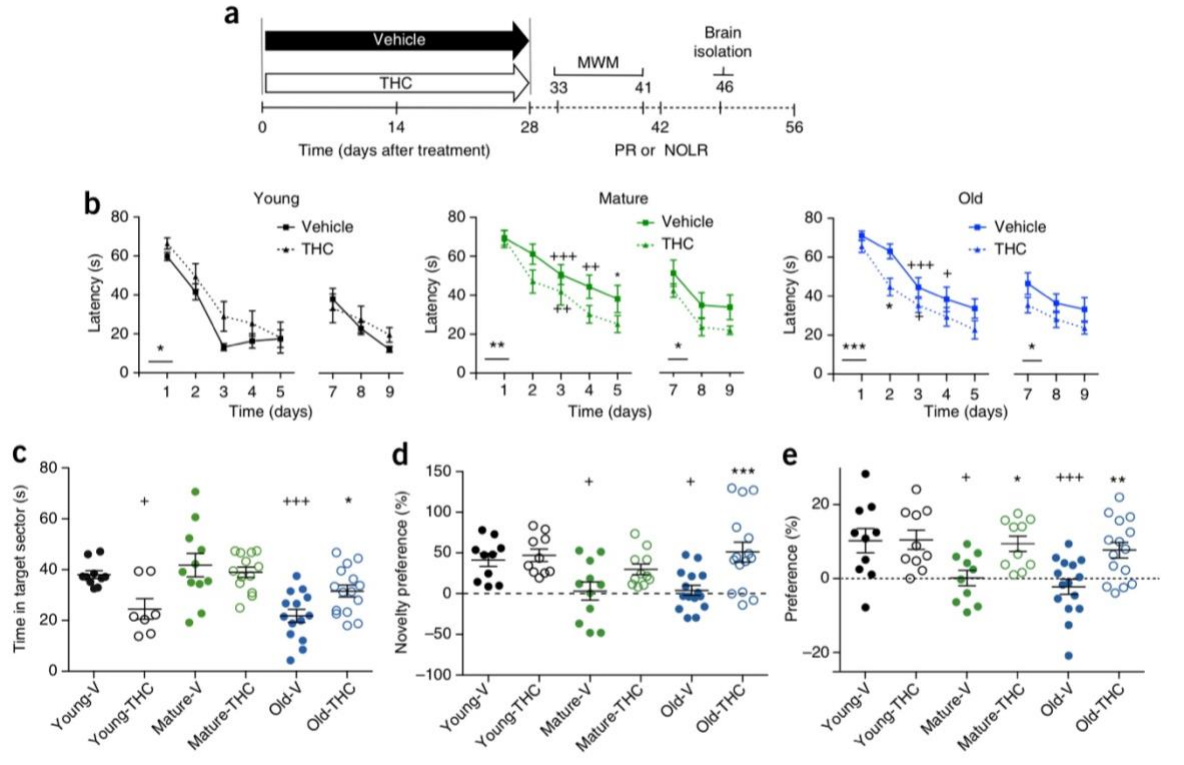

***Figure 7**. A) Timeline of treatment B) Morris Water Maze C) Probe Reversal Phase Testing D) Novel Object Recognition E) Social Recognition Test. (Bilkei-Gorzo et al. 2017)*

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NEUROPHYSIOLOGY

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 damage[1]. 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 life[2]. 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 CSPGs[3].

It has been seen for centuries that the central nervous system resists regeneration, with spinal cord injuries and brain trauma being permanent[4]. 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 CSPGs[5]. 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 phosphatases[6]. 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 (PTPσ) are both CSPG signalling receptors of oligodendrocytes in the CNS[7]. It's been seen that inhibition of PTPσ and LAR receptors promotes oligodendrogenesis, supporting recovery of the spinal cord[6].

The article in focus from Dyck et al., 2019 goes into more depth on how the LAR PTPσ pathway can be targeted in a potentially clinically beneficial manner[8].

The study used female Sprague Dawley rats and targeted LAR and PTPσ through LAR peptide (ILP) and intracellular sigma peptide (ISP); two peptides blocking the receptors for LAR and PTPσ[9]. 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.

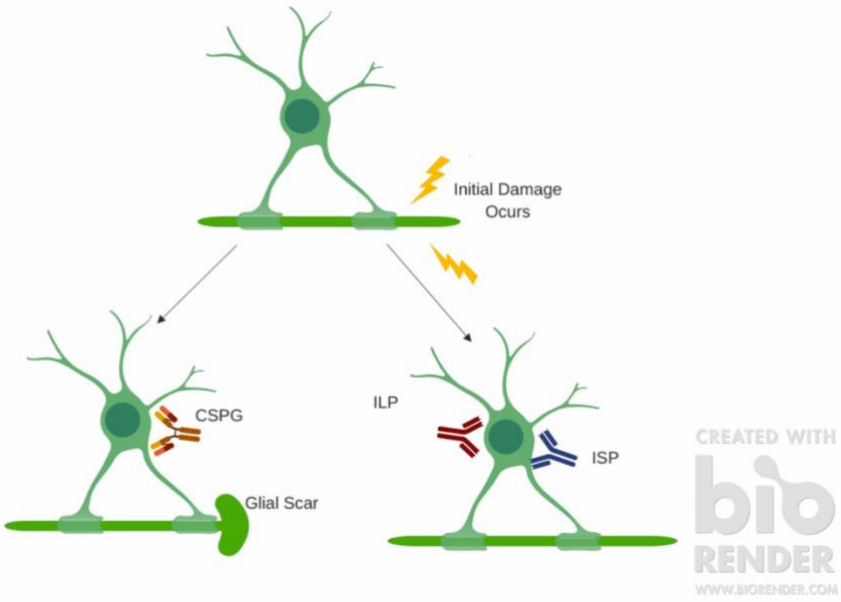

Figure 1. A cartoon model showing the divergence of CNS damage relating to CSPG binding presence. When ILP and ISPs block the receptor for CSPG, inflammation and damage-associated scars do not occur as easily.

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NEUROPHYSIOLOGY

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 disorder[1]. 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 rodents[3]. 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 addiction[4]. 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 event[9]. 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].

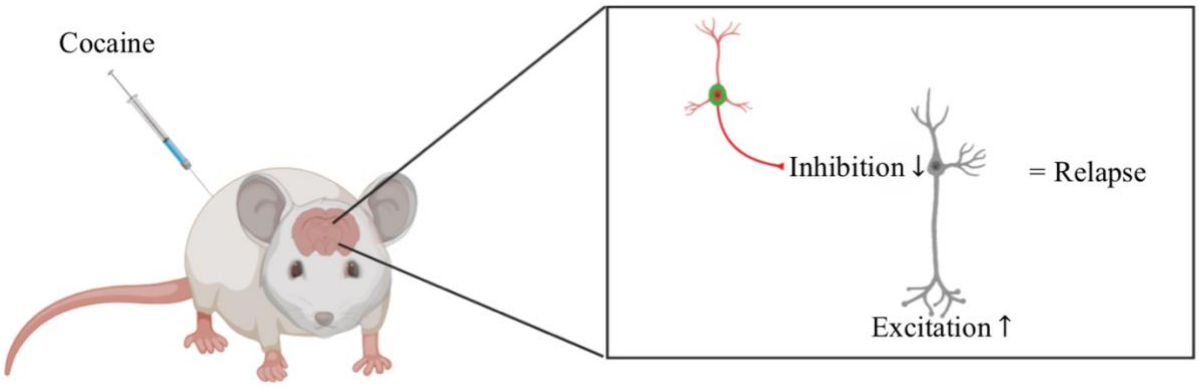

Figure 1. Cocaine administration induces changes in PNN (green) surrounded FSI (red) function, increasing excitatory output by principal neurons (grey) and promoting relapse.

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 networks[2]. 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 function[12]. 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.

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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 harassment[1]. 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 harassment[5]. These harassed animals may then be administered anxiolytics such as diazepam to attenuate the anxiety-like behavior[6]. 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, Delbecque, 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.

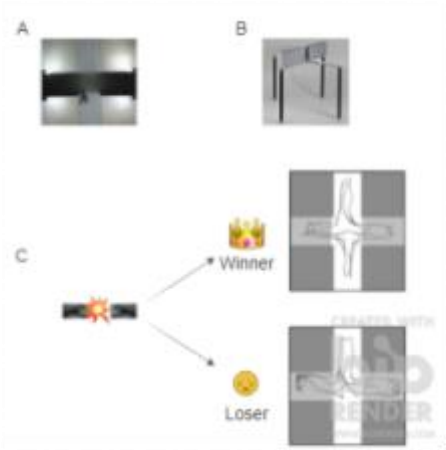

Fig. 1 **Light/dark plus maze. A)** Image of crayfish within the light/dark maze. **B)** Image of mouse within analogous maze. **C)** After social harassment period, crayfish routes within maze tracked with Ethovision software showed winning crayfish exploring the whole maze while losing crayfish stayed 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 antianxiety drug commonly used due to its low rate of intolerable side effects[10]. 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.

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NEUROPHYSIOLOGY

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 time[2]. As opposed to the standard 40 hours workweek, the average American works a 47-hour per week, which is equivalent to working an extra day[3]. Forty seven percent of workers argue that they do not have enough free time; American mothers only have an estimated 36 minutes of leisure time[4]. 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 nap[1]. 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 researches 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.

