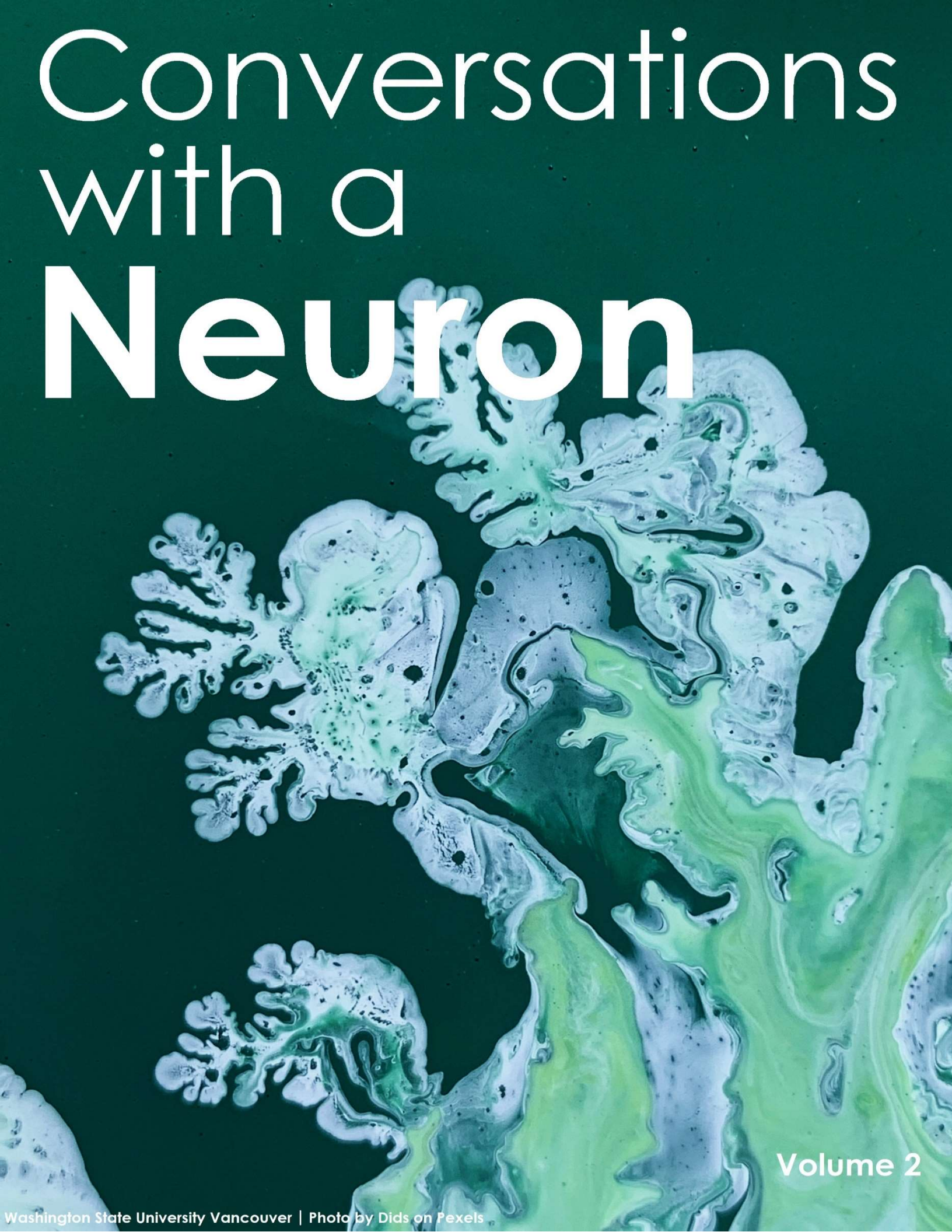
Conversations with a Neuron



Volume 2

Washington State University Vancouver | Photo by Dids on Pexels

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preface

Welcome to the second edition of Conversations with a Neuron! We launched this journal in 2019 to feature articles written by our Neuroscience majors at Washington State University Vancouver. The articles all started as class assignments for classes such as Neuroanatomy and the neuroscience Capstone course; upper-division courses that invite the students to dig into a topic in neuroscience that interests them, to think deeply, and to synthesize ideas. The end result, of course, is a graded assignment – we know our students work hard for their grades! In a traditional course, the grade would be the end – a paper written by the student, read and graded by the professor, and set aside.

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

I particularly want to thank Editor-in-Chief Sydney Wolfe, a gifted writer and current neuroscience major, for her excellent editing on this volume. Also, special thanks to Cheyanne Lewis (B.S. Neuroscience 2019), our inaugural editor who paved the way for this edition and those to come.

To learn more about our Neuroscience program, please visit our website at
https://cas.vancouver.wsu.edu/neuroscience.
We hope you enjoy this issue… and learn something you are glad to know!

Sincerely,

Allison Coffin

Dr. Allison Coffin
Associate Professor of Neuroscience

Adventurous monkeys: choosing to explore the unknown

Communication between the amygdala and the ventral striatum regulate decision-making behavior by determining whether new options outweigh a known option with a guaranteed reward.

Ella Cary

This article describes new research regarding the explore-exploit decision-making process of rhesus monkeys. While it has been previously documented that animal subjects will learn via motivational circuits through reward repetition,[1] this research dives into the neural circuits involved with choosing to explore a new option over selecting a choice with a known and guaranteed reward.

The amygdala is an almond-shaped structure in the temporal lobe of the brain. It is known to play a role in processing emotions and motivation.[2,3] The ventral striatum is a structure of the brain that is heavily linked to how the brain processes reward.[4] In a paper published in Neuron in 2019, Vincent Costa, Andrew Mitz, and Bruno Averbeck investigate whether or not motivational circuits in the brain of rhesus monkeys support activity related to decisions to explore rather than exploit a path with a guaranteed reward. It was found that motivational circuits were active and important in guiding the explore-exploit decisions of the monkeys. These findings further pose the need for research to be conducted in order to determine specific interactions between brain structures of the reward systems that regulate this exploratory decision making.

The explore-exploit dilemma refers to deciding when to give up a known immediate reward in order to explore a new option.[5] Essentially, is the potential value of the new option high enough that it is worth disregarding the guaranteed value of the known choice? This dilemma presents a problem in reinforcement learning where a subject is given consistent rewards upon completing tasks, thus conditioning them to perform the task on a regular basis.[6] Neural circuits associated with motivation which include structures that receive dopamine input like the amygdala and ventral striatum facilitate learning based on choices and outcomes, though it is unknown whether these same structures and circuits support in-depth processing that allows the subject to decide whether or not to explore.[7] Cortical activity is typically higher in humans and monkeys (compared to other mammals) when foregoing reinforced learning behaviors in order to explore novel alternative options.[8] This suggests that dopamine regulates the explore/exploit choices and therefore structures of the brain such as the ventral striatum and amygdala that receive dopaminergic input must also play a role in explore/exploit choices.[9]

In this study, three rhesus monkeys were taught to play a task-oriented game in which three options were presented that had different reward values. The monkeys were given time to learn choice-outcome relationships by selecting each option. The same set of visual choice options was presented to the monkeys repeatedly for a minimum of ten times. The time given to explore the three options was limited in that one option was randomly replaced with a new novel image, forming a new set of options that were then repeated for several trials. Whenever a novel image was first introduced, the monkey had to determine its value by choosing to explore

it over selecting one of the other two options where the outcome was already known. This process continued and a new novel image was introduced a total of 32 times. During each trial, the neural activity in brain structures was recorded while focusing on those that receive dopaminergic input like the amygdala and ventral striatum. Additionally, choice reaction times were also recorded.[10]

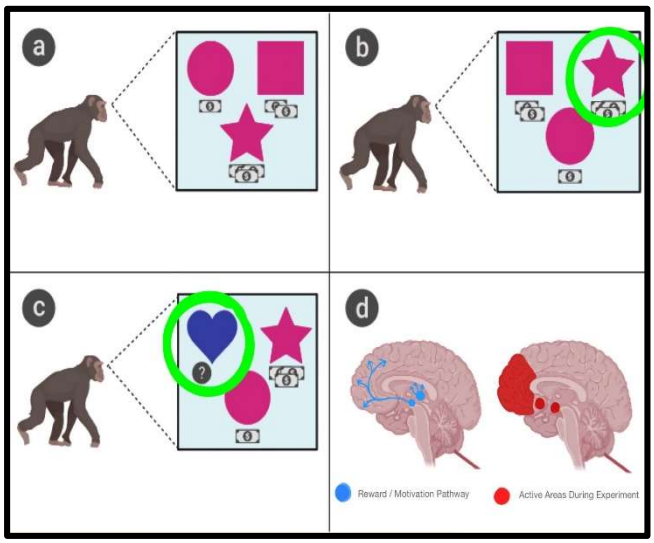

Figure 1. Each rhesus monkey was presented with three choices and given time to explore each option and the reward associated with it (1a). After learning what reward was associated with each the monkey would continue to choose the one with the most valuable reward even if the choices had shifted places (1b). After many trials with the same choices, one was removed and replaced with a novel choice of an unknown value. Each monkey preferred the new choice even though the associated reward value was unknown (1c). The areas of the brain active during this decision-making process (red) include the amygdala, ventral striatum, and prefrontal cortex and mimic the areas affected by the dopaminergic motivation and reward pathway (blue).

Overall, when the novel images were first introduced, the monkeys showed a preference for exploring it rather than exploiting the other two options with known rewards. After the initial exploration, however, they transitioned to choosing the best alternative option. The novel options were given lower values than the best alternative option, so the monkeys choice to forgo the novel option after initial exploration and instead select whichever of the remaining two options had the higher value demonstrates high levels of processing. As the value of the best alternative option increased, the selection rate of the introduced novel option decreased, indicating that at some point, exploration will be forgone in order to exploit a path with a high-value reward. Analysis of choice reaction times (RT's) show that when a novel image was introduced as a choice, the choice reaction times were longer when the monkeys ended up selecting the novel option over the best alternative option.[10]

Activity in 329 total amygdalar neurons and 281 total ventral striatum neurons were recorded. Overall, baseline firing rates were higher in amygdalar neurons compared to neurons of the ventral striatum, and spike-width durations were shorter for ventral striatum neurons.[11] When the monkeys learned the value of their choice there was more obvious activity demonstrating immediate reward value in the neurons of the ventral striatum compared to the amygdala. When a novel option was first introduced, the neuron activity remained the same whether or not it turned out to be high, medium, or low value. As learning progressed, the firing rate increased when the monkey selected novel options with high-value rewards and decreased when a low-value novel option was selected. Overall, brain activity was observed in high concentrations in the prefrontal cortex, amygdala, and ventral striatum when the monkeys chose to explore the novel options. These areas all correlate with those that receive dopamine input along the motivation and reward neural circuit.

This study demonstrated the similarities in brain activity during reward-motivated decision making as well as explorative periods where a reward was foregone. It was shown that the ventral striatum and amygdala both show activity during these decision periods though not at the same time or in the same way. In general, brain activity during explorative moments mimics the path of the known dopaminergic mesolimbic circuit that is key in the reward and motivation circuits of both monkeys and humans. While this level of complex processing and choice-making most likely is a result of several neural circuits at work it is known that projections

between the amygdala and ventral striatum are known to stimulate reward-seeking behaviors as well as positive reinforcements and have an effect on dopamine.[11] This research demonstrates a further need for the exploration into which specific connections and circuits between the amygdala and ventral striatum regulate exploratory decision making. Additionally, activity in specific brain structures and cell groups could be recorded in order to further understand the basis behind the advanced choice strategies used by the monkeys to further navigate the explore vs. exploit trade-offs.

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Cerebellum at a junction, now there's a function

This article provides new evidence that the cerebellum, a structure located in the rear of the brain, has a larger role in controlling reward circuitry and social behavior than previously thought.

Ashley Vega-Lazaro

In a paper recently published in *Science,* Ilaria Carta and associates investigate the cerebellum's function beyond its known role. This research broadens the understanding of mental disorders. It provides an explanation for why functional imaging experiments indicate a connection between the cerebellum and addiction. The primary finding being that the cerebellum sends direct excitatory projections to the ventral tegmental area (VTA) which is associated with reward. The implications of this finding are important. This shows that the cerebellum's functions are broader than the motor movements (including posture, balance, and speech) previously associated with it.[3] Additionally, this finding can later be used to possibly help treat mental disorders associated with addiction and reward. This research shows that there is still a lot to learn about the cerebellum and the brain as a whole.

Background

It was long known that the cerebellum played a role in motor coordination, and this experiment attempted to look beyond that.[8] Previous studies by Van Overwalle and colleagues have examined the connection between the cerebellum and social-cognitive processes. This research gave background information on connections that the cerebellum makes.[6] Additionally, the ventral tegmental area was focused on during this experiment because of its known connection to addiction. Research by Eric Nestler informed this knowledge and provided background data to support the idea of a VTA connection being significant and linked to reward circuitry related to the cerebellum.[5] In a study by Tiffany Rogers and colleagues, the neuronal circuitry by which the cerebellum modulates the medial prefrontal cortex dopamine release was investigated. Their findings give credence to the idea of cerebellar involvement in autism, schizophrenia, and other cognitive disorders in addition to dopamine reward circuitry. Some researchers hypothesize that the cerebellum may refine higher-order functions.[9,4]

Methods

A variety of methods was used during this experiment, including *in vivo* and in vitro electrophysiology, herpes viral tracing, self-stimulation, and histology. Three to four-month-old adult mice of both genders were used as the subject to determine the relationship between the cerebellum and reward circuitry/social behavior. The parallel rod floor test, behavior tests, and the three-chamber test were some of the main experimental procedures used. All behavioral experiments were analyzed by doing statistical comparisons in GraphPad and Matlab for electrophysiological experiments.

The three-chamber test used adult mice that were bilaterally implanted with a 200μm fiber-optic that targeted the VTA and was coated with molecular probes. After 2 weeks post-surgery, the mice were recorded in a clear 3 chamber plastic box (24x13in) where one side was a "social" side with another mouse, and the other side was empty. The quantity of social behavior between mice was recorded on video and timed by a computer and an individual who was

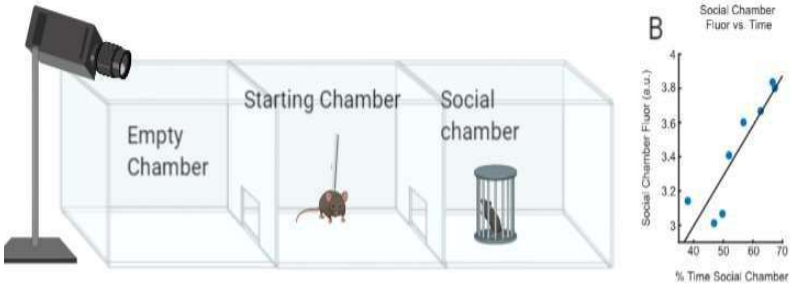
unaware of the experiment. This experiment was based off a similar one done by Anton Ilango and colleagues.[1] Fiber photometry was used to monitor changes in calcium in cerebellar axons located in the VTA as a method of determining neuronal activity.

Results

Using the behavioral tests, it was determined that stimulation of cerebellum-VTA projections was able to cause both short and long-term preference proving that the pathway was rewarding. Optogenetic inhibition during the 3-chamber test removed social preferencewhich indicates that the connection between the cerebellum and VTA is required for normal social behavior. Additionally, when undergoing optogenetic stimulation the cerebello-VTA projections increased the activity of VTA both *in vivo* and in vitro. Nevertheless, optogenetic activation of the cerebello-VTA inputs did not cause an increase in sociability. It was found that the monosynaptic excitatory projections from the cerebellar nuclei to the VTA activate reward circuitry and contribute to social tendencies.

These findings are significant. The new knowledge about the cerebello-VTA connections will open the doors for future research connecting the cerebellum to social behavior and reward. This research can help inform and add to the present role and understanding of the cerebellum. Additionally, the findings from this experiment connect to cognitive illnesses which researchers are attempting to address. This new research will inform further studies into the cerebellum and show that the cerebellum plays a larger role in mental illness than previously anticipated.