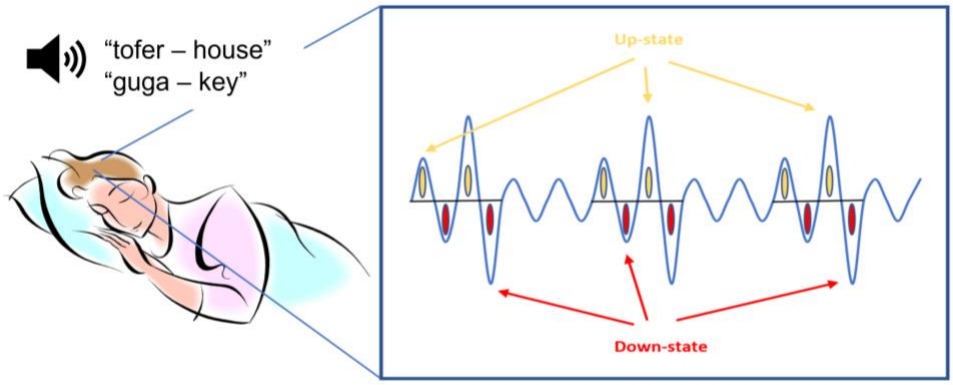

Figure 1. Representation of the EEG study during sleep. The figure represent sleeping person during deep sleep where brain electrical activity recording is performed using electroencephalogram (EEG). Left side of the image shows sleeping person being introduced to a pseudoword and a German translation 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).

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NEUROPHYSIOLOGY

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 is 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.

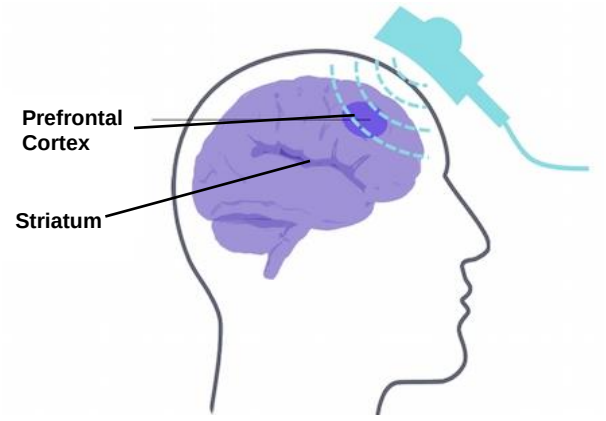
Substance use disorders (SUDs) represent a major public health concern worldwide and are a leading cause of disease and mortality.[1,3,4] The addiction becomes compulsive and difficult to control despite harmful consequences.[3,4] There are about 27.7 million users in the western world alone allowing 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 contain 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.

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NEUROPHYSIOLOGY

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) symptoms[1]. Recent estimates for the prevalence of PTSD in the U.S. suggest that approximately 3.5% of the population is affected by the disorder[2]. The symptoms associated with PTSD can have a detrimental effect on quality of life, putting individuals at a higher risk of suicide[3]. Common treatments for PTSD symptoms are medications, such as selective serotonin reuptake inhibitors (SSRIs), or psychosocial interventions, such as cognitive behavioral therapy[4]. However, even when these treatments are combined, the majority of patients do not experience significant improvement in their symptoms[5]. 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 disorders[6].

External trigeminal nerve stimulation therapy can be used to send signals to the brain by stimulating the trigeminal nerve with small electrical currents[7]. 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 PTSD[8]. 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 µsec, 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.

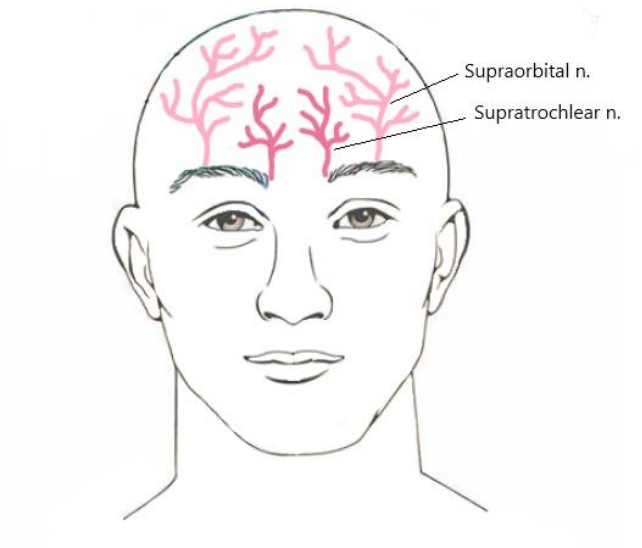

Figure 1. Surface electrode placement for eTNS therapy. During the 8-week eTNS intervention, participants applied stimulation to the supraorbital and supratrochlear branches of the trigeminal nerve for 8 hours each night. This non-invasive neuromodulation therapy has shown to be therapeutic for several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

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 implantations[11]. 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.

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NEUROPHYSIOLOGY

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 striatum[1]. 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 sleep[2].

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 impulses[3]. 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 stimulator[4]. 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 term[5].

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.

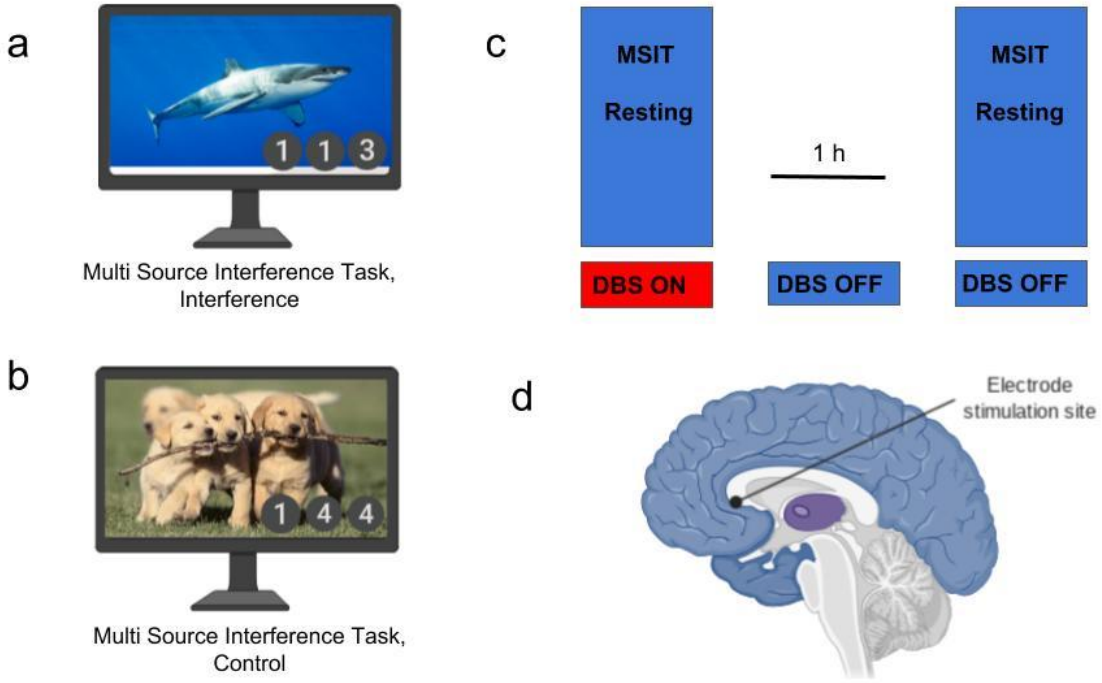

Figure 1. Experiment Design and Behavior. a,b.) Visual demonstration on participants perspective during multi-source interference task. Emotionally arousing images are displayed in conjunction with a series of three numerical values, one of which different from the rest. Distractors like the shark are 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.

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NEUROPHYSIOLOGY

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 females[1].

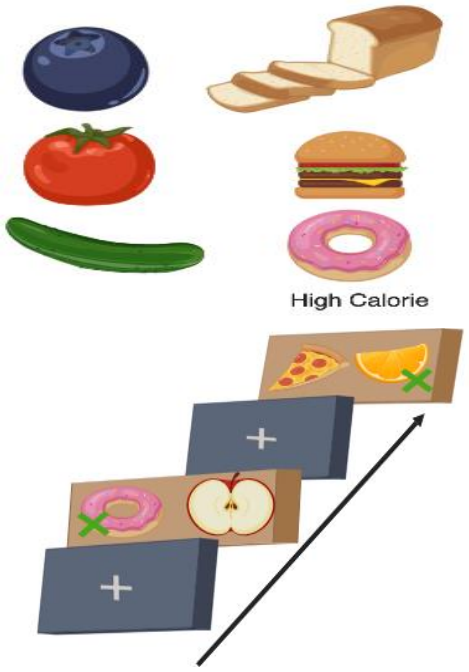
In the last decade more studies about food behaviors and food choices have become a higher priority due to the large availability of food in western countries[1,2,3]. This results in individuals to shift from eating for survival to eating for pleasure[1,2,3]. This style of eating has led people to choose 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 population[7]. The treatment took significantly longer for women and men that tested higher with severe concerns with body weight/shape[4]. As males who tested low for concerns with body weight/shape treatment was significantly lower[4]. This trend of women being more concerned with weight and body shape than men is common[4].