Figure 1: Results of Three-Chamber Test (2,7)



This figure shows the results of the study completed with adult mice, ranging in age from 3-4 months. The testing environment of the three-chamber test is shown (A). The three-chambered test results showed that fluorescence increased over time in the social chamber indicating that the VTA and cerebellum are connected to social behavior(B).

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Is your corpus callosum normal?

The left and right sides of your brain are connected via nerve bundles that make up the largest structure within your brain called the corpus callosum. Research highlighted here by Lischke et. al.,show that disruptions in this key brain structure may correlate with increased suicidal behaviors in people suffering from borderline personality disorder.

Deborah Rosca

Background

The corpus callosum is an important structure in the brain made up of white matter that connects both hemispheres. This structure contains four parts, including the genu, rostrum, body, and the splenium.[1] The corpus callosum is known to have an impact on cognition, emotions, and behaviors. Impairment to this structure has shown to cause many diseases like borderline personality disorder, attention deficit hyperactivity disorder, and suicidal behavior.[2,3] Research into this structure has been studied in-depth on the microscopic level regarding differences in the corpus callosum in patients with borderline personality disorder.[4] Lischke, Domin, Freyberger along with others, are just some of the people who researched at the University of Greifswald Germany, in the departments of psychiatry, psychology, and psychotherapy.

Diffusion tensor imaging was used to view the microscopic alterations in each of the groups: the healthy control, borderline personality disorder, and the suicidal patients. This imaging allows the movement of water diffusion in the white matter of the corpus callosum. Fractional anisotropy and mean diffusivity was used in characterizing the density and myelination of the fibers inside the structure.[5] Structures that the corpus callosum connect to that regulate emotions and impulse control in borderline personality disorder suicidal group showed to have decreased thickness than from the healthy control group; this indicated suicidal behavior.[9]

Methods

Two groups were tested, one healthy control group containing 20 women and another meeting the DSM-IV criteria of borderline personality disorder with emotional instability and impulsivity. Strict criteria must’ve been met, for example, no borderline personality disorder patients on medications of any sort, and schizophrenic personality and attention deficit hyperactivity disorder were to be excluded. The reason for this was shown to have an effect on the integrity of the structure of the corpus. All participants were women in the age range of 18-45 years old, with all right-handed women, being the same sex and age groups.[6,7,8] A Magnetic Resonance Imaging (MRI) was used in processing each participant in the groups, then used a JavaDTI to calculate the diffusion tensor and determine statistical analyses of the corpus. With this, researchers were able to conduct two sets of analyses, first testing whether the splenium of the corpus had associations in structural alterations in suicidal behavior, and two, seeing if emotional instability and impulsivity were associated in the same regions.[10]

Results

Structural alterations were found to be associated with suicidal behavior in borderline personality disorder participants in the corpus callosum. Fractional anisotropy and mean diffusivity were used to determine if specific regions in the corpus correlated negatively or positively with suicidal behavior. In the fractional anisotropy, splenium and genu correlated negatively with suicidal behavior, while in the mean diffusivity, the splenium correlated positively. This shows that regions such as the splenium and genu were associated with regions of the brain that are connected to emotion regulation and impulse control, showing an impact on suicidal thoughts and behavior in those with borderline personality disorder.

Conclusion

Research in the corpus and its structure helps in understanding how patients with disorders are affected and if structural support of the corpus is correlated to such disorders. It's a good start in understanding the neurobiological underpinnings of borderline personality disorder and suicidal behavior in patients with structural alterations. Further studies should be done in seeing how the splenium and genu connect to regions of the brain, such as the prefrontal and tempo-parietal and how both structures play a role in emotions and impulse control.

Reasons for suicide	Number	Percentage
Helplessness and desperation	13	100.00
Pain and sorrow	12	92.30
Worthless and guilt	4	30.77
Anger and rage	4	30.77
Loneliness and isolation	2	15.38
Other	1	7.69
Types of self-Injury		
Cutting	16	76.20
Scratching	16	76.20
Biting	5	23.80
Burning	3	14.30
Beating	8	38.10
Bone breaking	2	9.50
Hair pulling	3	14.30
Interfering with wound healing	8	38.10
Stabbing	2	9.50
Other	3	14.30

Figure 1. Chart for reasons of suicide and types of self-injury from borderline personality disorder.

	Splenium of CC	Genu of CC
Fractional Anisotropy	r= -0.45	r= -0.55
Mean Diffusivity	r= 0.45	r= 0.31

Figure 2. Correlation between structures in the corpus callosum using these methods.

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The power of music on the brain

A study on the way music can anatomically alter the brain found that, amongst identical twins, the twin who had at least 1,000 hours of training on the keyboard had more advanced gray matter and white matter when compared to the twin who had no musical training.

Ban Alamin

This study sought to determine the effects of training on neuroanatomy and discovered that extensive musical training can induce brain changes, including greater cortical thickness, more developed white matter microstructure, and an increase in gray matter volume. The results of this study are important to further understand how the causal effects of practicing a skill can impact the brain's ability to adapt and form new connections.

The relationship between expertise in a skill and regional brain anatomy is a topic of extensive investigation. However, the specific causal effects of training on the brain without the influence of genetic factors remains unclear. In this article published by *OxfordAcademic*, which describes a study conducted by Örjan de Manzano and Fredrick Ullén, sought to determine the differences in brain anatomy between musical experts and non-experts.[1] This study was completed by observing the structural differences in the brains of nine pairs of monozygotic twins where one sibling in each pair played a keyboard instrument, and the other did not. This experiment found that even when controlling for genetic and early environmental factors there were still significant observable differences in both gray matter and white matter microstructure between the twins. These findings are important because the ways in which the brain's structural plasticity is affected can be used to better understand why the brain changes as a result of different practices and can be utilized in further research regarding learning and brain development.

From previous research, it is known that playing an instrument changes how the brain interprets a wide range of sensory information. Due to this, musicians have been ideal subjects for the role of long-term plasticity in the brain.[2] The plasticity of the brain refers to the brain's ability to change its physical structure and form new connections in response to learning or brain injury.[3] This influences the functionality of the brain and can create new patterns of organization within the brain.[2] For example, it was found in a previous study that London taxi drivers' hippocampal gray matter volume correlated with the level of driving experience.[4] Similarly, another past study found that just 15 months of musical training in early childhood lead to structural brain changes that were different from typical brain development.[5] These studies have solidified the fact that there is a relationship between training and differences in brain structure both during development and throughout adulthood. In comparison, this study seeks to clarify whether there is a direct relationship and how genetic factors come into play.

In this study a set of 9 pairs of monozygotic twins between ages of 31 to 47 were analyzed. One sibling in each pair had been playing a keyboard instrument with at least 1000 hours of experience, while the other did not. The musical history of each sibling was determined through a survey. Structural and functional MRI (magnetic resonance imaging) data of each sibling was acquired, and cortical thickness was analyzed with a FreeSurfer brain MRI program.[6] In order to

localize regions of interest, each musical sibling played simple melodies, and the corresponding clusters of activity in specific regions were observed. Diffusion weighted images, fiber tractography, and VBM (Voxel-Based Morphometry) analysis were also completed. All data was analyzed through a linear mixed model, and within-pair differences were analyzed with paired t-tests.[1]

Overall, the data indicated a correlation between musical training experience and differences in brain structure. It was found that the musical twins had greater gray matter volume in their left cerebellar lobules, greater cortical thickness in their left cerebral auditory motor network, and more developed white matter microstructure in their bilateral hemispheres.[1] Additionally, these areas were found to be related to the auditory network of the brain.[7] Regarding cortical thickness, the within-pair differences would have been higher, but a discordance occurred due to one pair of twins who both had early childhood musical training. Later one sibling stopped playing while the other continued. Overall, the findings clearly support the claim that extensive musical training increases the development of the auditory-motor network.

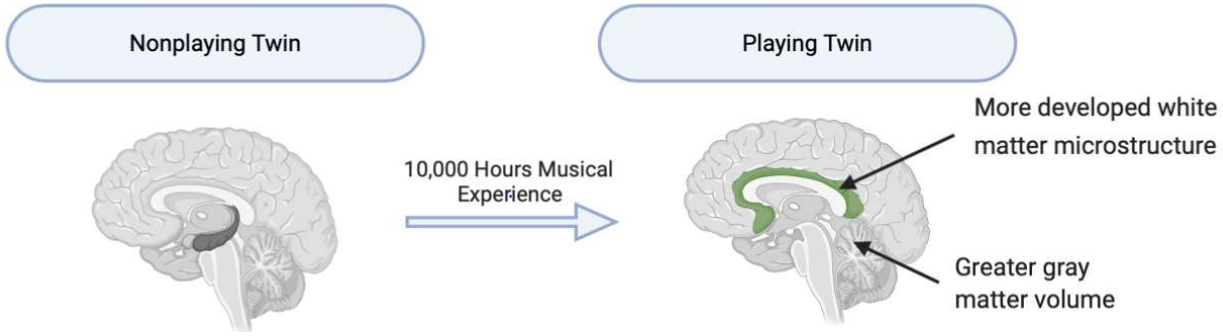

Figure 1. Anatomical differences between monozygotic twins were apparent with one twin having at least 1,000 hours of musical experience. This included more developed white matter in the corpus callosum and increases in gray matter along with cerebellar lobule of the auditory cortex. The anatomical differences were found to be influenced by causal effects of practice rather than genetic factors.

This study is significant because it shows how the brain can change and develop differently based only on the amount of musical training done and not including genetic predispositions. This is also significant regarding children with developmental disorders and adults with neurological diseases where the ability of the brain to undergo structural changes in response to the environment is hindered.[8] From this study, further research can be done to evaluate the learning process in people with disorders. However, this study could have been done with a larger sample in order to determine more accurate effects of training on a larger scale. Additionally, some of the discordances that occurred within the experiment could have been influenced by general developmental factors. It has been seen in previous research that differences occur in structural brain plasticity and behavior from early stages in development.[9] These factors are always present and indicate the unpredictable outcome of development that may have contributed to differences in brain structures between siblings, even those who are genetically identical. In conclusion, the study may have been influenced by early developmental factors, but the results indicate that there is an undeniable causal effect of expert musical training on the structural plasticity of the brain.

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Caffeine: does it improve our reading skills?

This study tests whether caffeine affects the global pattern processing, and more specifically, if caffeine could enhance text reading skills.

Cierra Bump

A recent article from the Journal of Psychopharmacology begs the question of whether caffeine improves text reading and global perception. Global perception is how someone overall perceives an object or situation and focusing on the totality rather than the parts. Therefore, it is arguably a very important human skill, along with reading, alertness, spatial attention, and executive functions. These skills were put to the test in these two studies and were the primary focus of the article. Through the experiments, it was found that a small amount (200 mg) of caffeine would improve a person's global processing, though it had little effect on alertness, spatial attention, or executive functions. This research could provide help for those with mental disorders that make it difficult for them to read in a timely manner. Though it is not the ideal drug to use, it could aid those with mild symptoms to slightly improve their text reading and global perception skills.

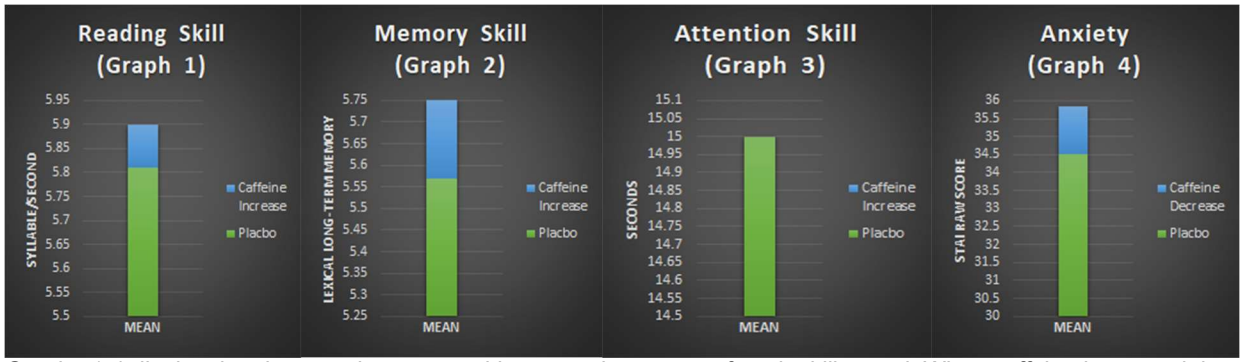
Even though reading and global perception are such important skills for everyone to have, there are unfortunately many disorders that prevent or make it very difficult for a person to perform at what would be considered a normal level. Dyslexia, attention deficit hyperactivity disorder (ADHD), or falling on the autism spectrum are a few among many examples of such disorders. However, if these people happened to have more mild symptoms, caffeine could prove itself as a candidate drug to help people with these disorders in intervention training. More on this, the article mentions several other studies also involving caffeine's effect on the body. These studies investigated how caffeine improved physical exercise performance, as well as being able to counter both the physical and cognitive effects of sleep loss. Knowing how caffeine affects the body in a physical sense helps pave the way for research on how the drug affects the body in the neurological sense.

Regarding the actual experiment, the participants of both studies completed a double-blind, repeated-measures test, which meant that information about the drug was unknown to the participants during the time it was taken and that several measures of the same variable were taken each with slightly different conditions. The first study included 24 participants, who were each tested twice, seven days apart. Before each test, the participants either consumed a small amount of caffeine (200 mg) or a drink containing a placebo. To ensure a double-blind procedure, half of the group took the caffeinated drink during the first test and the placebo drink in the second test, and vice versus for the other half. None of the participants knew which group they were in, and a bitter drink was used to disguise caffeine's taste. The second study, which included fifty-three participants, operated on the same procedure, though this time participants were required to report how many hours they had slept the night before without caffeine consumption so that sleep debt could be calculated.

Before the experiment began, participants completed the same tasks when they had not consumed caffeine in at least 12 hours, so that they acted as their own control group. Once the

beverage was consumed on the day of the first test, the participants had to complete tasks that tested their reading skills, memory, attention, and anxiety levels. The second study also tested these skills but involved different tasks for its participants to complete.

Based on the results of the studies, it could be concluded that caffeine had a significant effect on the reading skills of the participants, but no significant effect on their memory, attention, or anxiety levels. This is known through the P-value, which must be less than 0.05 to be significant, and the reading skill was the only test to produce such results. For the reading skill, the number of syllables per second that could be read was measured, increasing by 1.55% from placebo to caffeine (Graph 1) with a P-value of 0.037. For memory skill, the number of questions (up to 10) that could be answered correctly about a text they had just read was measured, increasing by 3.23% from placebo to caffeine (Graph 2) with a P-value of 0.532. For attention skills, the amount of time (s) it took the participants to identify the color of 30 squares was measured, which remained the same from placebo to caffeine (Graph 3) with a P-value of 0.253. Lastly, for anxiety levels, the scores achieved on an anxiety questionnaire (STAI) after consumption was measured, decreasing by 3.74% from placebo to caffeine (Graph 4) with a P-value of 0.136. Though it seems caffeine had a greater effect on everything but reading skills, the high P-values show that these findings are not significant. The results seem to match the previous studies that the article mentions at the beginning, where caffeine was shown to improve cognitive tasks, as well as having mixed results on how caffeine affects short term memory.



Graphs 1-4 display the changes that occurred between the means of each skill tested. When caffeine increased the mean (Graphs 1-3), the green bar represents the total mean of the placebo, while the green and blue bar combined represents the mean of the caffeine. When caffeine decreased the mean (Graph 4), the green and blue bar combined represents the mean of the placebo, while the green bar represents the mean of the caffeine.

With reading being such an important skill, finding anything that could help improve it is very useful. Now that this study has established that a small amount of caffeine can cause such improvements, more varied control sizes can be used in experiments to see what amount of caffeine is optimal for the best improvement. Questions such as whether a certain amount of caffeine can become detrimental or hit a plateau for improvement of reading skills can be answered through further research. Because caffeine is shown to have a positive effect, it could also be used to treat the before mentioned disorders, dyslexia, ADHD, and autism, for those who have more mild symptoms. Personally, as someone who drinks a lot of caffeine (about 80 to 120 mg per day) it's good to know that drinking it isn't all bad. I usually drink it before I do homework, so I wonder if this habit developed unconsciously due to the positive symptoms it gave me.