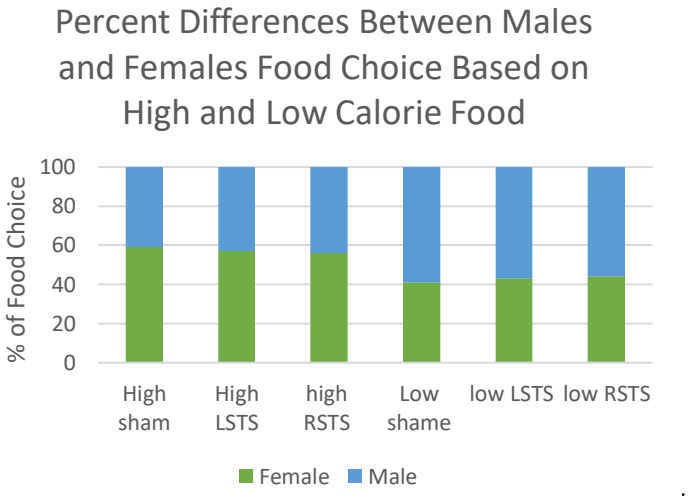
A study conducted by Uccula and Nuvoli found that women tend to be more invested in food choices resulting in women to overestimate their weight and decrease amount of meals (also limiting themselves to healthier food options such as higher intakes of dietary fibers and low intake of fats and salts)[1,8]. 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 cognition[8]. In other studies, evaluating brain region correlation to food choices found that the superior temporal sulcus (STS) was activated

during force 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 foods[1]. 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 secs[1].





***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

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/ 0 = very healthy)[1].

These choices were used to independently create food pairing for each participant to be used during the tRNA task[1]. During the tRNA task, the selected photos for each participant were presented in a random order[1]. Each participant was told to choose the product they most wanted to eat[1]. 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 STS[1].

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.

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NEUROPHYSIOLOGY

Amyloid-β betta 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 disease[1]. 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 cases[2]. Furthermore, it is estimated that by 2050, diagnoses will increase to every 33 seconds[1]. Currently, Alzheimer's disease is the 6th most common cause of death and has already deaths caused by stroke, heart disease, and prostate cancer combined[1]. This results in over $236 billion dollars a year in health care fees[1]. 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**-secretases[6] (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 neurodegeneration[7]. 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 short[2] (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 vesicle[9] (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 disease[2]. 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.

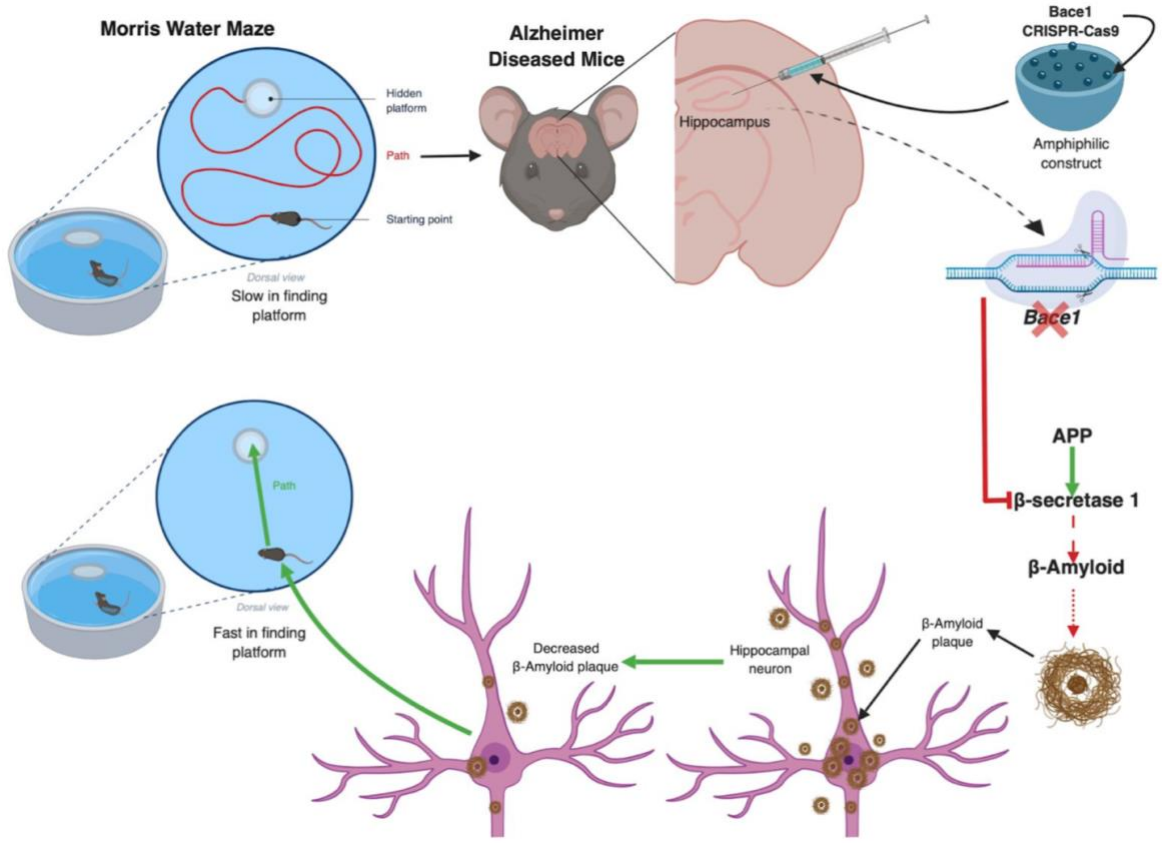

Figure 1: Nanoparticle injection into the hippocampus of Alzheimer diseased mice significantly reduced β-amyloid plaques and improved time to find platform with Morris Water Maze Test. Use of amphiphilic-CRISPR-Cas9 construct to genetically silence the function of **β**-secretase 1 expression significantly 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* expression[2]. 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 mice[2]. 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-injection[2]. Lastly, after the injection there was no observed increase in inflammation, cell death or microglia recruitment[2].

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.

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NEUROPHYSIOLOGY

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.

Cheyanne 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 other[1]. 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 wakefulness[2]. 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 stress[3]. 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 night[6]. 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 threshold[9]. The cingulate cortex as well as the insula are responsible for the higher order

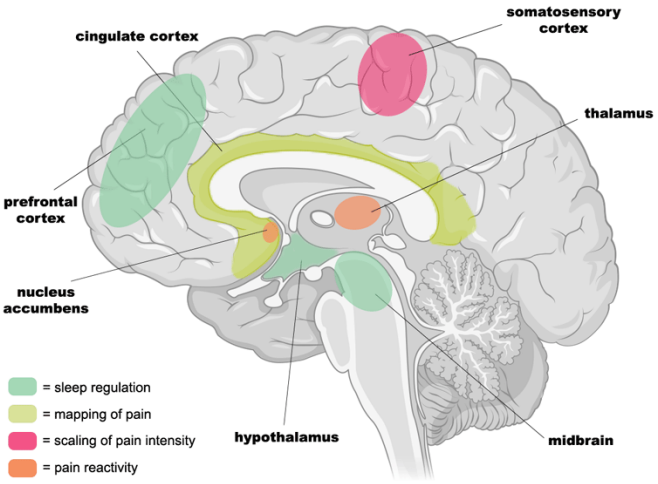

Figure 1. Anatomy of sleep and the pain reactivity network. Following sleep deprivation, various cortical and limbic regions change activation patterns in relation to both the perception and processing of painful stimuli.

processing of internal state changes in response to pain[10] while the nucleus accumbens encodes for emotional value and saliency[11]. 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.

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NEUROPHYSIOLOGY

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 before[1]. 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 solution[2]. Research has attempted to find what causes this Eureka moment, with various theories about its neural basis[1]. 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 answer[3]. 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 manner[1]. 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 theta[4]. 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 processing[5].

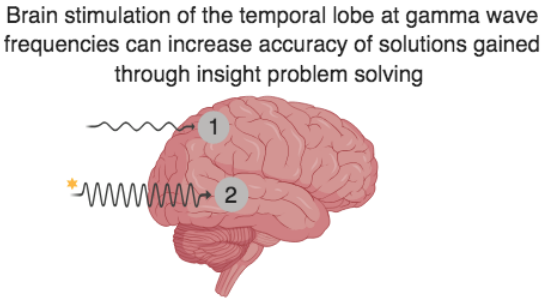

Figure 8. Brain stimulation was applied at the parietal-occipital region (1) and the temporal region (2). The star indicates the area stimulated to improve accuracy of CRA puzzles.

Santarnecchi 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 activity[6].

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 saying[8]. 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.

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NEUROPHYSIOLOGY

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.