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Neurons in the dorsomedial striatum play a critical role in excessive alcohol consumption

Exciting D1 medium spiny neurons and Inhibiting D2 medium spiny neurons leads to excessive alcohol consumption in mice.

Vicky Nganga

Approximately 14.4 million people ages 18 and older were diagnosed with Alcohol Use Disorder (AUD) in 2018. Of these people, only 7.9% received treatment. The striatum is a brain area that has been implicated in drug and alcohol addiction. The dorsomedial striatum (DSM) controls goal-directed behaviors. Neurons are the basic working unit of the brain. Specifically, the DSM contains specialized neurons called medium spiny neurons. MSN are medium-sized units that contain many little spines. These neurons express either dopamine D1 receptors, which are excitatory, or dopamine D2 receptors, which are inhibitory. There have been gaps in how these D1 and D2 expressing neurons are involved in alcohol addiction. The goal of this paper was to look at the role of D1 and D2 MSNs in alcohol consumption to identify a target for the development of a new therapeutic approach to alcoholism.

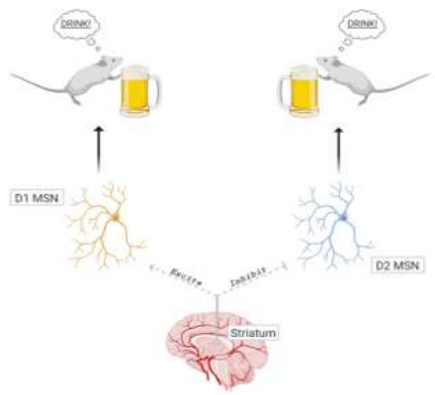

Figure 1. Excitation of D1 MSN and inhibition of D1 MSN promotes alcohol consumption.

A paper published in the Biological Psychiatry (2017) by Yifeng Cheng and colleagues investigates the role of MSN expressing different dopamine receptors, D1 and D2, on alcohol consumption in mice. They used twelve-week old male mice that were housed individually under 12-hour light and dark cycles. The mice were given intermittent accesses to two bottle choices: one containing 20% alcohol (ethanol) and another containing water. The bottles were alternated every session to control for side preference among the mice. This was done for 8 weeks. 24 hours after the last alcohol consumption, the mice were sacrificed, and their brains were sectioned sagittally or coronally.

The first part of their analysis included observing N-methyl-D-aspartate receptor (NMDAR) transmission since these receptors are the main target for alcohol. What they found is that NMDAR transmission was dramatically increased in D1 MSN where mice exposed to alcohol. However, D2 MSN did not have such a drastic increase. Instead, they found that these D2

MSNs increase the effect of GABAergic transmissions. GABA is a type of chemical signal in the brain that inhibits or blocks signals. So, what this means is that these D2 MSNs are sending inhibitory signals. The second finding was that D2 receptors can inhibit these GABAergic signals from D2 MSN through the use of GSK3β (an enzyme) activity. This means that D2 receptors and GSK3β play a significant role in alcohol consumption.

The main finding was that exciting D1 MSN in the dorsomedial striatum increased alcohol consumption in mice, as well as inhibiting D2 MSN. These findings revealed that D2 and D1 expressing MSN play a critical role in alcohol abuse. They also provided a mechanism for alcohol consumption in the dorsomedial striatum. Excessive alcohol consumption results in excitatory strengthening of D1 MSN and inhibitory strengthening of D2 MSN. Therefore, increasing and decreasing the strength of their correlating pathways. This information gives insight into a new target site for future treatment approaches.

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Predicting nausea using AI

A new study shows that artificial intelligence may be able to predict an individual's susceptibility to nausea.

Alex Jensen

Nausea leaves individuals in pain, feeling weak, with little to no appetite, and often leads to vomiting. There can be several underlying causes for nausea, leaving many suffering from this discomfort.[1] Nausea is an adverse feeling that some individuals are more prone to than others. This increased or decreased susceptibility to nausea may contain underlying biomarkers within the brain.[2] This study investigates functional and anatomical biomarkers within the brain that may predict nausea susceptibility in individuals. This research is then applied to machine learning to investigate the possibility of computer programs predicting susceptibility or resistance to nausea.[3] The resulting model will then be able to assign nausea susceptible or nausea resistant to any individual accurately.

There are many available treatments for nausea but knowing the cause of this nausea is essential. The knowledge of whether or not an individual is prone to nausea due to anatomical or functional differences in the brain allows a doctor to accurately treat nausea in a patient.[4] Those susceptible to nausea require different treatment from those with underlying medical causes of nausea.[5] Machine learning used to predict nausea susceptibility will let doctors diagnose and treat nausea accurately.

Machine learning allows researchers to use technology to find statistical patterns in data. This use of technology simplifies large sets of data and increases the scope and complexity of problems that can be solved through research.[6] A machine can be trained to solve problems and analyze data quicker and easier than humans, broadening the amount of issues that can be solved, including nausea susceptibility.[7]

28 healthy participants between the ages of 18-65 were used for assessing nausea. In order to induce nausea, participants wore MR-compatible goggles that would project a 10-minute video during an fMRI scan. This video was comprised of still images of a landscape, each tilted and rotated to simulate the perception of spinning about a tilted axis. This was shown to enhance and induce motion sickness. A second MRI was taken while the participants watched a single, still image; this was used as a control. Nausea was assessed using a 4-point visual analogue scale (VAS), where 1 means there are no symptoms of nausea and 4 indicates severe nausea[8]. Two questionnaires were also used, the motion sickness sensitivity score (MSSQ) and the motion sickness assessment questionnaire (MSAQ), to assess susceptibility to nausea and symptoms of motion sickness respectively.[9]

Resting autonomic parameters, including heart rate, were established for the participants before MRIs were taken. Machine learning was used to identify regions of interest within the brain to extract data. Regions identified include the bilateral nucleus accumbens, the amygdala, caudate, hippocampus, pallidum, putamen, and thalamus. The hypothalamus, bilateral insula, bilateral

orbitofrontal cortex and anterior (ACC), middle (MCC), and posterior cingulate cortices were also added for additional analysis. Computer programs were then used to identify statistically significant differences within these areas that are contingent on nausea severity, comparing the nausea-induced MRIs to the control MRIs of the participants.

A model was created to predict nausea susceptibility using machine learning. This model was designed as a binary, giving results of either 0 or 1. A 0 indicates nausea resistance in an individual while a 1 indicates nausea susceptibility in an individual. Neuroanatomical features, connectivity features, and autonomic (parasympathetic and sympathetic) features are used to predict nausea susceptibility.[10] These steps are summarized in figure 1 below for reference.

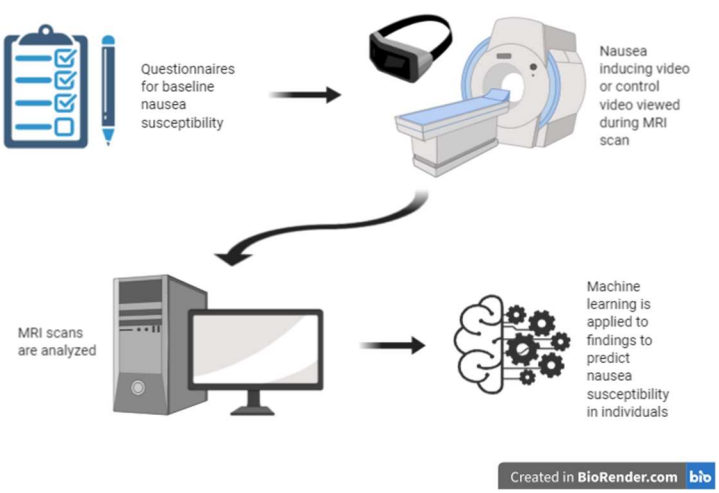

Figure 1: This image describes the process by which machine learning was used to predict nausea susceptibility. Questionnaires were used to assess nausea susceptibility in participants before MRI scans are taken. While the MRI scans were being taken, participants watched a nausea-inducing video and later watched a stable video as a control. MRI scans were then analyzed using computer programming to identify differences in the nausea susceptible brain. This information was used to teach a computer how to identify an individual susceptible to nausea versus an individual resistant to nausea. Machine learning was then used to predict an individual's susceptibility to nausea.

From this research, it was found that the MSSQ score was not an effective predictor for nausea sensitivity. The MSSQ score showed no significant correlation with the VAS score or the MSAQ score. This means that, in response to this stimulus, the MSSQ score was a poor predictive measure for nausea severity in the participants. In regard to these results, the VAS score is used as an effective prediction for nausea. The severity of the VAS score during the fMRI showed a significant correlation with sympathetic and parasympathetic activity, showing the ability of VAS to predict an individual's nausea susceptibility.

The model for machine learning was trained using 70% of the susceptibility data and obtained an accuracy of 80% when predicting nausea susceptibility in this data set. The model was then used to predict nausea susceptibility of the remaining 30% of the data. The model was able to accurately assign nausea susceptibility or resistance to 100% of the individuals in this remaining section of data when compared to the respective individual's VAS score.

Small anatomical and functional differences in the brain can correspond to struggles in daily life, as seen through susceptibility to nausea. Research in nausea susceptibility is needed in order to fully understand the inner workings of the brain.[11] This research uncovers underlying differences that impact an individual's life and physical comfort by affecting susceptibility to nausea. This will lay the foundation for understanding the causes of nausea susceptibility and resistance.

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New imaging technique for assessing damage to brain cells

The study examines a novel myelin imaging technique (REMyDI) that was used to discover the amount of myelin in patients with multiple sclerosis and how the quantity was correlated with their physical and cognitive disability ratings.

Susannah Schloss

There has long been a need for studying the damage done to brain cells during autoimmune and neurodegenerative diseases. These brain cells, called neurons, are protected by a fatty insulating tissue called myelin. This protective insulation is often both visibly and invisibly compromised in a variety of diseases, such as multiple sclerosis (MS). Current testing for damage to the brains of patients with MS is fairly limited to what a conventional magnetic resonance imaging (MRI) technique displays, and lacks the specificity of illuminating damage to myelin itself.[1,2] Along this vein of thought, Ouellette et al. (2020) examined a new technique called Rapid Estimation of Myelin for Diagnostic Imaging (REMyDI) with regards to its efficacy in assessing the myelin composition of healthy brains and of those compromised by MS. The researchers found that REMyDI was validated by the many tests performed. There is a need to study such fundamental aspects of debilitating diseases, and research which helps validate new techniques that aid future efforts to diagnose and treat MS.

To better understand why the REMyDI technique requires validation, one must first understand its components and the logic behind what it measures in the brain. REMyDI was conceived first by understanding conventional MRI displays and then transferring that understanding to create quantitative MRI displays.[3] This was further explored by integrating Magnetic Transfer Imaging (MTI) in order to differentiate between singular unbound protons and those bound to fats; this is also related to the identification of myelin by the water present in the fatty tissue itself and the water present not inside of it.[3,4,5,6] This presence of water allows the structure to be closely examined, especially when there are no visual deficits in the fatty tissue.[7,8,9] Some of the unique facets of previous studies were T/T and proton density mapping, which are generally limited by time and budget constraints, and describe the interaction of magnetic field properties within both quantitative MRIs and MTIs and have been integrated into REMyDI.[1,8,10,11] The concerns which were addressed in previous articles, including low-resolution displays and time consuming unreliable tests, have been mitigated in REMyDI to allow for a 7 minutes comprehensive scan.[1] The constraints on the utilization of intensive myelin imaging are now far less likely to hinder further exploration and eventual use of REMyDI in clinical practice.

The study by Ouellette et al. (2020) had many parts, the first of which was multiple forms of staining allowing colored dye to reveal the presence and location of myelin within sections of brain tissue examined under a microscope. The next step was recruiting 21 healthy individuals and 71 individuals with MS to participate in the study. As stated earlier, REMyDI combines ideas from many previous studies aimed at measuring myelin, such as quantitative MRI, MTI, T /T relaxometry, and proton density mapping. The simplest way to understand what is being measured by these tests is to understand the nature of water within myelin. The specific

properties of water in contact with fatty tissue, as opposed to water in an environment where fatty tissue is not present or is negligible, allows a ratio to be composed, which compares these two situations. This is done using the properties of magnetic fields and the tendency of molecules to spin as they interact with one another, giving a measurable way to assess the state of the myelin present in their brain cells. All MS patients were also given tests by a qualified physician to determine their physical and cognitive disability levels at the time of the study and also approximately 1.5 to 2 years after. The results of the myelin stains and the REMyDI scans were compared, and then quantitatively analyzed in the context of the cognitive and physical disability scores.

The results of the study showed that the myelin found in the staining procedure and in the REMyDI scans had significant correlation in that both methods produced accurate representations of myelin distribution within the brain. Likewise, the disability tests required for the MS patient group aligned with the composition and amount of myelin present at the time of testing and two years after for the follow-up.[11] In other words, low myelin amounts in the brain correlated with impaired physical and cognitive ability. The researchers posited that because there appeared to be less myelin in the unlesioned fatty tissue of the brain of MS patients, REMyDI could assess the status of myelin within the tissue, which appeared normal upon cursory visual analysis.

When researching myelin and its relationship to MS, it is inevitable to come across speculation of repairing the damaged myelin as a treatment. The amount of myelin and visible lesions in the brain are also taken as indicators of MS using conventional MRI.[7] However, this does not account for the invisible damage to myelin which often accompanies MS. While other studies have explored each aspect of myelin imaging, REMyDI is the first to combine all of these facets into a single comprehensive testing scan which takes 7 minutes and has proven consistent when tested against itself in multiple trials.[1,3,5,8] As most scans are expensive and time-consuming, a single scan will prove useful for future diagnostic and treatment efforts related to myelin diseases. This study was extremely thorough and addressed all previous research done in similar areas while making sure to note differences and explain why they occurred. Comparing multiple myelin stains with actual images from REMyDI, and showing the correlation between them, allows for confidence when addressing the quantities of myelin within specific areas of the brain. The correlation of less myelin with impaired physical and cognitive disability shown by REMyDI and the disability test itself aligns with expectations of MS damaging myelin within the brain, which bodes well for the use of REMyDI in any future studies exploring MS.

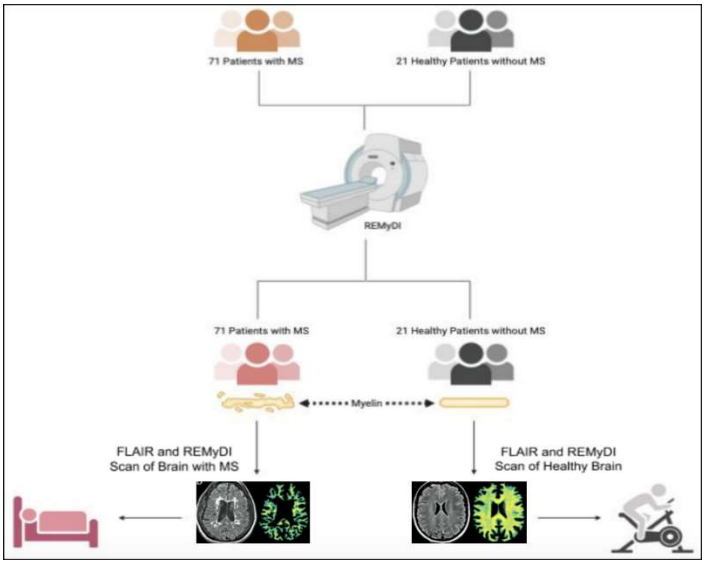

Figure 1. Amount of myelin present in the brains of patients with MS and without MS measured with REMyDI. The general outline of the experiment included 71 patients with MS and 21 healthy patients undergoing the REMyDI technique. Black and white MRI scans are shown alongside fluorescent yellow-green REMyDI scans to better display the location of lesions caused by MS (which were represented by the white portions inside the MS brain) and also the myelin distribution. The REMyDI image, in particular, shows the significant decrease in myelin of the brain in MS, leading to various consequences for the cognitive and physical abilities of the afflicted. The use of REMyDI in assessing invisible damage to brain cells can therefore lead to

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Opening the blood-brain barrier

The study examines a novel myelin imaging technique (REMyDI) that was used to discover the amount of myelin in patients with multiple sclerosis and how the quantity was correlated with their physical and cognitive disability ratings.

Alex Tomceac

The blood-brain barrier prevents most drugs from entering the brain due to its protective elements and the way it is structured.[1] In a recently published article, Nguyen and colleagues developed a technique of using a newly developed biomedical material to utilize for monitoring intra-organ pressure but more importantly, to open the blood-brain barrier.[2] The motivation behind this research was to find a more effective device to deliver drugs through the blood-brain barrier, but also a safer way to implement these medical devices without requiring multiple aggressive surgeries that are harmful to the tissues to get to the target location. The primary finding of this research was that the biodegradable device that was created by biomedical engineers is a better way to assist with the delivery of drugs to the brain in localized areas. This is also a new path to avoid invasive surgeries and greatly impact the development of different medical devices in various fields of medicine.[2]

The human brain has evolved great lengths to keep itself safe and protected from damage. One of the protective elements that the brain has is the blood-brain barrier (BBB). This barrier, as the name, suggests is the physical wall is composed of different cells and cellular components that separate the blood vessels and neuronal tissue. The endothelial cells that line the walls of all blood vessels are structured differently in the vessels of the brain.[3] These cells in other parts of the body are fenestrated in the blood vessels, while in the brain, they form tight junctions that only allow small molecules and a few volatile gasses to pass through to the tissue.[4] This barrier is also supported by astrocytes and extracellular matrix proteins that are produced by pericytes to further maintain the barrier making it highly selective.[5] The BBB is a major obstacle for delivering drugs to the brain for different neurological disorders because these compounds are classified as foreign molecules, therefore, are prevented from passing through to the target tissue.[6] Many techniques have been utilized to get passed the BBB, including developing drugs that are lipid-soluble to get delivered to their targets.[7] Other new techniques involve using ultrasound are much more effective at opening the BBB by using the vibrations created by a transducer device.[8] There are shortcomings with the current ultrasound technology because it is very expensive, and ultrasound waves must be localized on the tissue from multiple sources.[9] Another problem with the current transducers devices that are much better at localizing in a specific area is that they require surgery and contain toxic materials such as lead.[10] The new device that the bioengineers developed is biodegradable and made of safe materials. It produces localized vibrations to open the BBB for the drugs to pass to the brain tissue successfully.

The piezoelectric transducer is composed of poly L-lactic acid (PLLA) polymer aligned into a specific pattern, and it is activated by an electric circuit that causes the nanofibers to move and vibrate at a high intensity.[2] This transducer is then placed through surgical procedure into the

brain of a mouse which is connected to erodible wires that are attached to an electrical input to stimulate it. The engineers split the mouse brain into three equal coronal sections (C1, C2, C3). The device was placed in section 2 (C2) and the other sections (C1 and C3) were used as to serve internal control. The control variable in the experiment used a different transducer that was nonpiezoelectric (different material) and had a lower intensity vibration. The effectiveness of the transducer was measured by the concentration of blood protein in neuronal tissue which was coded with fluorescent coloring. A trial consisted of the transducer getting stimulated to vibrate for 30 seconds then a 30-second break.[2]

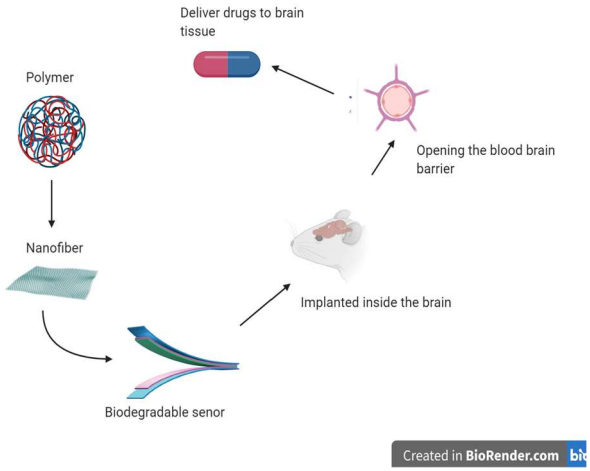

Figure 1: PLLA nanofibers implanted into a biodegradable device which is then placed surgically into the brain. The transducer utilizes ultrasound to open the blood brain barrier to deliver the necessary drugs for treatment.

Autofluorescence analysis showed higher concentrations of the labeled protein in neuronal tissue in the mice that had the new device implemented. The control produced no similar results showing only low concentrations of the protein. The experiment further ensured that the results are significant and valid by using another drug which also produced the same results. The experimenters analyzed the histology after 2 and 4 weeks after implementation, and results showed that the biodegradable transducer caused a minimal immune response.

This research has many implications, including utilizing these biodegradable materials to engineer medical sensors and transducers for other areas of medicine. The surgical procedure to implement this transducer is much more favorable because the device degrades and does not need to be removed like other transducers. The use of batteries can be eliminated in medical devices in the future if these same materials are used. One of the negative aspects of this is the device requires an external output to produce the desired outcomes. This is still a new field which needs to be further tested to solidify the validity of this experiment.

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Can't sleep? You might need some stimulation

Scientists discover the therapeutic potential of electrical vagus nerve stimulation in the treatment of primary insomnia

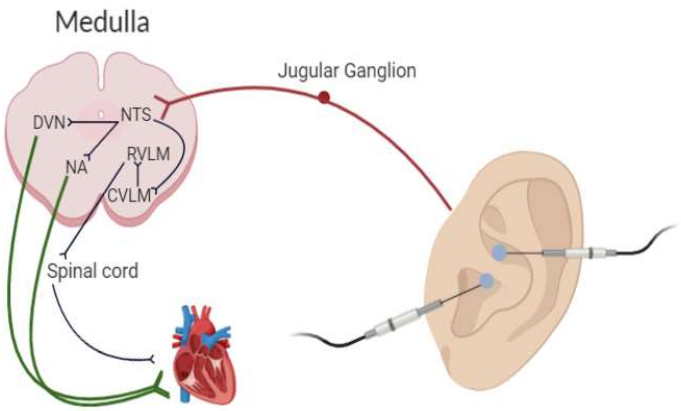
Sydney Wolfe

The therapeutic effects of transcutaneous auricular vagus nerve stimulation (taVNS) have only recently been explored in the last few years. TaVNS involves the application of electrical stimulation to the external portion of the ear to activate an essential cranial nerve called the vagus nerve.[1] In a recent article published in Frontiers in Neuroscience, researchers from the China Academy of Chinese Medical Sciences revealed the immediate effects of taVNS on the brains of patients with primary insomnia (PI).[2] They found that patients with primary insomnia showed higher resting-state neural activity than healthy patients, particularly in areas in the cerebral cortex. Interestingly, the application of taVNS to PI patients caused a significant decrease in neuronal activity in these abnormally hyperactive areas. These results provide a promising non-invasive treatment for patients with primary insomnia.

Primary insomnia is becoming an increasingly common issue, with as high as 10% to 15% of the world adult population classifying for a PI diagnosis.[3] PI is classified as sleep disturbances that are unrelated to other medical disorders, substance use, or prescription use and must persist for three nights per week for at least three months.[3] While most patients are treated using pharmacologic drugs, including benzodiazepines, non-pharmacological methods are being explored as alternatives, such as cognitive-behavioral therapy and taVNS. The therapeutic effects of taVNS have been primarily studied in the context of treatment-resistant depression.[7] Clinical studies have shown that taVNS can alleviate many adverse symptoms associated with depression, including anxiety, hopelessness, and sleep disturbance.[4,6,8] If taVNS can reduce depressive symptoms, one of which includes insomnia, taVNS can be a viable method for treating PI. While the exact mechanism for PI is not entirely understood, many scientists believe that "hyperarousal" is to blame. This theory suggests that patients with insomnia express abnormally high amounts of neurological activity. TaVNS induces a regulatory effect on patients with hyperactive sympathetic activity through stimulation of the vagus nerve.

The experiment

The study observed twenty-two participants with primary insomnia and twenty healthy patients without insomnia. Bilateral electrodes were placed on the auricular concha area for both ears.[2] The stimulus frequency was set at 20 Hz, and stimulus intensity varied from patient to patient (4 mA to 6 mA).[2] Stimulation was applied for 30 minutes.[2] Then, fMRI scans were taken of the PI patients before and directly after taVNS treatment.[2] The brains of healthy participants were also imaged once using fMRI.[2] The researchers performed two sets of analyses on the brain image: amplitude of low-frequency fluctuations (ALFF) and resting- state functional connectivity (RSFC) analysis. [2] These tests aimed to identify any statistically significant differences between brain activity between healthy controls and PI patients.



A proposed schematic for the hyperarousal theory (created in bioRender). Bilateral electrodes are placed on the ear's Auricular Branch of the vagus nerve (ABVN). The signal travels to the peripheral jugular ganglion, and then to the nucleus tractus solitarius (NTS). Projections are made to various medullary structures, including the dorsal vagal nucleus (DVN), nucleus ambiguus (NA), the rostral ventrolateral Medulla (RVLM), and the caudal ventrolateral medulla (CVLM). The DVN, NA, and RVLM can then send neuronal projections to the spinal cord and heart, causing a regulatory effect on sympathetic activity.[1] Vagal afferent projections are denoted by the red line, while vagal efferent projections are denoted by the green lines.

What they found

Significance was measured using a t-test, incorporating voxel quantification from the fMRI scans. Patients with PI had significantly increased ALFF in the right precuneus when compared to the control subjects.[2]. Following taVNS treatment, ALFF was remarkably decreased in the right precuneus and raised in the left middle occipital gyrus.[2] These findings implicated the right precuneus and the right cerebral cortex as regions of interest (ROI). After taVNS, RSFC analysis showed significant decreases in connectivity between the right precuneus and the right superior frontal gyrus, the right middle frontal gyrus, and the right angular gyrus. These results indicate that taVNS may have an unarousing effect on the brain.

Future directions

The exact mechanism by which taVNS operates in patients with primary insomnia is unknown. Studies have uncovered a general mechanism for the pathway of taVNS and how signals project from the ear to the medulla. However, the number of brain areas and pathways activated are far too numerous to attribute one mechanism to insomnia with our current body of knowledge. Further studies will need to explore the functions of the right precuneus and cerebral cortex in sleeplessness and hyperarousal. Additionally, studies will need to identify what medullary structures project to neurons in these ROIs to understand better why taVNS decreases (or increases) frequency fluctuations in these areas. While vagal nerve afferents (to the brain) are known to project to the medulla, vagal nerve efferents (away from the brain) are known to project to the heart, spinal cord, and other vital organs. Studying how these projections might regulate autonomic function may give insight into its therapeutic effects on primary insomnia.

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Mutation in receptors of the corpus callosum causes developmental issues in children

A mutation in the receptor DCC, needed for guidance of axons of the corpus callosum during development, is found to cause isolated agenesis. This provides information that can be used for prenatal detection of agenesis and the risk factor of an abnormal neurodevelopmental outcome.

Jordan Hill

The complete or partial absence of the corpus callosum is called agenesis and affects about 1 in 4,000 newborns and 3-5% of children with intellectual disabilities. Many genetic mutations can cause agenesis and intellectual disabilities, but the genetics of isolated agenesis is not fully understood. In a paper recently published in Nature Genetics, Ashley Marsh and her team investigated the formation of the corpus callosum and the effect that a mutation on a specific receptor plays on isolated agenesis. Marsh concluded that a mutation on the ligand-receptor DCC caused isolated agenesis in humans. This observation has implications for prenatal diagnosis and parental counseling.

The corpus callosum plays an important role in the communication between the left and right hemispheres of the brain. It is crucial to processing motor, sensory, and high-level cognitive signals. When the corpus callosum has been damaged or is missing, the communication becomes interrupted and can cause epileptic seizures, vision impairments, hearing impairments, difficulty reading facial expressions, difficulty understand abstract concepts, and more. When this occurs in the fetus or growing child, agenesis can cause developmental and behavioral delays and intellectual disorders. There are several ligands in the corpus callosum. The primary ligand that this recent study focused on was Netrin. Netrin is a class of proteins that are responsible for guiding axons as they grow to their proper locations. The receptor DCC is a netrin receptor that is important for guiding callosal axons at the midline.

46 individuals from 9 different families volunteered to have blood samples taken. They also consented to diffusion MRI and tractography, which allowed the research team to confirm agenesis. Once the data was collected the research team was able to run a linkage analysis and whole-exome sequence. This provided the team with the ability to create a family tree and mark who were carriers and who suffered from agenesis and who suffered from congenital mirror movements. They also took the blood samples to amplify and sequence the DCC coding region of everyone's DNA.

Previously, mutations in DCC were known to cause congenital mirror movement, but with this study it was also found that mutations in DCC cause isolated agenesis in humans. They were not able to determine the exact factors behind the mutation because this most likely includes hormonal context during development, the type and location of DCC mutation, and the genetic background of the individual. Heterozygous mutation of the Netrin receptor DCC resulted in isolated ACC but only had a very mild outward effect and the cognitive outcome was favorable. The exact opposite occurred with syndromic agenesis, or when agenesis was passed from parent to child.

This study was fascinating as they looked at the genetic aspect of agenesis of the corpus callosum. They were able to find a significant difference between agenesis being passed down and agenesis being isolated to the one individual. The work that was performed could easily be used to screen fetuses, much like how they do prescreen of other genetic disorders. This is important for preparing parents who may not be used to caring for a child with developmental delays. This work is also important for understanding the next step in how these specific genes play a role in the development of a fetus.

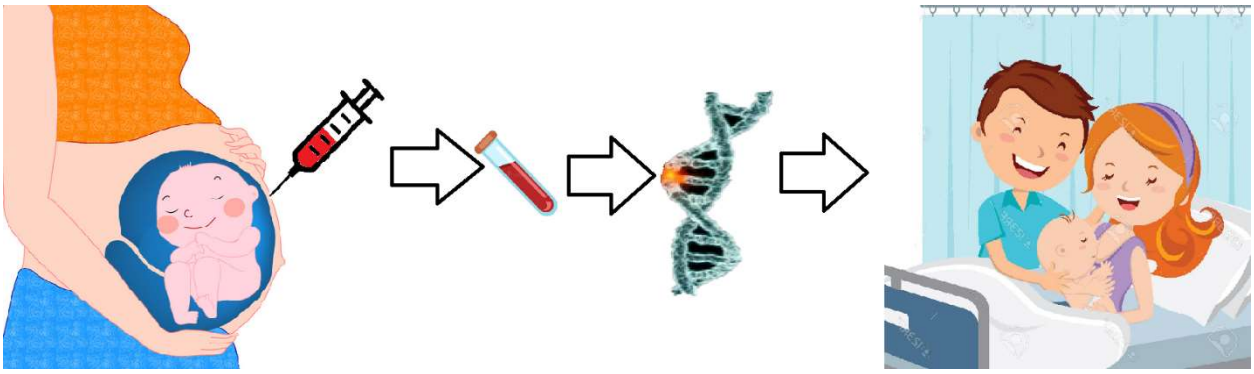

Figure 1: This study can lead to prenatal screening of mutated genes. This allows parents of newborns to be happy because they will have a better understanding of what to expect from their newborn and how to best help them.

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PTSD and its effects on the amygdala and the anterior cingulate cortex

The study aims to show that amygdala hyperactivity following an acute trauma is indicative of chronic post-traumatic stress disorder (PTSD) symptoms. Alternatively, the inability to sustain ventral anterior cingulate cortex activation when being exposed to similar trauma can be linked to difficulty recovering from ongoing PTSD symptoms.