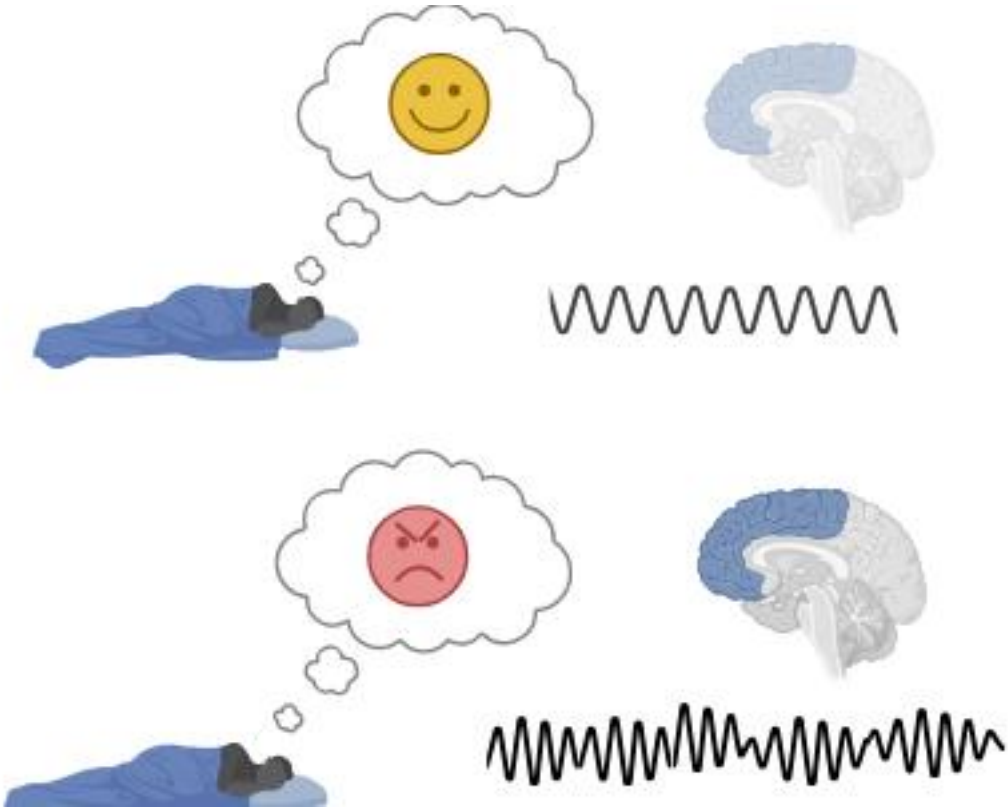

Figure 9. This figure demonstrates that the relationship between REM sleep FAA and anger during dreaming were driven by increased alpha power in the right frontal cortex. People dreaming with non-negative emotions had reduced FAA.

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NEUROPHYSIOLOGY

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 neurons[1]. Specifically, the excitatory neurons in areas of the brain important in executive function and memory formation[1]. This is formally known as the "Glutamate Hypothesis" of depression. Depression is also associated with loss of dendritic spines[2]. Dendritic spines are tiny protrusions coming off of neurons. Additionally, another hallmark of depression is chronic stress[3]. 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 effect[4]. Severe symptoms of depression include suicidal ideation and self harm. Thus, therapeutic onset of antidepressant medication is extremely important in these circumstances[5]. In humans, ketamine has shown promise in acutely reversing depression symptoms within 24 hours[6]. 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

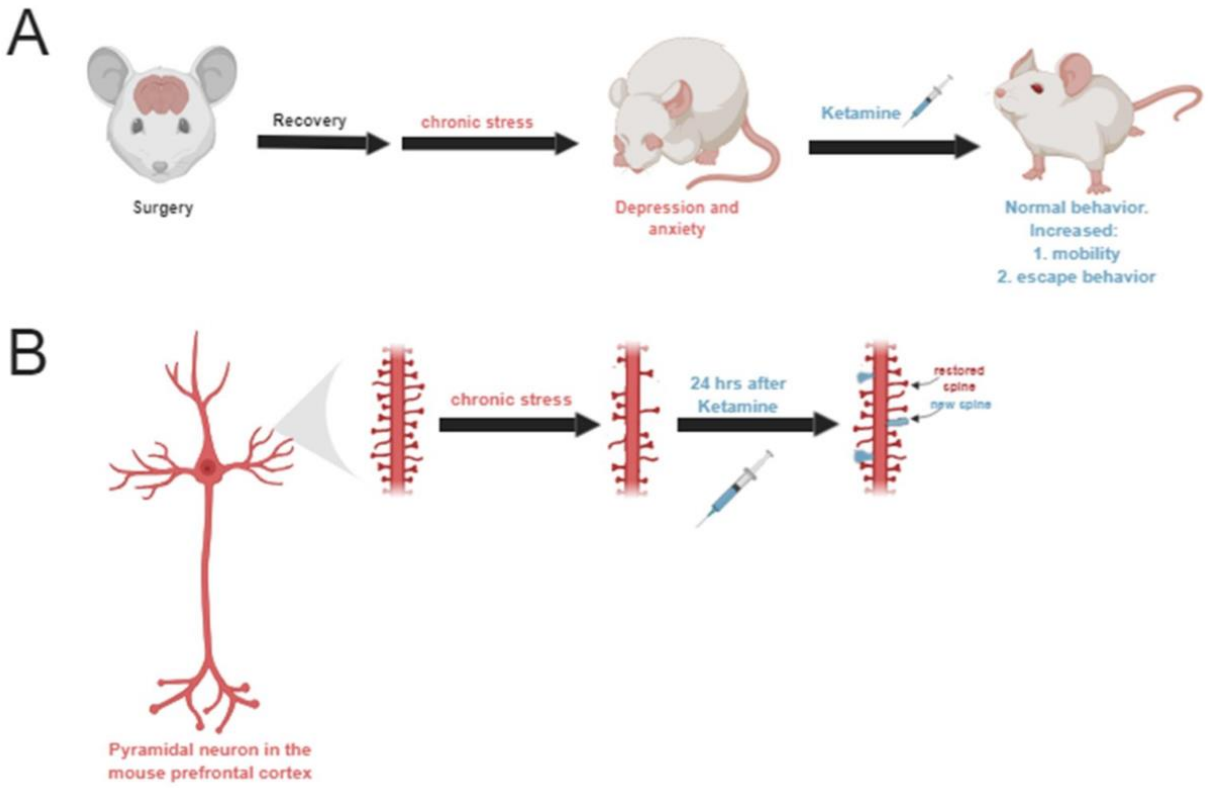

Figure. **Behavioral and structural changes of pyramidal dendritic spines after exposure to chronic stress and ketamine. (A)** General timeline of the experiment. Surgery with implantation of prism allowing for a 2 week recovery time. Next, the mice were subjected to 21 d of chronic stress treatment (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.

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NEUROPHYSIOLOGY

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 unclear[1]. 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 diet[2]. 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 neurons[3]. 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 conditions[7]. 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.

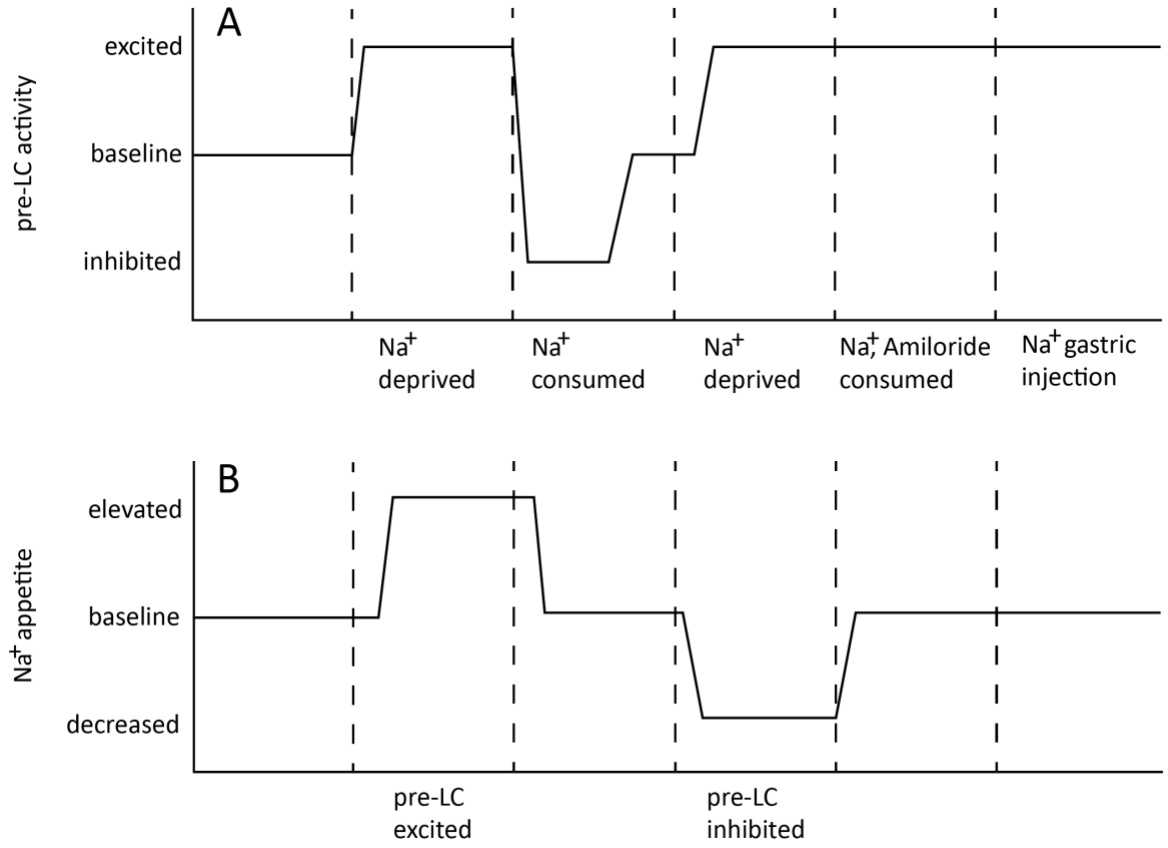

Figure 1. Schematic summary of observed effects. A. Level of Ca^{2+} fluorescence in pre-LC region. A prolonged low-sodium diet resulted in increased pre-LC activity. Subsequent oral consumption of sodium inhibited pre-LC activity, but gastric injection of sodium did not. Treatment with Amiloride blocked 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.