Tori Davis

The results of this study work to identify the risk factors that are present in an individual prior to trauma exposure that could potentially predispose them to later developing PTSD. In a paper published in 2017 by Stevens et al., the brain function of individuals who were exposed to a trauma underwent fMRI to analyze the neural reactivity and habituation to fearful and neutral facial stimuli to understand the correlation between the development of PTSD symptoms and amygdala hyperactivity.[12] The study showed that there was a strong correlation between the hyperactivity of the dorsal anterior cingulate cortex (dACC) and the amygdala when compared to the symptoms of PTSD. Additionally, the study showed that decreased ventral anterior cingulate cortex (vACC) activity is indicative of more severe PTSD symptoms and difficulty recovering from those symptoms. With this information, providers can work to decrease the activity of the amygdala and/or the dAAC as well as increase the activity of the vACC to help treat PTSD symptoms early before the development of more severe PTSD symptoms.

On average, 50-60% of people are exposed to a trauma in their lifetime, and out of those people, 6-8% end up developing PTSD.[1,2] Several studies suggest that connectivity between the amygdala and the dACC is increased when comparing pre-trauma to post trauma as well as when comparing soldiers before going to war and after returning from war.[3,4,5] The amygdala can be related to the human response to a fearful stimulus as well as the stress responses to emotional events like trauma that can have an effect on the development of mood disorders like PTSD and depression.[6,7] Alternatively, the dACC can be related to cognitive function, learning, and memory as well as arousal, which can function alongside the amygdala.[8] By studying these structures and understanding the role they play in individuals with PTSD, the symptoms of these conditions can be minimized before it worsens.

Participants included individuals that had been admitted to an emergency department immediately following a trauma that classified as a criterion A trauma per the DSM-IV-TR and had occurred within the past 24 hours.[9] Prior to the study and at the 1-, 2-, 3-, 6-, and 12-month marks, PTSD and depression symptoms were assessed using the PTSD Symptom Scale and the Beck Depression Inventory respectively.[10,11] Three weeks following the initial trauma, the participants underwent fMRI imaging to view the amygdala, dACC, and vAAC when viewing randomly alternating 15 fearful and 15 neutral faces. Reactivity was measured by comparing fearful vs. neutral faces using a random effect analysis, and habituation was measured by comparing the first third of the fearful and neutral faces (5 faces) to the last third (5 faces) of the fearful and neutral faces.

The results found that not only are the symptoms of PTSD and depression associated with the hyperactivity of the amygdala, but they are also associated with brain areas that regulate the amygdala's activity like the vACC, which ultimately is the cause of the amygdala's hyperactivity. PTSD symptom severity was reassessed 1 month after the initial trauma, which was when the severity was at its worst, and then there was a steady decline by the end of 12 months. However, the symptoms were will moderately present and important to note.

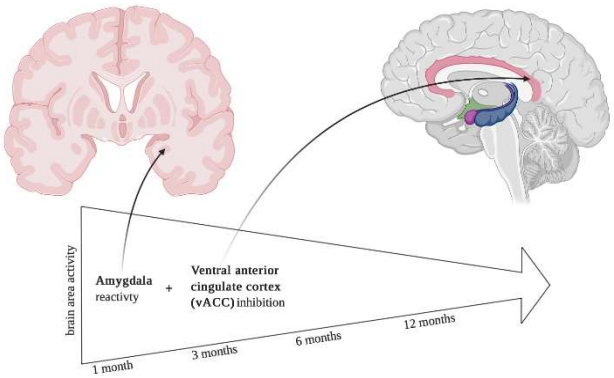

Figure 1: The results of the study showed that following the acute trauma, the increased reactivity of the amygdala and the decreased reactivity of the vACC is associated with the severity of the resulting PTSD symptoms. These brain area associations with symptom severity are more prevalent within the first few weeks following the trauma and decreased with time. By decreasing the reactivity of the amygdala and increasing the reactivity of the vACC within the first month following a trauma, the symptom severity of the resulting PTSD can potentially be decreased overall. Image created by BioRender.

Other findings indicated that the increased amygdala reactivity was present early on following the trauma alongside increased reactivity of the dACC and decreased reactivity of the vACC. The more time passed, the less prevalent the increased reactivity of the dACC and the decreased reactivity of the vACC was noticed. Additionally, there was no effect regarding amygdala habituation, however habituation of the vACC did indicate a worsened recovery time over a 12-month period. This finding is only significant for fearful stimuli. Regardless, overall the data did show that higher amygdala reactivity immediately post-trauma indicates a higher level of PTSD symptom severity after 12 months, and these individuals tended to maintain their PTSD symptoms for a longer period of time in comparison to their counterpart.

In conclusion, this data is important, because it can help providers treat neurological differences in those predisposed to PTSD symptoms and depression immediately following the trauma to help reduce long-term symptoms. Due to the data that showed that the following changes were observed only immediately following the trauma, these negative symptoms can be reduced by treating the increased amygdala reactivity, the increased dACC reactivity, and the decreased vACC reactivity early on after the trauma occurs. This is life-changing news since soldiers and trauma victims in the ED can be treated within three weeks of the exposure and can potentially show less long-term effects, resulting in a higher quality of life for those affected.

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Broccoli can prevent psychosis

A new study shows intake of a glucoraphanin dietary prevents sfi1 expression in the medial prefrontal cortex for adult offspring of maternal immune activation.

Vanity Garcia

Abstract

Schizophrenia can be determined from genetic vulnerabilities and environmental insults in order to be diagnosed as a neurodevelopmental disorder.[1] A genetic abnormality of maternal immune activation can lead into the severe symptoms of schizophrenia disorder.[2] Maternal immune activation (MIA) occurs in pregnant women who are at a higher risk of their neonate's functional connectivity being stressed, inflamed, and weakened.[3] MIA increases in glutamatergic synapse and expression of suppressor of fermentation-induced loss of stress resistance protein 1 (Sfl1) mRNA from the medial prefrontal cortex.[4] In a paper recently published in Scientific Reports, Matsuura and Hashimoto investigated the intake of glucoraphanin prevents sfi1 expression in the medial prefrontal cortex for adult offspring with maternal immune activation.[5]

Background

Glucoraphanin is the precursor of a sulforaphane antioxidant and found in cruciferous vegetables such as broccoli and cauliflower.[5,6] Adults that were offspring of maternal immune activation have been demonstrated to reduce psychosis symptoms with glucoraphanin dietary.[5] Psychosis symptoms are a higher risk to develop for adult offspring such as delusional, hallucinations, and cognitive functions.[7] Cognitive dysfunctions and environmental stressors have shown to impair prefrontal functions such as maternal immune activation.[8]

Methods

Pregnant mice with embryos of 9-10 weeks old were placed under a controlled polycarbonate cage with a 12/12-hour light-dark cycle. The mice were injected intraperitoneally for 6 consecutive days with poly(I:C) in order to contract MIA in figure 1.a.[5] The offspring from their mothers after 3 weeks and caged into 3-5 per group.[5] Food pellets contained 0.1% of glucoraphanin were prepared with broccoli sprout extract powder and fed to the offspring in figure 1.a.[5] The Sfi1 mRNA expression levels of the offspring's prefrontal cortex were measured by an RNA extraction using an RNeasy Mini kit.[5] Western blot analysis of Sfi1 protein in the medial prefrontal cortex from offspring after

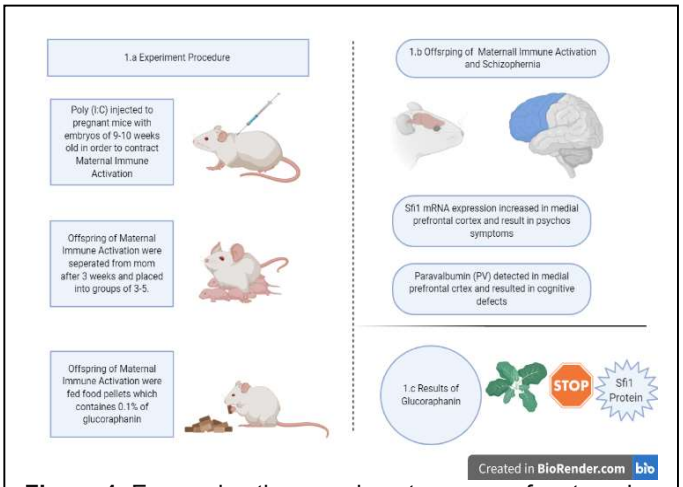

Figure 1. Expressing the experiment process of maternal immune infection being expressed from pregnant mice 1.a. The common results within the medial prefrontal cortex of offspring with maternal immune activation and schizophrenia, causing the similar psychosis symptoms of the neurological disorder in 1.b. The final result of dietary intake of glucoraphanin blocks the sfi1 expression within the medial prefrontal cortex which increases the psychosis symptoms of the disorder in 1.c.

maternal immune activation was performed and compared to postmortem brain samples from schizophrenia.[5,9] Immunohistochemistry analysis of parvalbumin (PV) in the medial prefrontal cortex from offspring after MIA were observed for cognitive defects in figure 1.b.[5]

Results

Expression of prenatal poly(I:C) showed a reduction of parvalbumin immunohistochemistry in offspring's medial prefrontal cortex with MIA compared to the control group. The reduction of PV resulted to cognitive impairment and psychosis.[5] The effects of dietary intake of 0.1% glucoraphanin food pellets on adult offspring after MIA resulted in improved cognitive deficits. Another PV immunohistochemistry was performed on adult offspring of MIA with a dietary intake [5]of glucoraphanin had a significantly higher amount of PV within the medial prefrontal cortex than the control group.[5] The RNA sequencing of the prefrontal cortex in adult offspring resulted in a significant change of suppressor of fermentation-induced loss of stress resistance (sfi1) expression.[5] The sfi1 mRNA expression in the prefrontal cortex was increased by the poly(I:C) injection and the dietary intake of glucoraphanin blocked the increase of the sfi1 gene expression shown in figure 1.c.[5] The Sfi1 protein in schizophrenia was significantly higher than the control group.[5] Sfi1 mRNA expression in hair follicles of schizophrenia were significantly lower than the healthy control group.[5]

Conclusion

Maternal immune activation is an infection-induced during pregnancy from a cascade of cytokines and immunologic alterations are transmitted to the fetus. MIA strikes the central nervous system and causes a neurochemical and anatomic changes in the brain.[11] These changes result into psychosis symptoms and schizophrenia disorder in the adult offspring after MIA.[10] This study explored how to reduce the psychosis symptoms in the adult offspring after maternal immune activation by using a dietary intake of glucoraphanin. Intaking 0.1% of glucoraphanin for offspring after MIA prevented cognitive deficits and reduction of parvalbumin in the medial prefrontal cortex. The Sfi1 expression aided in the development of behavioral abnormalities in adult offspring, which were blocked by the glucoraphanin intake.[5] Comparing the sfi1 mRNA in hair follicles of schizophrenia mice to sfi1 protein in the postmortem brain expressed how sfi1 associates to the pathophysiology of schizophrenia.[5] This study reveals how adolescences are more vulnerable to psychiatric disorders and how psychosis symptoms can be prevented during this time period.[5] Looking into more nutritional antioxidants that can prevent psychosis for schizophrenia disorder can benefit future research.

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Size does matter

The article discusses the difference in the size of the pineal gland between those with obsessive-compulsive personality disorder (OCPD) and those without it. The size difference matters as those with smaller pineal gland have consistently been diagnosed with OCPD.

Cori Dougher

The motivation behind this research is that our knowledge of the pathology of personality disorders is not very vast, especially regarding OCPD. Our knowledge of personality disorders is limited to psychoanalytical approaches alone. This is why performing the brain imaging of those with OCPD is crucial to gain knowledge on the biological aspects of this personality disorder to better understand how this disorder comes about. In the study by Murad Atmaca and Colleagues, they studied the size of the pineal gland of patients diagnosed with OCPD and healthy subjects by sending them through magnetic resonance imaging (MRI) and comparing the images of these patients. The findings showed that those with OCPD have a pineal gland volume 20% smaller than the normal patients. Also, to note was that hormonal changes of the pineal gland by melatonin and pineal calcifications were not included in the volumes of the pineal gland size, which could skew the results in a different way if these were considered. The findings of the study still show that those diagnosed with OCPD have significantly smaller pineal glands. This could prove that OCPD falls on the OCD spectrum of disorders due to similar results of MRI scans.

This study was based on other research done by Atmaca and colleagues. In an unpublished study done by Atmaca and colleagues, their team examined the size of the orbitofrontal cortex or the OFC, along with the thalamus in patients with and without OCPD. The study found that those who had been diagnosed with OCPD their MRI image showed much smaller left and right OFC's compared to those of normal patients, but those with OCPD had a greater size thalamus than those of normal patients. This unpublished study by Atmaca is what drove them to also study the pineal gland of those same patients that are normal and those that are diagnosed with OCPD.

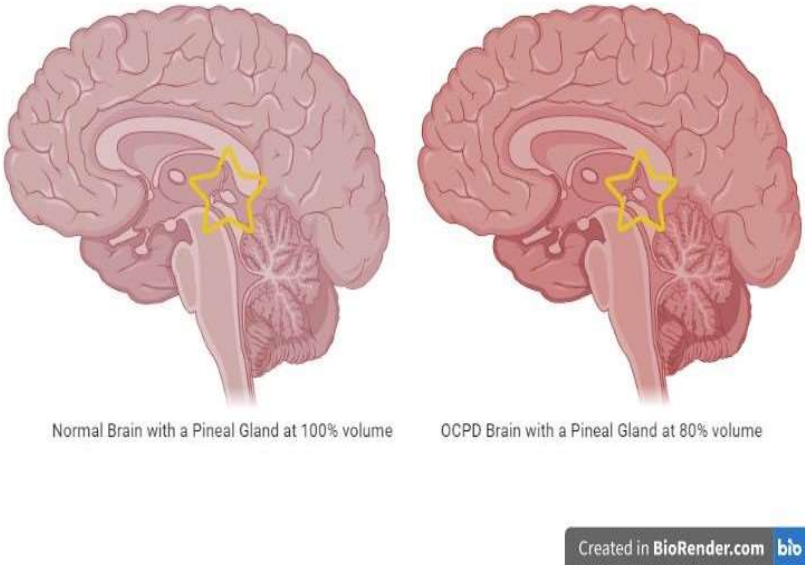
The pineal gland, as stated by Acer in "Pineal Gland and Melatonin: Recent Advances in Development, Imaging, Disease and Treatment" is a small, pinecone shaped organ of the brain that has a part in the circadian rhythms and sleep cycles by secreting melatonin at the appropriate times. The secretion of melatonin is altered in those with schizophrenia, bipolar disorder, and major depressive disorder as mentioned by Brown and their colleagues in their study on melatonin secretion. Catapano and Monteleone performed a study to track the melatonin release in normal patients and Obsessive-compulsive disorder or OCD over a 24-hour period. The patients with OCD had abnormal secretion of melatonin leading to troubles falling and staying asleep. This study was the other driver of the study by Atmaca as the pineal gland size was observed in OCD patients and normal patients and it was noted that those with OCD had a pineal gland volume that of 80% of the volume in comparison with the normal patients. This led Atmaca to think about how pineal gland sizes differ in those with not only OCD but OCPD as well.

The group utilized for this study was composed of consenting patients who were diagnosed with obsessive-compulsive personality by the clinics of the Firat University School of medicine Department of Psychiatry as well as ones diagnosed as healthy. These patients were between the ages of 18-65 and also participated in the previous study by Atmaca where they studied the size of the thalamus and the OFC in OCPD and normal patients. There was a normal patient for every diagnosed patient by matching based on age, sex, education, and handedness. If any of these patients were found to have any other psychiatric disorders, medical problems, malformations of the brain, or any substance abuse within 6 months of the study they were emitted from the study to prevent any discriminations. The procedure consisted of taking MRI images of the pineal gland for both the diagnosed and normal patients. The pictures were taken by a neuroradiologist researcher who was ignorant of who had OCPD and who was normal along with being verified by a second researcher who was ignorant as well. The boundaries of the pineal gland were determined by Jackson, Patel, Yuh, Sumida, Findikli, and their colleagues which were the superior colliculus, the quadrigeminal cistern, and the posterior portion of the third ventricle. Sagittal, Coronal, and Axial views were all taken of the pineal gland to see its entirety. The height and width of the pineal gland were measured on the coronal images and the length was measured on the axial images. These images were then transferred to a semi-automated program to obtain the volumetric results. To perform statistical analysis, the statistical package for social sciences was used. A t-test was used to compare the mean ages and volumetric differences between the patients with OCPD and the normal patients. A chi-square test was done to assess the gender distribution among the patients. A volumetric comparison of age, gender, and total brain volume was performed. The last statistical analysis to be performed was the Spearman's correlation test which detects correlations between pineal gland volumes and the demographic/clinical parameters as well.