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NEUROPHYSIOLOGY

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 at al., discusses that when pesticides are used in an outside location they can easily contaminate inside buildings[1]. 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 pesticide[2]. 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 trout[3], carp[4]), "increased carbonyl levels" (In freshwater fish[5]), "DNA Fragmentation'' (in Male albino rats[6]), and "DNA damage" (Female Wistar Rats[7]).

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 C[8]. Cytochrome C then acts to increase ROS production. ROS are dangerous to the cell since they can cause DNA damage, and apoptosis[9].

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.

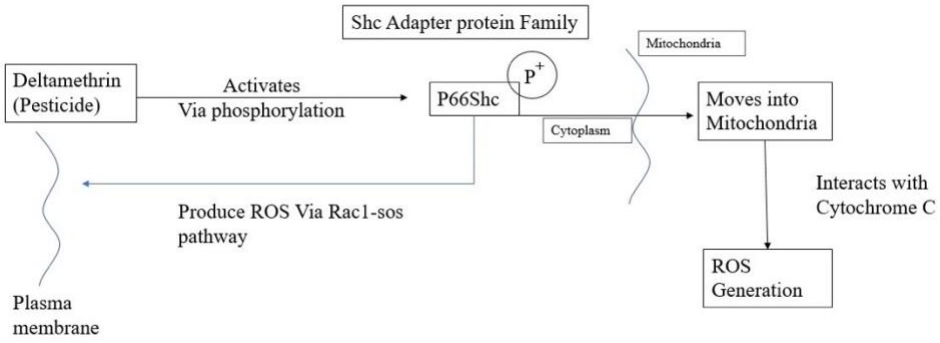

Figure 1.

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.

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spotlight

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!

CAPSTONE

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 adulthood[1]. 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 disorders[2]. 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 humans[5]. 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 drug[6]. In humans, stressful situations such as harassment result in abnormal, fluctuating serotonin levels and short-term anxiety[7]. 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 impacts[8]. 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 behavior[9]. Similarly, *C. elegans* are used in neural development research, as they have accessible and plain nervous systems[10]. 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 can[11]. 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 experiment[12]. However, the social behaviors of a model organism are an important variable to account for, especially when humans are also a social species[13]. 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 memory[14]. 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 pallium[22]. Sleep is also essential to avian memory consolidation, although it follows an unknown, alternate consolidation pathway that is thought to end within the avian pallium[23]. Do honeybees, with their circadian rhythms and mushroom bodies functioning as analogs to mammalian sleep patterns and vertebrate cortices, also utilize sleep for memory consolidation[24]?

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 response[25]. 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 environment[29]. 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 ants[30]. 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 nestbox[31].

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 pathway[16]. 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 memory[32]. 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 habitation, 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 training[15]. 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 environment[35]. 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 crustaceans[36]. 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.

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CAPSTONE

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

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INTRODUCTION

Sensorineural hearing loss accounts for about 90% of reported hearing loss[1] 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 lobe[4] 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 (AI) 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 representations 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.

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CAPSTONE

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 cancer[1]. 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 sounds 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 sepsis[4]. 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 adult[8]. 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 loaded[11]. 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 time[12]. The retention of aminoglycosides and the cells' higher metabolic rates are likely the reasons for the cells' increased susceptibility to aminoglycoside-induced damage[12]. 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 channels[13]. The liquid surrounding hair cells, called endolymph, has a strong positive membrane potential, whereas the cytoplasm of hair cells has a strong negative membrane potential[14]. This creates a strong electrochemical force that drives cations, including aminoglycosides, through open non-selective cation channels, such as MET channels[14]. MET channels are complexes composed of TMC (transmembrane-channel) proteins forming the pore[15], myosin VIIA in the hair bundles[16], and Cadherin 23/Protocadherin 15 proteins forming the tip links[17], 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 cells[18]. 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 death[20]. 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 cell[20]. 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 function[23]. Calreticulin is a chaperone protein within the endoplasmic reticulum[24,25] responsible for protein folding and degradation[26]. Calreticulin is highly expressed in hair cells' stereocilia[25]. Aminoglycosides act as competitive antagonists, preventing calcium from binding to calreticulin, resulting in a loss of calreticulin function[25]. This disruption likely results in cytotoxicity[27,28]. CLIMP-63 is another protein within the endoplasmic reticulum[23], responsible for anchoring microtubules to the endoplasmic reticulum[29]. Aminoglycosides bind to CLIMP-63, and CLIMP-63 then binds to 14-3-3 proteins[23]. 14-3-3 proteins then bind to and inactivate MDMX, a negative regulator of p53. p53 is a well-known tumor suppressor protein[30]. 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 pathways[31]. 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 IP_3 (inositol triphosphate) receptors[32]. This increases mitochondrial calcium, resulting in increased oxidative phosphorylation and consequent accumulation of cytotoxic levels of reactive oxygen species[33]. 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 apoptosis[34]. Another mechanism of aminoglycoside-induced hair cell death includes the activation of the stress-induced c-Jun N-terminal kinase pathway[35]. 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 gentamicin[38]. All eight drugs shared a similar quinoline structure, and are thought to reduce aminoglycoside uptake into hair cells[38]. In another study, four plant alkaloid compounds with bisbenozquinoline 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 exposure[39]. These include berbamine, E6 berbamine, hernandezine, isotetrandrine – with E6 berbamine being most protective[39]. 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 channel[39]. In a following study, berbamine was examined further, along with another otoprotectant compound d-Tubocurarine (dTC), in zebrafish and neonatal mice[40]. 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 death[40]. 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 channels[41]. 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 either[41]. These results indicate protection downstream of aminoglycoside entry[41]. 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 activity[42]. 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 bactericidal[43]. 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 death[35]. 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 gentamicin[31]. 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 prevention[30]. 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

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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.

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CAPSTONE

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 tumors[1]. About 15% of these cases occurs in children between 0-15 years old and 85% in adolescents, adults, and elderly[1]. GBM (GBM in pediatrics are called high grade glioma, HGG) in children are much rarer to occur compared to adults[1]. 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 patients[3]. 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 7[4]. The key role EGFR in GBM, is thought to be involved in pathogenesis, longevity, and recurrence of the tumor[5].

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 deleted[4]. Overexpression of wtEGFR or EGFRvIII has been found to increase activation of EGFR proteins[4]. 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 EGFRvIII[8]. 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 protein[1]. This causes constitutive phosphorylation of tyrosine by EGFRvIII[1]. Since EGFRvIII cannot bind to the receptor, the receptor is internalized more slowly[1]. 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 EGFRvIII[6]. Thus, they concluded that this mechanism may be the same mechanism seen in adult EGFRvIII mutation[7]. 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 p27[2]. The EGFR that are altered in GBM are p16 and p27[2]. As in HGG, alteration in p16 and p27 are rare and a majority of the p53 are deleted[2]. Also, they found that deletion in p53 results in transcription of EGFRvIII in HGG due to missense mutation[2]. 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 knowns 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.

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CAPSTONE

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 lifetime[1]. 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 alone[2]. 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 brain[6]. 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 mice[7]. The absence of PNNs allow for freer formation of synaptic connections and creation of associations between previously unrelated stimuli[8]. For this reason, it is thought that the construction of perineuronal nets indicates the closure of various critical periods, such as the visual critical period[6]. 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 dominance[9]. 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 Cajal[10]. 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 OD[9].

OD is a deficit marked by the disproportion allocation of cortical neurons to one eye over the other[9]. 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 adults[9]. 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 animal[12]. 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 density[12]. 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' lifetime[15]. 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 administrating 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 alone[13]. 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 control[6]. 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 timepoint[6]. 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 association[14]. 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 CSPGs[17]. 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 PNNs[18]. 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 integrity[19]. 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 disorders[20]. The administration of fluoxetine to adult rats helps shift cortical neurons to monocularly deprived eyes in OD trials, like the results seen by PNN degradation[20]. 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 one[21].

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.

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CAPSTONE

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 year[1]. Parkinson's disease (PD) is a disease that is defined by dysfunction in motor movement as well as the existence of Lewy bodies[2]. 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-1[3]. 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 nigra[2].

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 cortex[2]. 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 cortex[2]. 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, exasperating 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 dysregulation[2]. Not only can DNA play a role, but mitochondrial dysfunction and oxidative stress[4] 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 dysfunction[5]. 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 cases[2], 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 families[2].

While there are different genetic mutations that can cause PD, one common mutation is in the gene SCNA. SCNA codes for the protein alpha synuclein which is found within Lewy bodies[2]. The higher the number of copies of SNCA the more alpha synuclein produced within the brain[2]. The higher number of copies of SNCA and therefore alpha synuclein concentrations correlates with a higher risk of developing PD[2].

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 life[2]. 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 SCNA that causes familial PD is A53T. The A53T mutation has shown to cause misfolding of Alpha synuclein[6]. The second mutation, G2019S is in the gene coding for LRRK2, which leads to increased kinase activity[6]. Both mutations have been correlated with an increased risk of PD[6].

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 AF3T paired with G2019S caused a significant amount of deaths of neurons. When both mutations occurred together significantly more neurons died then when there was just the AF3T mutation. While the G2019S mutation did not cause a mutation on its own it did amplify the effects of the AF3T 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 SNCA[7], 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 dendrites[7]. Dendritic blebs are associated with toxicity within the neuron[8]. 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 model[2]. MPTP is quickly converted to MPP^+ by glial cells, which then can cause oxidative stress within the neuron[2].

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 mitochondria[9]. 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 cell[2]. 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 micrometer[10]. 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 MPTP[11].

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 II[12]. PD can be caused by a change in protein production, PD can also be caused due to mitochondrial dysfunction[12].

Parkinson's disease is a widespread neurological disorder, that affects half a million people[1]. 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.

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CAPSTONE

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 body[1]. 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 activity[2]. 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 gene[3]. 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 formation[4]. 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 levels[7].

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 survival[8].

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 excitation[9]. 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.

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CAPSTONE

Visual and auditory perceptual load applied to distracted driving

Janee Meengs

INTRODUCTION

There are three types of distracted driving: manual, visual and cognitive[1]. 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 not[2]. 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 awareness[3] 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 rate[4]. 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 resources[7]. 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 modal[6]. 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 meaning[8]. 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 later[7]. 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 provided[7]. 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 test[9]. 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 stimulus[8].

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 load[7]. 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 voluntary[7].

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 road[3]. 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 modal[3]. 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 task[10]. The fMRI revealed a decrease in activation of the bilateral parietal lobes and the superior extrastriate, which are associated with the driving task[10]. When the sentence task was added there was increased activation of temporal and prefrontal language areas involved in hearing and language processing[9]. A decrease in overall visual attention is shown through the decrease in spatial processing as well as the reallocation of visual attention[10] 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 area[11]. 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 stimulus[6].

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 cortex[12].

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.

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CAPSTONE

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 systems[1]. 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 invader[2]. 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-threating conditions called "cytokine storms," which can ultimately lead to death[3]. A cytokine storm is an overproduction of immune cells and their activating compounds – cytokines[4]. 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 cells[7]. 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 defense[8]. 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 response[9] 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 models[10]. 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 donor[11]. In the early 2000s, more papers began to use the term "cytokine storm" to describe infectious diseases such as the influenza virus[12]. 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 chemokines[13]. 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 storm[14]. 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 load[15]. 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.

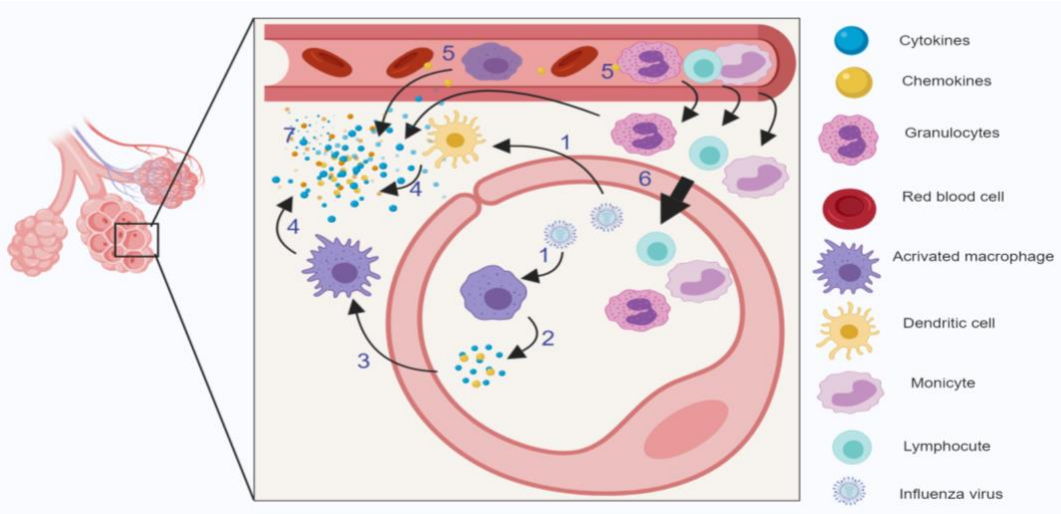

Figure 1. Cytokine storm in the lung following severe influenza infection. **(**1) Viruses infect lung epithelial cells, alveolar macrophages, and dendritic cells to produce progeny viruses and release cytokines/chemokines (2). (3) Cytokine/chemokine-activated macrophages and virally infected dendritic 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 defenses[16]. 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 (PPAPs), 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 pneumonia[18]. 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 mice[20].

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 mice[21]. A triple combination therapy of zalamivir, 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 compilations 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.

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CAPSTONE

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 used[1]. 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 rectification[1]. 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 presented[2]. 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 interest[3]. 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 processing[4]. 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 frequencies[1]. 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 cochlea[1].

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 value[1]. When including this output nonlinearity, the model as a whole is often referred to as the linear-nonlinear spectro-temporal receptive field (LN STRF) model[1]. 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 synapse[5]. 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 cortex[4].

The model for contrast-dependent gain control does not attempt to recreate a specific mechanism since one has not been definitively identified[14]. 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 sounds[14]. 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 model[3]. 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 performance[7]. 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 power[7]. For example, a weight matrix consisting of 18 spectral channels and 15 temporal bins would normally consist of 18x15 = 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 parameterization[7]. 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 process[7]. 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 alone[7].

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 layers[6]. 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 plasticity[6]. 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 cortex[6].

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.

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CAPSTONE

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, respectively[1]. 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 100μm region[2]. GABAergic interneurons may also provide inhibitory input onto other GABAergic interneurons[1]. 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 manipulation[1]. PV^+ interneurons regulate the output of prefrontal cortex pyramidal neurons[3]. 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^+ interneurons[5]. Understanding the ability of PNNs to modulate PV^+ interneuron function is important as many disorders, such as schizophrenia, are characterized by diminished PV^+ interneuron activity[3]. 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 neocortex[7], 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 sources[1]. 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^+ interneurons[8]. 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 synapses[9]. 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 neurons[1]. 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 inhibition[7]. 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 interneuron[10]. Action potentials are initiated in the axon initial segment for PV+ interneurons[11] and propagate with rare failure and relatively high speeds[1]. 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 branches[12]. 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 proteoglycans[13]. The majority of proteoglycans composing PNNs are chondroitin sulfate proteoglycans (CSPGs) including aggrecan, brevican, versican, and neurocan[5]. CSPGs and the hyaluronan backbone are joined via hyaluronan and proteoglycan link proteins while CSPGs are linked by tenascin-R[5]. 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 proteins[5].

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 signaling[3]. This regulation by inhibitory interneurons is important as there exists a balance of excitatory and inhibitory responses to a given neuronal event[14]. For example, rodent models of epilepsy are marked by loss of interneurons and inhibitory synapses[15]. 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+ interneurons[16]. 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 intensity[17], 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 intensity[18]. 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 staining[18]. This is consistent with the ability of PNNs to restrict new synapse formation[19].

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 neurons[4]. Because PNNs are highly colocalized with PV+ interneurons[4], 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 treatment[20]. 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 hippocampus[21]. 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 brevican[21]. 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 behavior[4]. 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.

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CAPSTONE

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 exposure[1]. 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 mammals[2]. 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 cells[7]. ROS are natural biologically harmful products derived from molecular oxygen during production of ATP within cells[8]. Attempts at attenuating cytotoxic levels of ROS with nonspecific ROS scavengers have been somewhat successful and currently there are clinical trials underway with mixed results[9]. 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 ROS[10]. 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 cells[8]. Therefore, it is hypothesized that the primary location for ROS accumulation after AG exposure is within mitochondria of mammals[9]. Further investigations have shown that there is a dose-dependent relationship between rapid calcium flux from ER to mitochondria after AG administration[13]. This finding led to the discovery that elevated

mitochondrial Ca^{2+} is necessary for cytotoxic levels of ROS accumulation during neomycin-induced ototoxicity[12]. 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 tips[17] (Figure 1). Each stereocilia tip is physically connected to each other with tip links, proteins that synchronize stereocilia deflection by direct tethering[17]. AG primarily enter through non-selective MET channels[18] (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 cells[19] (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.

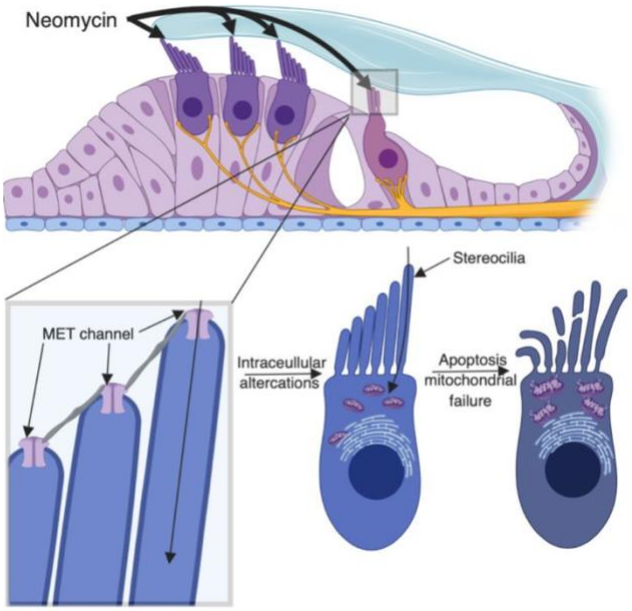

Figure 1. Neomycin entry into hair cells of *Homo sapiens* through MET channel. Neomycin entry induces intracellular dysfunction and subsequent apoptosis. MET = mechanotransduction channel.