Analyzing the demographics of the 16 patients with OCD and the 18 patients in the control group (the normal patients) showed they are pretty similar overall, which was the intent so it wouldn't be a major factor. The age of the OCPD group averaged 32.5 years old plus or minus 8.9 years while the average age for the control group is 29.5 years old plus or minus 5.1 years. There is a ratio of 11 females to 5 males in the OCPD group while there are 10 females and 8 males. For the OCPD patients, 9 of them at least finished high school while 15 from the normal group finished high school, while the rest from both groups (7 and 3 respectively) did not. Everyone from both groups were right-handed. On the depression rating scale, the OCPD group had an average score of 9.8 plus or minus 2.2 points while the normal group had an average score of 3.3 plus or minus 1.7 points. The OCPD patients had been diagnosed with their illness on average about 6.4 years from the start of the study plus or minus 2.2 years.

While there were no differences in the gray and white matter volumes of the brain between the two groups, the volumes of the pineal glands differed greatly. The average size of the pineal gland in the group diagnosed with OCPD was 20% smaller than the normal group. Even when controlling for gender, age, and overall brain volumes, those diagnosed with OCPD still had significantly smaller pineal gland volumes. When utilizing a correlation test, there was no correlational relationship between any of the demographical or clinical variables and pineal gland volumes among the OCPD patients and normal patients.

This was the first study on the volumes of the pineal gland in those diagnosed with OCPD so the information gathered from this study can help to grasp a better understanding of OCPD. While OCPD is normally classified as a personality disorder there has been increasing evidence stated by Fineberg and colleagues that it could actually be more like a neurocognitive function disorder. Stein states how OCPD when in comparison with OCD based on phenomenology, comorbidity, heritability, risk factors, the course of the illness, and response to treatment shows OCPD falls among personality disorders, obsessive-compulsive, and related disorders, which is conflicting with the previous statement by Fineberg. This study done by Atmaca and a previous study of theirs also listed in this paper, shows that those with OCPD has significantly reduced sizes of OFC, the thalamus, and the pineal gland, which was also found to be true in patients with OCD in previous studies done by Atmaca. The sum of these studies shows that OCPD and OCD could fall onto the same spectrum of disorders based on neuroanatomy. While normally studying such a small sample size would not permit these drastic of claims, since OCPD normally has comorbid situations it can be hard to perform studies on those with OCPD so this small sample size is acceptable. Considering all of this, I believe further studies are necessary to draw conclusions that could change the way we associate and diagnose OCPD. While OCD and OCPD are similar in nature, I believe this study does prove that they fall within the same spectrum of mental disorders, but a study with a larger sample size is necessary to draw any further conclusions.



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NEUROANATOMY

Apathy or empathy: the systems behind guilt analyzed

What makes us feel guilty when we silently walk past a panhandler or say "no thanks" when asked to round up our purchase for charity at the grocery store? Brain scanning of individuals in guilt-causing situations implicate the amygdala, insula, and superior temporal sulcus.

Nicholas Le

The neural regions that affect our feelings of guilt and the emotions that follow have largely been unknown. Guilt can be described as one of the cornerstones and a large contributor to our behavior in society.[1] Altruism, the act of selflessness for others, is a belief that many of us practice today. In recent studies, altruism has been concluded to be a factor in why we feel guilt in situations such as declining to give charitable donations.[2] Guilt proneness can also be a factor in individuals' personalities, where those who are more prone to feelings of guiltiness are calmer in angering situations and more willing to accept their past mistakes.[3,4] However, while we have known the emotional causes behind feelings of guilt, there has been a lack of imaging studies done to determine the neural regions and structures behind it as well as how it affects further decision making.

In an article published in 2017, Ambrose Ty, Derek Mitchell, and Elizabeth Finger conducted an imaging study in which they observed individuals' brains during a guilt-causing situation. The study's participants were asked to donate to fictitious charities; however, if they chose not to donate, the fictional money would go towards their compensation for participating in order instead to intensify emotional tension in the situation.[5] Observations from the study conducted by Ty, et al. in 2017 observed that "the amygdala, insula, and [superior temporal sulcus] were activated during choices to not-help when compared to choices to help." The insula was previously found to activate when experiencing guilt-causing events again or anticipating guilt.[6] It was also observed that ventrolateral prefrontal cortex (vlPFC) and dorsomedial prefrontal cortex (dmPFC) activity were associated with individuals' proneness to guilt and how they responded to guilt-causing situations.[5] Ty, et al.'s (2017) research implicates that neural structures related to our personality can be responsible for how we as individuals can process guilt differently, and structures related to how we experience emotions determine how we respond and react to guilt.

Previous studies have suggested that the vlPFC and dmPFC, STS, insula, amygdala, and the anterior cingulate cortex all play a part in how we feel guilt and attempt to avoid it.[7,8] The studies' methods varied from presenting guilt-causing situations with witnesses and without, to reading guilt-causing statements to participants. Increased activation of the medial prefrontal cortex (mPFC), a brain region involved in morality and decision making, was also related to guilt in Takahashi, et al.'s 2004 study of guilt and embarrassment through participants reading sentences. A study by Steven Greening, et al., also suggested that the dmPFC is involved in charitable acts, where people can view losses to charity as greater to losses for themselves.[9]

Ty, et al.'s study began with gathering 23 healthy participants with written consent. The participants were placed in a functional magnetic resonance imaging (fMRI) machine and shown

a website that asked them to choose if they wanted to donate to any fictional charities for people in need. If the participants chose not to, the sum of money that would have gone to the charity would instead be added to the participants' compensation money for the study (all participants received an additional $15 after the study regardless of their choice in this situation). If the participant declined, a negative feedback screen would be shown and ask the participant again but with only half of the donation value. If the patient did choose to donate in any situation, a positive feedback screen would be shown. The fMRI machine measured activation levels of participants' brain structures during the scenarios. Following the imaging process of the study, participants were asked to rate their feelings of guilt or compassion during any of their decisions in the scenarios from 1-5, with 1 being "not at all guilty/compassionate" to 5 being "very guilty/compassionate".[5]

Increased activation in the insula, STS, and the amygdala was observed in situations where the participant chose not to donate, suggesting that areas of the brain relevant to emotional processing and negative feedback contribute to guilt. They also found increased activation in the dmPFC and vlPFC which correlated with guilt-proneness when participants were presented with a second opportunity to donate after initially declining.[5] The study implicates the insula, STS, and amygdala as primary factors in creating feelings of guilt following a decision, whereas the vlPFC and dmPFC are responsible for how likely individuals are to experience guilt and how they make decisions following feelings of guilt. This study was important in replicating results found in previous studies such as Takahashi's while following a different experimental technique. The study further verifies that brain structures relevant to emotion also play a role in guilt, and those involved in personality are involved in restitution and proneness to guilt.[5] This study contributes to the research on finding exact answers for how guilt is processed in the human mind. Everyone is different, and that is the most interesting part about neuroscience and psychology: how everyone is structured similarly but can function and react so differently to similar situations.

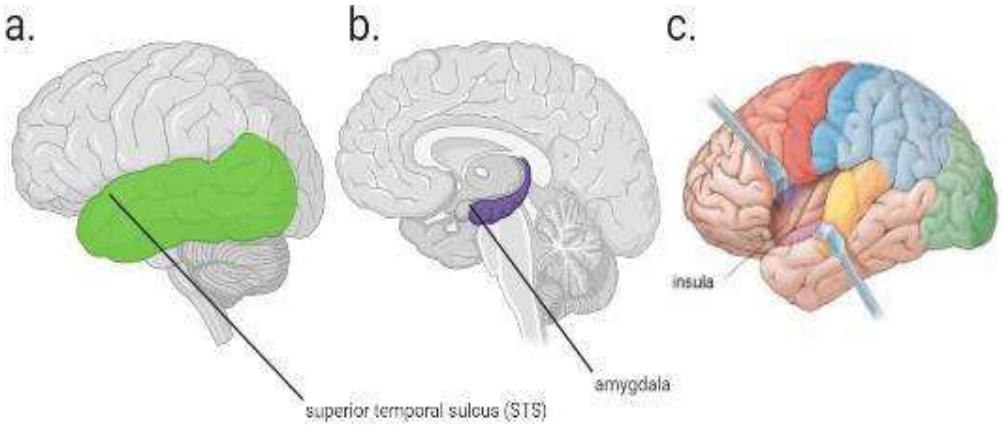

Figure 1. Brain structures implicated in guilt
The superior temporal sulcus is the first sulcus inferior to the lateral fissure and has been implicated in theory-of-mind behaviors (1a).[10] The amygdala is a structure responsible for emotional responses (1b). The insula, or insular cortex, performs "sensory and affective processing to high-level cognition" (1c).[11]

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The obesity epidemic: one more reason to be anxious

Consuming a high-fat Western diet during adolescence can alter the development of brain circuitry involved in fear. This can put an individual at a higher risk for anxiety-related disorders.

Forrest Fearington

Childhood obesity is a rising problem in the United States, affecting over 13.7 million adolescents in the U.S.[1] Adolescent obesity is known to have numerous adverse effects on mental health – especially anxiety and stress.[2,3,4] However, the reason for this correlation is not fully understood, making prevention and treatment difficult. In a paper recently published in *Brain, Behavior, and Immunity,* Vega-Torres and colleagues investigated whether a high-fat Western diet impairs the development of neural circuits associated with fear responses.[5] Using a rat model, they found that consuming a Western diet during adolescence does indeed alter the development of brain regions and brain circuitry involved in fear. The implications of this finding are significant because they can help neuroscientists better understand the mechanisms of obesity-facilitated anxiety, potentially leading to better, more informed methods of treatment.

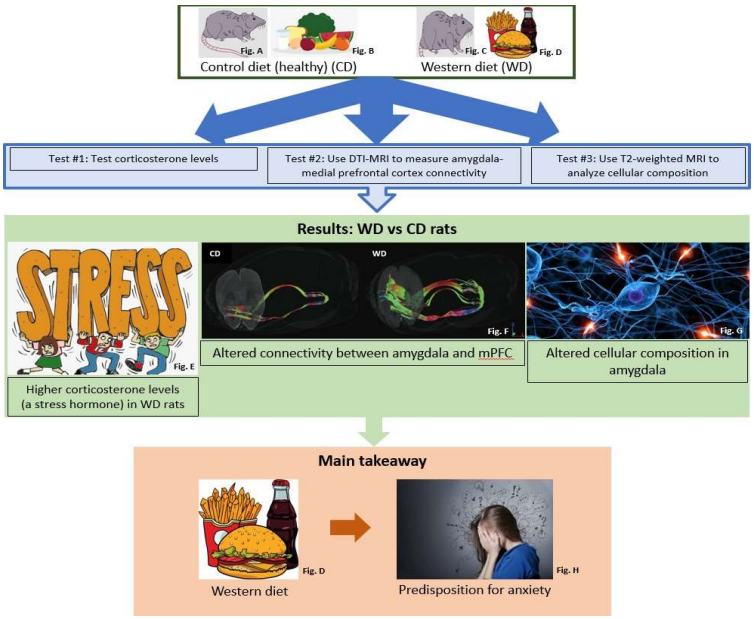
Numerous studies have suggested that obese children are at a higher risk for anxiety and impaired emotional processing.[2,3,4] However, these studies have failed to demonstrate whether social frowning on obesity is the main cause of anxiety or whether the obesogenic diet itself predisposes individuals to abnormal anxious responses.[5] From a physiological perspective, anxiety is generally associated with abnormal activity in the amygdala and medial prefrontal cortex.[6,7] These two brain structures undergo critical development stages during adolescence, and any interference during these stages can have detrimental long-term effects on one's emotional responses.[8] These pieces of evidence have led to the hypothesis that overconsumption of the high-fat Western diet during adolescence affects the development of brain cells and brain circuitry in the amygdala and medial prefrontal cortex, potentially predisposing obese individuals to anxiety-related disorders.[5] Using cutting-edge MRI imaging and chemical labeling techniques, Vega-Torres and colleagues set out to investigate this hypothesis using a rat model.

In the study, thirty-six 3-week-old rats were split into two equal groups and fed either a high-fat Western diet (41% fat) and allowed to become obese or a healthy control diet (13% fat) for 9 weeks. Rats then underwent a 2 day "stress program" to simulate exposure to traumatic events (e.g. electric shocks, loud noises). Post-mortem T2-MRI and DTI-MRI scans were obtained from the WD and CD groups, which provide information regarding cellular composition and connective pathways, respectively. Also, rat feces were collected at three points during the experiment and analyzed for corticosterone, which is a stress hormone similar to cortisol in humans.[9]

The WD rats had significantly higher corticosterone levels than their CD peers at all stages of the study. Corticosterone is closely involved in regulating stress responses, so the higher levels

found in WD rats indicate they were experiencing more stress compared to CD rats.[9,10]

The results of T2-MRI analysis showed more densely packed brain matter in WD rat amygdala. This suggests a different cellular composition between WD and CD rat amygdala, although histological analysis would be needed to confirm this. In addition, DTI-MRI analysis found that connectivity between the mPFC and amygdala (a pathway known as the uncinate fasciculus) differed greatly between WD and CD rats (Fig. F). WD rats showed more abundant connections to certain areas and less abundant connections to other areas in the amygdala and mPFC. This implies the neurocircuitry between WD and CD rats was significantly altered by diet alone.



Summary of methods and results. Rats from two groups were fed a healthy control diet (CD) and a high-fat Western diet (WD). Following 9 weeks of diet consumption and stress exposure, three tests were performed. Results showed higher stress hormone levels in WD rats, altered connectivity between the amygdala and medial prefrontal cortex (mPFC), and altered cellular composition in the amygdala in WD rats. This indicates that consuming a high-fat Western diet can predispose individuals to anxiety-related disorders.

The study clearly found that while keeping all other factors constant, consuming a high-fat Western diet alters brain connectivity, cellular composition, and hormone levels related to stress, likely predisposing these individuals to anxiety-related disorders.[5] The experiment could have been improved by investigating histological changes between WD and CD rat amygdala via staining and microscopic examination, which would have provided more insight on the cellular differences beyond a simple MRI. Interestingly, the article briefly mentioned another study by Paternain et al. that came to contrary conclusions – that is, a high-fat diet could actually reduce stress hormone levels.[10] However, Vega-Torres and colleagues merely discounted this as contrary evidence and never elaborated on possible explanations for the discrepancy, which weakens the reliability of their own findings. While it has its shortcomings, this study advances our understanding of the neural networks and structural changes that predispose obese adolescents to develop anxiety-related disorders, which may inform treatments of mental illness in the future.

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A drug-free solution

Researchers show that stimulating the nucleus accumbens, a brain structure involved with reward, may offer a new treatment option for those suffering from severe depression and anxiety.

Alexandra Pederson

Introduction

Depression and anxiety-related mood disorders are often treated using methods such as psychotherapy, counseling, pharmaceuticals, or any combination of these. When defining depression, it can often be described as a chemical imbalance, but while this is true, it is much more complex than that expression.[1] There are multiple brain areas and chemicals associated with depression and anxiety. When treating this with pharmaceuticals such as antidepressants, it is hard to target these specific brain areas as the drug cannot be directed to a specific area of the brain. Studies conducted in recent years offer a new potential treatment for these disorders through the use of deep brain stimulation (DBS). This type of treatment aims to do what antidepressants cannot by stimulating the specific brain areas associated with these chemicals to generate long term potentiation or neuroplasticity.

What is DBS and how does it work?