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 death[22]. 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 mitochondria[13]. Approximation of ER and mitochondria have been recognized for roles in bioenergetic signaling, cytotoxic events, and apoptotic signaling[11].

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 mitochondria[24]. 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 levels[22] (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 conductances[27]. 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 OMM[28]. Upon large ER calcium efflux, VDAC creates a bottleneck of calcium micro-domains allowing for large amounts of calcium to pass the OMM[27] (Figure 2).

Under physiological conditions, another OMM protein, $Na^{+}/3Ca^{2+}$ 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 overload[30]. Beyond entry of calcium through the OMM, transduction across the inner mitochondrial membrane (IMM) is less characterized[11]. 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 conditions[11] (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 seconds[32]. 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 cytosol[32] (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 entity[11]. 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 upregulated[11] (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 production[35] (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 cytosol[36] (Figure 2). Accumulation of cytosolic cyto C activates the cyto C-dependent caspase signaling pathway[37] (Figure 2). Caspases are cysteine-dependent/independent proteases which participate in the cascade of events signaling apoptosis[37]. Activation of caspases specifically leads to apoptosis through recruitment of P53 mediated apoptosis[38] (Figure 2). P53 is another apoptotic signaling molecule associated with AG-induced ototoxicity[39]. 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.

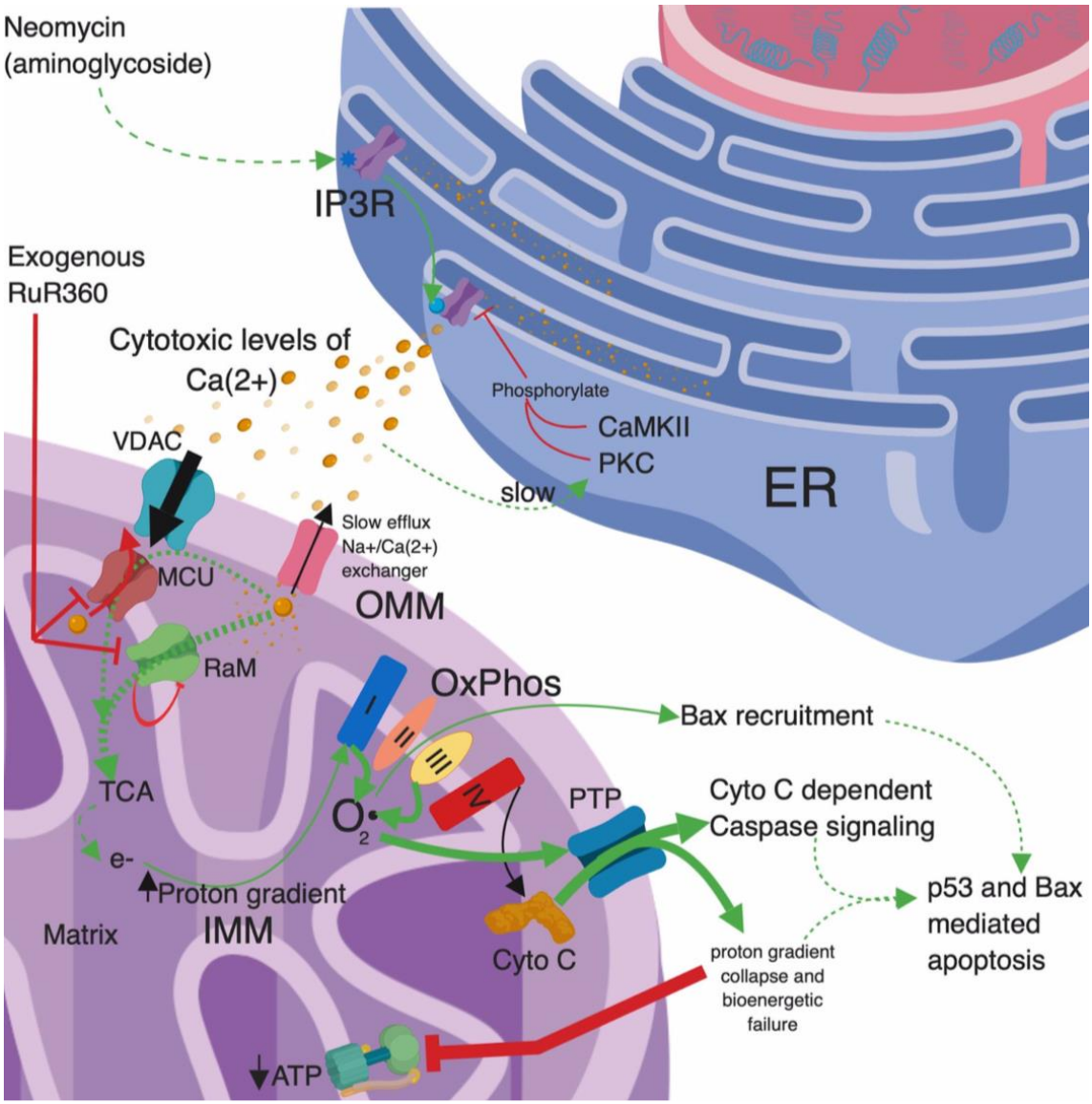

Figure 2. Current proposed mechanism of neomycin-induced toxicity on mitochondrial function. Neomycin likely acts as an agonist on the IP_3R causing a prolonged release of cytotoxic calcium levels onto the OMM. Calcium is readily transduced into the intramitochondrial space through VDAC. The Na+/Ca(2+) 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.

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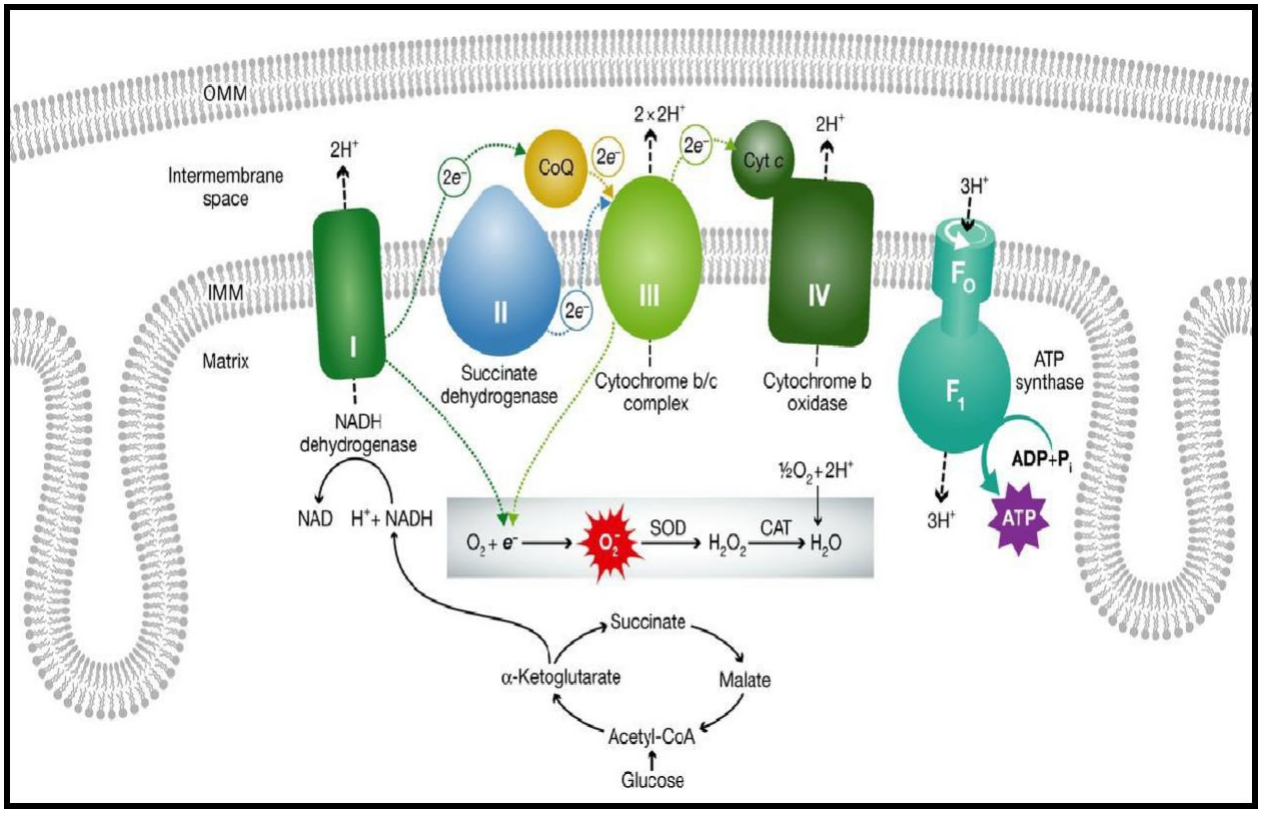
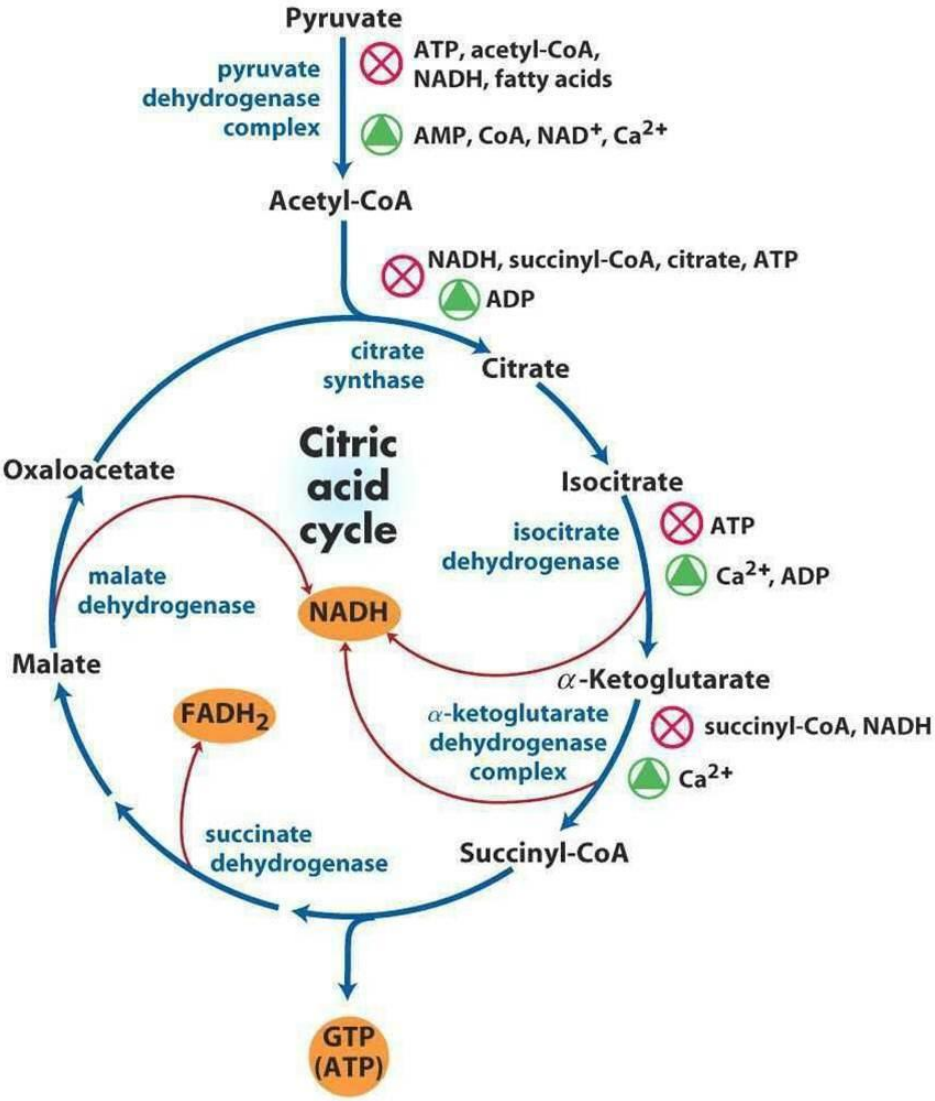
SUPPLEMENTAL INFORMATION



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 (H2O2). 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. GTP = Guanosine triphosphate. NADH nicotinamide adenine dinucleotide. FADH2 = Flavin adenine dinucleotide. CoA = Coenzyme A.

CAPSTONE

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 career[4]. 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 controls[1].

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 damage[8]. Football players are thought to experience mostly straight blows from the front to back[7]. This is believed to especially damage areas with mixed cell types such as blood vessels directly next to neurons and astrocytes[7]. 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 well[1]. 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 fetus[11]. The membrane fuses together for most people before they reach sixth months of age[11]. CSP does not cause of many symptoms itself but is present in several conditions including traumatic CTE[7]. 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 hematoma[5]. 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 fight[3]. 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 student[1]. 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 old[9]. 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 martials 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 fatal[10]. 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.

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CAPSTONE

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

Cheyanne 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 year[1]. 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 own[2]. 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 child[3]. 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) axis[4]. 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 complications[7]. 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 postpartum[4]. 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 conditions[8]. 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 connectivity[9]. 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 child[15].

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 formation[6]. 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 adrenocorticotropic 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 levels[19]. This process alters the mechanisms of the HPA-axis, and it is suspected that high levels of cortisol secretion may lead to hypercortisolemia[20]. 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 receptors[21]. As a result, the hippocampus becomes metabolically stressed[22]. 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 network[23].

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 slope[24]. 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 women[24]. 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 year[26]. 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 PPD[27]. 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 support[28]. 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 factors[29]. 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 older[3].

FUTURE DIRECTIONS

For women who suffer from postpartum conditions such as depression, anxiety and psychosis, there 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 heterogenous 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.

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CAPSTONE

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 worldwide[1]. The majority of those affected are young adults aged 18-25[1]. Depression is a complex mental disorder which results from a combination of different triggers such as stress, genetic predispositions, and environmental factors[1]. Roughly 20% of adolescents with major depression are treated with antidepressants such as selective serotonin reuptake inhibitors[2]. Unfortunately, traditional antidepressants can have adverse effects on adolescents potentially increasing the risk of self-harm and suicide[3]. 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 neuroplasticity[6]. A 2011 study found that germ-free (GF) mice showed decreased levels of BDNF[7]. Importantly, these GF mice exhibited anxiety and depression-like behaviors[7]. Similarly, decreased

levels of BDNF have also been observed in depressed human patients[6]. 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 tract[8]. Interestingly, a recent study by Mandic and colleagues discovered that *Clostridium ramosum* is capable of increasing expression of peripheral 5-HT-producing enterochromaffin cells[9]. Thus, the gut microbiome is able to directly influence levels of 5-HT. One hallmark of depression is low levels of 5-HT[10]. 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 levels[11]. 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 stress[12]. 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) alpha[13]. Fascinatingly, bacteria found in the GI tract such as *Fusobacterium nucleatum* mediate levels of TNF gene expression in humans[15]. TNF can induce fever, cell apoptosis, and the release of other proinflammatory cytokines[13]. TNF also increases expression of indoleamine (2,3)-dioxygenase, an enzyme which converts TRP to kynurenine[16]. Thus, chronic inflammation in the gut can trigger the degradation of TRP via the kynurenine pathway[17].

Kynurenine is able to influence the brain since this compound is able to cross the blood brain barrier[18]. Kynurenine is then converted to KYNA by kynurenine aminotransferases found in astrocytes[18]. 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 symptoms[19]. In fact, patients with depression in remission and currently depressed both showed disproportionately low levels of KYNA compared to healthy individuals[20]. Furthermore, dysregulated NMDARs have been linked to decreased levels of BDNF and dysfunctional serotonergic signaling[21]. 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 probiotics[22]. 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 treatments[23]. The gut microbiome provides a powerful clinical tool for treatment of mental disorders.

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CAPSTONE

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 damage[1]. The central nervous system (CNS) complicates clinical recovery due to its lack of significant natural regeneration; when damage occurs it is generally permanent[2]. 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 CNS[6]. 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 CSPGs[4] . 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 properties[11]. Based on binding affinity, sulfonation pattern, and

binding location, CSPGs will regulate a neuron differently, making them highly diverse molecules[10]. 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 connection[4]. 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 formation[4]. 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-functional[8].

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 proteins[19]. NgCAMs works similarly to NCAM stimulating the movement of both neural and glial cells within the CNS[20]. The core proteins of CSPGs have high affinities for NCAMs and NgCAMs, neutralizing these growth factors through occupying what would be the site for connection[21].

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 regenerate[10]. The glia found in the PNS functionally change the ECM to be one that promotes the repair of axonal injury and prevents scarring from occurring[10]. Through a handful of PNS specific cell adhesion and guidance molecules neural reinnervation is supported[26]. 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 recovery[30]. 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 recovery[29].

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 PNS[24]. 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 factors[24]. The CNS lacked many growth-promoting and counter-inhibitory genes found within the PNS[24]. 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 regrowth[13]. 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 movement[31].

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 tissue[22]. Melatonin has been shown to have anti-inflammatory, antioxidant, and neuroprotective effects; combining melatonin and ChABC can promote CNS regeneration[23]. This result was achieved by reducing oxidative damage and inflammatory reaction in the injury area and inhibiting glial scar formation and allowing for neural regrowth[23].

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 importance[1]. 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 reconnect[2]. This lack of regeneration is largely attributed to the extracellular matrix of the CNS, specifically CSPGs inhibiting axon extension[3]. It has been seen in previous research that enzymes such as ChABC successfully degrade CSPGs to help alleviate the inhibitory effects[15]. This method combined with other neural supportive procedures such as PNS grafts have been shown to be successful in restoring neural function in rodent models[22]. These tested treatment methods provide opportunities to do further research into primate models in hopes to provide progress towards human clinical use.

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