Deep brain stimulation is a type of treatment that has been used to treat people with movement disorders such as Parkinson's Disease. For this treatment, an electrode along with its wire is surgically implanted into the brain and can either be placed bilaterally or unilaterally on the desired site of stimulation.[2,3,4] The amount, strength and duration of the stimulation can be controlled and set based on the specific needs of the patient. This treatment method works by regulating abnormalities in specific brain areas through electrical stimulation.[2,3,4] In the case of mood related disorders, a specific area is targeted by these stimulations and over time the synapses in that area will strengthen and new pathways will generate. In addition to DBS there are other alternative treatment options for those who suffer from depression and anxiety related disorders. Transcranial magnetic stimulation (TMS) is a non-invasive treatment that targets and stimulates a specific brain area similar to DBS but instead uses magnetic fields rather than electricity. TMS treatment for depression often includes stimulating the dorsal lateral prefrontal cortex of the brain in a specific pattern. The results of this treatment are generally similar to the results from DBS treatment.

Why the nucleus accumbens?

The nucleus accumbens is a structure in the brain that is part of the basal ganglia. This structure is often referred to as the reward circuit and is comprised of two main parts, the shell and the core.[5,8] The shell is involved with pleasure and fear behavior while the core is related to addiction and drug behavior.[6] The primary neurotransmitters associated with the nucleus accumbens are norepinephrine and dopamine. In order to understand why the nucleus accumbens would be a viable brain area to stimulate for the treatment of depression, it is important to understand the relationship between these neurotransmitters with anxiety and depression. When talking about depression and neurotransmitters its often about a lack thereof. However, when you throw anxiety into the mix as well, there are other neurotransmitters involved other than dopamine, such as norepinephrine. That is when noradrenergic neurons

come into play. Neurons that express norepinephrine are involved with functions such as alertness and readiness for action. However, this neurotransmitter can often be in excess in people suffering from anxiety. Similarly, too much dopamine in your system can lead to anxiety. The nucleus accumbens, therefore, is a viable brain area to stimulate for the depression and anxiety treatment via DBS. And more specifically, the shell of the nucleus accumbens, which is the target for a study that was conducted recently involving DBS in a depression animal model involving adult male rats.

What was done

This study was conducted by taking three different groups of fifteen adult male rats and surgically implanting electrodes into the right side of the brain onto the nucleus accumbens. The three different groups received varying amounts of stimulation with the sham group receiving none, the intermittent group receiving stimulation for three hours per day and the continuous group receiving constant stimulation. This was conducted for a total of two weeks. Behavioral tests were conducted both before and after the two-week stimulation period followed by analysis of the brain tissue through high-performance liquid chromatography and Golgi-Cox stainings.

What happened as a result?

The behavioral analysis conducted at the beginning of the experiment showed no difference between the three rat groups whatsoever. However, once the two-week stimulation period was over the behaviors between the three groups differed making it apparent which rats received the stimulation series and which did not. The sham group stood out the most from the intermittent and continuous stimulation groups. The sham group of rats had more recorded anxiety-like behavior than the first behavioral analysis test. This is probably due to the rats being kept separate from each other, which induces stress. However, the two groups that received the stimulation showed a reduction in anxiety-like behaviors and exhibited more exploratory behaviors with a small difference between the intermittent group and the continuous group. The group that received continuous stimulation had the most positive result overall.
With the high-performance liquid chromatography analysis, the levels of dopamine and norepinephrine were measured for the three rat groups. The sham group had increased levels of both neurotransmitters while the other two groups saw a decreased amount of these neurotransmitters. Similarly to before, the group that received the continuous stimulation saw the most decrease in these neurotransmitter levels compared to the other groups. Along with neurotransmitters, the levels of tyrosine hydroxylase were measured. Tyrosine hydroxylase is a precursor to dopamine and the findings were similar to that of the neurotransmitter levels found for each group. The sham group saw increased levels of tyrosine hydroxylase, while the two groups that received stimulation had a decrease with the continuous group seeing the largest decrease overall. The Golgi-Cox staining was done to measure the arbors of the dendrites from each rat brain. Once this was done, it was shown that the rat groups that received stimulation saw an increased length in these arbors indicating that neuroplasticity had taken place during this study. The shame group saw no change in dendritic arborization with the two stimulated groups showing an increase. Comparably to all the data gathered from this experiment listed previously, the group that received continuous stimulation over the two-week period saw the most increase in dendritic arborization.

What does this mean?

Based on the results of this experiment, it can be said that deep brain stimulation of the nucleus accumbens is an alternative treatment method for anxiety and depression-related disorders. This experiment “demonstrates that DBS of the nucleus accumbens not only halted the progression of the disease process but improved it.”[7] Deep brain stimulation is already an available and verified treatment option for various types of disorders. Therefore, it

can be deduced that stimulating a specific brain area would also serve the same purpose when treating anxiety and depression-related disorders. Deep brain stimulation not only strengthens the synapses in the stimulated brain areas, but it also generates neuroplasticity, which is essential in alleviating the symptoms of depression and anxiety long term. Similar to deep brain stimulation, transcranial magnetic stimulation offers the same relief from these symptoms through stimulating areas of the prefrontal cortex. These type of treatment options are perfect for those who do not want the side effects of pharmaceuticals and for those who simply do not want to put foreign chemicals into their bodies.

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Levels of blood proteins within the brain may be linked to major depressive disorder

A new study found that asymmetry of the frontal areas of the brain was positively correlated with thoughts of suicide in patients with Major Depressive Disorder (MDD).

Ashlynn Dean

Although there are many studies over depression and related markers within the brain, there are no studies that specifically test the link between depression and asymmetry in certain brain regions. In a paper recently published by Diagnostics, Seung et al. investigates frontal areas of the brain and how it affects the relationship between depression and suicidal thoughts in patients with MDD.[1] This study found that prefrontal asymmetry controlled the effects of depression severity on suicidal thoughts while patients were performing the Verbal Fluency Task (VFT). There was also a positive correlation between prefrontal asymmetry and suicidal thoughts in patients with MDD, along with a lower reported level of asymmetry in patients with MDD compared to those without.

Introduction

Depression is a common mood disorder that can affect the way people think, feel, and perform activities in their daily lives.[2] Although this disorder is common, it is also a very serious brain condition that is difficult to diagnose. Some studies have focused on using blood and tissue samples to measure proteins and analyze gene expression.[3] Currently, there are no non-invasive, qualitative procedures for diagnosing MDD.[4] Fortunately, a recent technique was developed which makes it easier to look at brain activity, called functional near-infrared spectroscopy (fNIRS). This technique is an easy, portable, non-invasive method of monitoring brain activity by detecting a protein within the blood, called Hemoglobin (Hb).[5] More specifically, this imaging system tracks changes in the amount of oxygenated (oxy-) and deoxygenated (deoxy-) Hb using light, which then reflects brain activity.[1] Although much research is devoted to finding markers within the brain that relate to risk of depression and suicidal thoughts, none have looked at asymmetry within the frontal areas of the brain.

Methods

A group of 106 participants were used in this study, 42 with MDD and 64 without. Each participant went through three different cognitive tasks while their Hb levels were measured, including the VFT, Korean Stroop Task (KST), and N-Back Task (NBT). The VFT involves generating as many words with the same first letter as possible in 30 seconds. The KST challenges the patients to choose the correct color of a word when the word itself names a different color. Lastly, the NBT was used to assess working memory by presenting a number (1-5) every second and requiring the participants to identify the number they saw two numbers prior. Participants filled out a questionnaire to determine personal levels of anxiety, depression,

and suicidal thoughts (Scale of 1-4, 1 = absent, 4 = suicide attempts). During these tasks, the participants were to sit still with an fNIRS sensor attached to them. Prefrontal asymmetry was assessed by calculating the changes in Hb between the left and right parts of the brain. The scientists also analyzed the differences between the participants with MDD and their healthy counterparts of similar age and sex (controls).

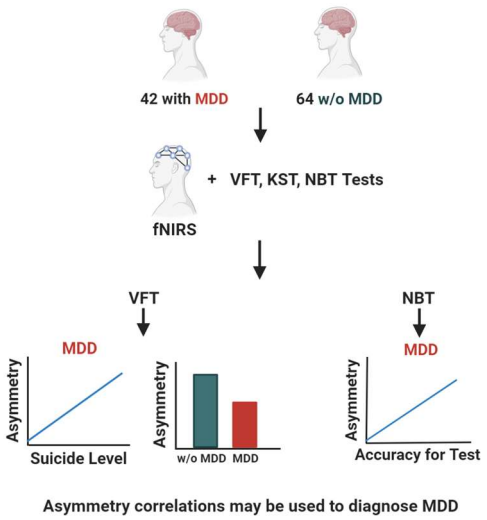

Figure 1: Methods and Results of MDD Patients and non-MDD Patients. This figure shows the methods of this experiment within boxes and the results near the bottom of the figure. A total of 106 patients were subjected to the VFT, KST, and NBT test while hooked up to an fNIRS to measure blood oxygenation. The VFT showed a positive correlation between asymmetry of oxygenation and suicide level and showed a higher amount of asymmetry for patients without MDD than patients with. The KST showed a negative correlation between asymmetry and anxiety level, and the NBT showed a positive correlation between asymmetry and accuracy during the test.

Results

Throughout the study, no significance was reported for deoxy-Hb, so all results are referring to the asymmetry of oxy-Hb. The study revealed that patients with MDD showed a lower prefrontal asymmetry during the VFT compared to the controls (Figure 1). Patients with MDD also showed a positive correlation between asymmetry during VFT and the self-reported level of suicidal thoughts, while the controls showed a negative correlation between asymmetry and both the KST and anxiety levels (Figure 1). For the NBT, a positive correlation was found between asymmetry and accuracy during the task in MDD participants (Figure 1). Lastly, the VFT indicated a moderation of the relationship between depression severity and suicidal thoughts by prefrontal asymmetry. The moderation of this relationship by prefrontal asymmetry was higher when levels of oxy-Hb were greater in the left side of the brain.

Discussion

These data indicate that asymmetry in the prefrontal regions of the brain may show different levels of depression or suicidal thoughts when performing certain tasks. Currently, the methods for diagnosing MDD and minor forms of depression are not with scientific techniques but rather with checklists and questionnaires.[6] Using checklists is not accurate and cannot properly quantify the severity of people's symptoms. There have been recent updates to the methods of diagnosing and treating depression, including animated systems, but still fall short of long-term treatments for depression.[7] Analyzing brain activity using fNIRS is an easy and possibly more effective method of diagnosing depression and suicidal tendencies. The use of fNIRS in neuroscience has grown rapidly in the previous years due to its non-invasive, portable system that is less likely to be affected by bodily movements.[8] The use of fNIRS in conjecture with basic cognitive tasks could be a simple, yet promising way of accurately diagnosing depression and risk of suicidal actions. Although this method seems promising, more research is needed on the reliability of the correlation between asymmetry and severity of depression.

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A new target for depression treatment?

It was found that overexpression of Regulator of G Protein Signaling 8 (RGS8) protein is linked to resistance of depression. This could lead to a new drug target for treatment of depression.

Edward Hernandez

Introduction

In a recent study published in *Neuroscience,* researchers at Hiroshima University investigate a protein, known as Regulator of G Protein Signaling 8, involvement in depression.[1] Depression is a mental disorder that affects many people. In 2015, there were around 215 million people worldwide diagnosed with depression.[2] The Diagnostic and Statistical Manual of Mental Disorders' criteria for depression diagnosis are various symptoms that include sad mood, fatigue, weight loss, or worthlessness for at least 2 weeks.[3] The cause of depression is currently unknown, but it is thought to be caused through a variety of factors as proposed through the biopsychosocial model.[4] Examples of these factors are genetics, psychological trauma, and other illnesses. One major biological cause of depression is thought to occur through the monoamine hypothesis. The monoamine hypothesis suggests the predominant physiological cause of depression is an inadequate function of monoamine neurotransmitters, such as serotonin and dopamine.[5] However, this study found when mice with significantly increased RGS8 were compared to regular mice, the overexpression of RGS8 helped to promote resistance to depressive symptoms in the mice.[1] This was discovered through a comparison in a forced swimming test, use of a monoamine antidepressant and use of an antagonist of MCHRI, a receptor involved in emotion processing and energy homeostasis and that is also affected by RGS8.[1] This study suggests RGS8 and MCHRI are other physiological factors that have a relationship with depression without the involvement of monoamines. This could lead to new treatment targets for people who are resistant to drugs that target monoamines.

Background

G proteins are important in transmitting signals from the exterior of the cell to the interior. G proteins are activated through G protein-coupled receptors and inactivated through regulator of G protein Signaling (RGS) protein. RGS8 is a specific RGS that has previously been found to be densely populated in the brain and to be antagonistic to Melanin-concentrating hormone receptor 1 (MCHR1).[6] Stimulation of the receptor, MCHR1, has been found to lead to depressive behaviors.[7] Furthermore, antagonism of the MCHR1 receptor, results in anti-obesity and antidepressant effects.[8] The theory is that increased RGS8 will inhibit MCHR1, reducing depressive behaviors.

Methods

In the study, mice were genetically modified to form transgenic mice with increased RGS8 (tmRGS8). These mice appeared healthy with no abnormal behaviors or anatomy. Biopsies of their tails were tested using PCR to confirm the presence of the gene for increased RGS8. An open field test was performed to measure the difference in spontaneous locomotion. A forced swimming test (FST) was performed with the mice to test for depressive behavior. The FST was performed by placing the mice in a cylinder (24 cm height x 17 cm diameter) full of water up to 13 cm, to force the mice to swim, for 6 min. The water was changed after every test. The mice were filmed while they were in the swim tank, and the total duration of immobility was then

measured afterward. The mice were then tested on the FST, 1 hour after being given SNAP94847 (10mg/kg), a chemical used to inhibit MCHR1. They then were tested with Desipramine (3mg/kg), a tricyclic antidepressant that inhibits norepinephrine and serotonin, that was injected intraperitoneally before the test.[1]

Figure 1. The hippocampus is part of the limbic system, functioning in memory and emotional responses. The CA1 region of the hippocampus is involved in autobiographical memory. In this region, the transgenic mice were found to have RGS8 expression 2.3x the amount of wild type mice (Fluorescence intensity of RGS8 - WT: 100+/- 11.8; tmRGS8: 233.9 +/- 23.1; $p<0.01$)

Results

Immunohistochemistry staining for RGS8 was used to analyze the cerebellum and hippocampus, where the strongest signal of RGS8 was found by using in situ hybridization and RT-PCR. It was found that tmRGS8 mice had about 2.3x the amount of RGS8 expression compared to WT mice in the hippocampal region, CA1. RGS8 expression in both mice was similar in other areas.[5] For the open field test, there were no significant differences found in spontaneous locomotion. However, the tmRGS8 mice did have a significantly lower immobility time when compared to the WT mice. When the FST was performed with SNAP94847, the immobility time for tmRGS8 did not significantly decrease, but it did significantly decrease with WT mice. When the FST was performed with Desipramine, the immobility time for tmRGS8 was further decreased, while the effect for the wild type mice was similar to the effect of SNAP94847 (Kobayashi, 2018).

Discussion

Based on the results of the study, it can be concluded that increased expression of RGS8 may cause decreased depressive symptoms. This results from RGS8 possibly inhibiting the activity of MCHR1. This was found as tmRGS8 mice decreased immobility time with a monoamine antidepressant, Desipramine, but did not with the MCHR1 antagonist, SNAP94847. This suggests that the increased RGS8 was already acting on MCHR1. For the WT mice, SNAP94847 and Desipramine had similar effects on decreasing immobility time. This suggests that treatment of RGS8 and MCHR1 may be as effective as a monoamine antidepressant. This is important for future research as most of the current medications for depression have been modeled to act after the monoamine theory.[9] This is a problem because around 10-30% of people with major depression, do not improve with this antidepressant treatment and some even have a poorer life quality, as well as taking a few weeks to work adequately.[10] It is also known that SSRIs may increase the risk of suicide in adolescents and are not as effective in treating depression.[11] Further research into

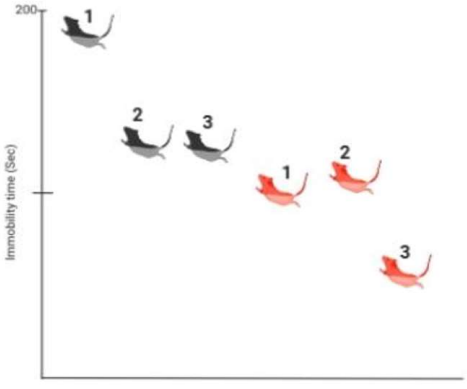

Figure 2. Effects of desipramine and SNAP94847 on the immobility time of mice. The black mice (BM) represent wild type mice, and the red mice (RM) represent tmRGS8 mice. 1= no treatment, 2= treatment with SNAP94847, 3= treatment with desipramine. All mice had significantly less immobility than BM1. RM3 had significantly less immobility than RM1, but RM2 did not.

RGS8 and MCHR1 may result in new treatment discoveries that may be as effective as monoamine antidepressants and may help those that are resistant to current treatments.

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The “hunt” for an answer continues

Functional deficits have been identified in striatal neurons of mice expressing the mutation for Huntington’s disease. This research identifies a potential target for therapy treatment.

Shelby Brock

Huntington’s disease (HD) is a terminal neurodegenerative disorder which is the result of a repeating sequence of three nucleotides, CAG, in the huntingtin (HTT) gene.[1] Repeats in this sequence are normal, but when it is repeated more than 40 times, the carrier is almost guaranteed to develop HD. Symptoms begin with difficulty concentrating and tremors and progress to involuntary movement (called chorea) and psychiatric decline.[2] This is an autosomal dominant disease, meaning that the offspring of someone with Huntington’s has a 50% chance of developing the disease themselves. While there is no cure for this disease, most therapies focus on the treatment of symptoms.

In the pursuit of mechanistic knowledge of this disease, McAdam et al.’s team at the University of Edinburgh published a paper in *Neurobiology of Disease* which focused on the striatal neurons of mice with and without the HTT mutation.[3] The striatum was chosen because it is rich in excitatory medium spiny neurons (MSNs), which are known to cause chorea (uncontrollable movement) when degenerated because the striatum is associated with voluntary movement.[4] The researchers chose to study cell properties during high and low stimulation to identify latencies or defects. They hypothesized that MSNs are vulnerable to presynaptic dysfunction because they receive high amounts of excitatory input but would be unable to sustain those inputs overtime.

In the study, three groups of mice were used: wild type, homozygous $HTT^{Q140/Q140}$ mutants, and heterozygous $HTT^{Q140/+}$ mutants. The mutant mice have human mutations of the CAG repeat inserted into their genome, making them “knock-in” mice. A culture of striatum neurons was made from each mouse and the firing properties were assessed.

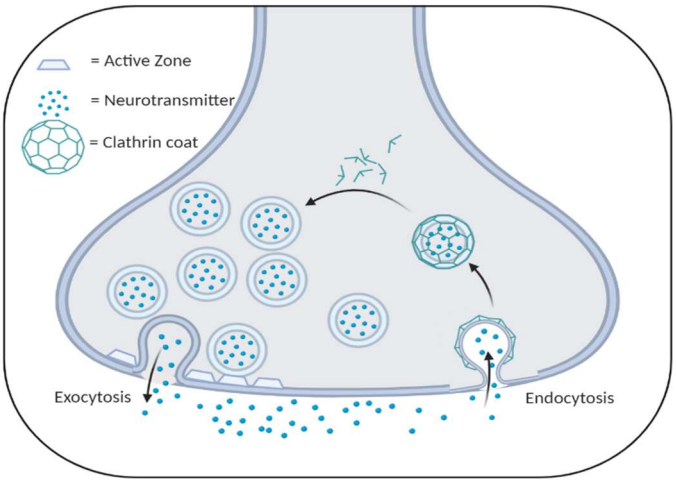

Figure 1-Normal vesicle recycling in a presynaptic cell. Created using BioRender.

Normal synapses have synaptic vesicles which release neurotransmitter into the synaptic cleft (exocytosis) and are taken back into the presynaptic cell (endocytosis) to be recycled into the vesicle pool to be released again. This process is illustrated in Figure 1. McAdam’s team found that the cells of the homo- and heterozygous mice had significantly lower amounts of vesicle endocytosis, which would result in lower vesicle availability, lower neurotransmitter release, and, eventually, synaptic failure because of an inability to sustain the high volume of input being relayed through the striatum. However, this discrepancy in vesicle fusion timing only occurred at high-frequency stimulation. This coincides with the author’s hypothesis that synaptic dysfunction stems from an inability to maintain high levels of input.

Activity patterns stimulate the release of dopamine from medium spiny neurons, which is responsible for expected reward behavior and smooth coordination of movement. Some medium spiny neuron function relies on constantly active neurons to temporarily cease firing to encode salient environmental cues.[5] If these cells do not fire consistently or cease firing at the wrong time, then the information input from other regions cannot be properly encoded, which includes voluntary movement.

Since this study's results were found in both the homo- and heterozygous mice before physical symptoms of HD occurred, this suggests that those with the mutation have inherent dysfunction of their cells for their entire life. Slowed vesicle endocytosis may serve as a possible mechanism that might compound over time to promote the full manifestation of HD. Since this identifies a discrepancy in function before degeneration begins, it can be inferred that it is involved with the pathology of HD.

These findings could potentially stem future research on how vesicle timing leads to degeneration and potentially target the striatal excitatory neurons (specifically, vesicle fusion) when considering pharmacological ways to slow the progression of Huntington's. Future experiments should be conducted *in vivo* to verify results and test how vesicle fusion changes over time with full inputs from other brain regions.

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Gene mutation heals traumatic brain injury

Traumatic brain injuries heal differently for different people. That is because of an apoptotic gene, P53 which is mutated in some people and leads to higher resilience of programmed cell death. Thus, increased recovery in severe TBI patients with the mutated P53 gene[1].

Victor Cosovan

In a paper recently published in *Biological Research for Nursing*, Kaleigh Mellett and her team of researchers investigated 429 patients who suffered a severe brain injury and they monitored their DNA from blood to discover if they had any gene mutations. They found that there was a gene with changed language and thus the proteins made from it acted differently. They compared the mutated DNA to the regular human gene for programmed cell death.[1] They discovered the mutated gene, it is more successful in stopping programmed cell death leading brain injury, thus giving brain cells the opportunity to recover. Traumatic brain injuries are a leading cause of death with more than 5-million people dying or suffering a disability from it each year.[2] Currently, it is not known how to properly treat severe brain injuries and the mechanism of brain healing and recovery needs extensive research to better the outcome of patients who suffer from brain injuries. By looking into the gene for programmed cell death, they found a mutated form of the gene which is more successful at giving damaged cells the opportunity to heal.[1]

Background

PT53 is the name for the gene that codes programmed cell death.[4] When a cell detects a harmful environment(s), faulty DNA, or other cell-deadly factors, it sends out proteins (transcription factors) to wake up DNA and make signals to kill the cell (cell suicide).[6] Once the P53 gene is activated it makes proteins which then cause the cell to die and be eaten up as resources by neighboring cells or immune cells. In this study, only subjects who had traumatic brain injuries (TBI) were accessed. TBI is brain damage from force or other harmful environmental causes like drugs or severe temperature. When there is structural damage to the connections between neurons and the cells themselves. That is why when cells detect severe damage, P53 is activated and releases proteins to set the pathway for programmed cell death.[8] When a cell is set for programmed cell death, excess proteins and enzymes will flow through the brain circulatory pathway (cerebral spinal fluid) and drain into the blood to be metabolized and discharged as waste.[10] Researchers are able to then collect blood samples from severe TBI patients and locate and analyze P53 genes and proteins. The gene mutation is called Arg72Pro TP53 because on the long amino acid strand (DNA alphabet and language), there is a typo on the 72nd amino acid where a proline got replaced by an arginine molecule. By finding two different types of P53, scientists are able to compare the outcomes in patients who have the mutated version and how well they heal because of reduced cell death.[1] Neurons take a long time to heal and build new connections and pathways when one is damaged. They function as a series of bridges that move and form other bridges too. When there is structural change, there is mass inflammation which then needs to be controlled because the brain is encapsulated in a tight compartment, the skull.

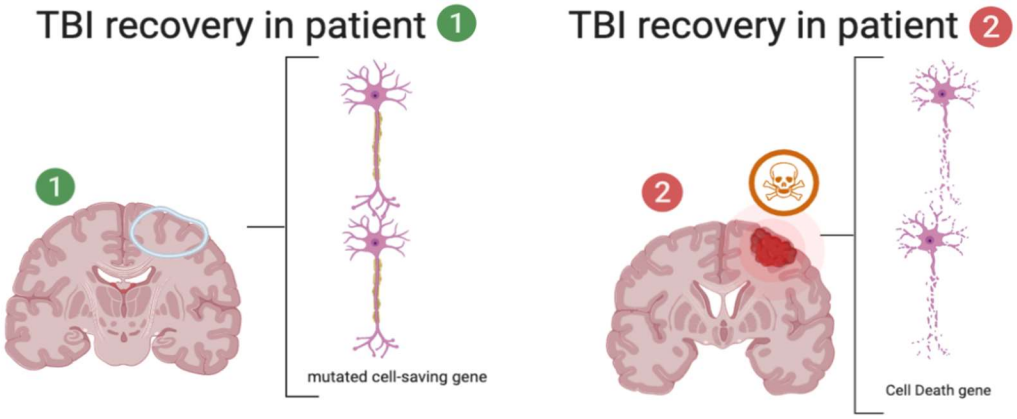

Figure 2. Patient 1 has the mutated programmed cell death (apoptosis) gene, Arg72Pro TP53. Patient 2 has the regular non-mutated human gene, TP53 for regular functioning apoptosis. Patient 1 & 2 are both recovering from Traumatic Brain Injuries (TBI) and each expresses a different P53 gene. Patient 2 has dead brain cells due to high functioning apoptosis process. Patient 1 has a mutated gene leading to less apoptosis and better recovering brain tissue.[1] Figure was made using BioRender.

Methods

This study took blood or cerebral spinal fluid samples from 429 participants suffering from a severe traumatic brain injury (TBI). They selected participants off of a particular criterion. The participants were adults ages 16-80, taken only with a Glasgow Coma Scale (GCS) score ≤ 8.[3] The participants couldn't have any brain injury related to any form of food or drug. During 3, 6, 12, and 24 months, a team of neurophysiological technicians performed a class of neurological exams. They tested using mortality and scores on the Glasgow Outcome Scale (GOS), Neurobehavioral Rating Scale (NRS), and Disability Rating Scale (DRS). They monitored and tested; alertness, attention, fatigability, orientation, memory, motor behavior, expressive/reception language, mood disturbances, disinhibitory behavior or agitation, and capacity for self-insight.[1]

Results

In this study, they found two forms of the P53 gene, a wild type, and a mutated type, Arg72Pro TP53.[1] They found that the mutated gene patients had a significantly lower mortality rate than those with the wild type activated P53 gene. They found that in their subjects, after 24-months post TBI event, there was a significant difference in NRS and DRS score outcomes in the two patients groups using X^2 tests. They also found a significant difference in outcome of patients who started off with a GCS 3-4 vs those who started at a better 5-8 score at 24 months post-TBI. Age was also significant for NRS and GOS at 24 and 3 months post-TBI. The study found that patients with the proline (wild type) gene and had significantly worse outcomes than those with the arginine mutation using GOS scores to compare and analyze statistically.[7] The mutated TP53 gene is more efficient at inducing apotosis.[4]

Significance

There were many important findings in this article.[1] They study how the mutated gene compares to the wild type gene in multi-variable analyses. The first finding was the significant decrease in mortality rate across mutated patients and wild type patients. This is an important finding because it shows that treating apoptotic functions in patients with severe TBIs can lead patients to have an increased survival rate.[11] This opens the door to medicine to treat genotype-based precision medicine in patients with varying neurodegenerative illnesses. Patients with the wild type gene were 2.7 times more likely to have a poor outcome compared to patients[10] with the induced apoptotic function.[1] Meaning if we can use genotypic medicine or pharmacological inhibition of apoptosis in cells, there can be a 2.7 times greater chance of survival in severe TBI

patients. This is a great study with a large sample size and many well-though-out positive and negative control tests. The study holds great validity and took extra time to compare all variables and statistically evaluate all of them. They also plugged from previous studies and filled holes by using multi-variable analysis to assess the differences in sex and age in patients with severe TBIs. This study now opened the door to new studies searching for ways to efficiently induce apoptosis to increase neurophysiological outcomes. I only wish the study specified how many patients they collected blood samples from and how many patients they collected CSF from. Then do control analysis on the two sample types. I consider this a well written valid study.

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A study of neuronal distribution within the brain of epileptic patients shows the preservation of a certain neuron

A new study finds a link between distribution patterns of neurons within the brain and tissue samples from epileptic patients, indicating that a certain interneuron is mainly preserved in the epileptic brain.

Ashlynn Dean

Although epileptic brains are commonly studied, the specific distribution patterns of biological markers within these brains have not been highly studied. In a paper recently published by Neuroscience, Zhu et al. (2018) investigate the distribution of neurons and certain biological markers within the brain of epileptic patients.[1] This study found that somatostatin (SST) and neuropeptide Y-containing (NPY) neurons were reduced in the epileptic brain while Parvalbumin-positive (PV) interneurons were mainly conserved within the brain of epileptic patients, indicating that these neurons do not experience damage in the majority of the brain.

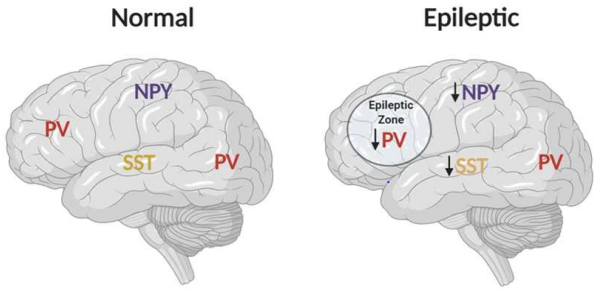

Figure 1: Difference in Biological Markers Between Normal and Epileptic Brain. This figure shows the difference in SST, PV, and NPY-containing neurons within a normal and epileptic brain. NPY and SST are lowered in the epileptic brain compared to the normal controls. PV is the same throughout the entire brain but is lowered in the epileptic zone of the affected brain. Created with BioRender.

Introduction

Epilepsy is a broad spectrum of brain disorders that can range from benign to life-threatening and disabling.[2] There are multiple mechanisms to seizures, but all mechanisms revolve around the idea that there is an imbalance in neuronal activity within the brain.[3] The effect of seizures on neurons, especially severe or repeated seizures, has been highly researched and debated for many years.[4] It is widely accepted that neuronal death can result from prolonged seizures.[5] There is also evidence that brief seizures can cause some form of neuronal death, but this knowledge is not as well accepted.[5] Some studies have focused on a specific set of neurons, called interneurons, and found that this group was highly involved in seizures and were especially susceptible to damage from epileptic seizures.[6,7] Interneurons are a subset of neurons that have the purpose of inhibiting or exciting other neurons. Much research is devoted to finding markers within the brain that relate to the cause and severity of epilepsy, but not many have researched the distribution and conservation of certain neurons within the epileptic brain. In this study, the neurons focused on are Parvalbumin (PV), somatostatin (SST), cholecystokinin (CCK), tyrosine hydroxylase (TH), and neuropeptide Y-containing interneurons (NPY).

Methods

Brain tissue was removed from 9 patients while undergoing surgery to treat an epileptic condition and 3 patients undergoing non-epilepsy related brain surgery. This tissue was then frozen and sectioned into thin slices. These slices were stained with substances that made each type of interneuron a different color, indicating different cell components including PV, SST, CCK, TH, and NPY. Each type of interneuron was counted within each brain layer and the distribution of each of these interneurons was noted as well. The number of neurons with more than one labeled cellular component was also recorded (double-labeled cells).

Results

It was found that SST, PV, and CCK cells were found in the middle and deep layers of the brain, while TH and NPY cells were generally found deeper. Groups of neurons contained similar double-labeling patterns including PV + TH, PV + CCK, SST + NPY, and SST + TH. Both SST and NPY cells showed reduced densities than in the average non-epileptic brain (Figure 1). As for PV cells, it was found the overall density and number of PV cells remained the same between epileptic and non-epileptic brain tissue. In the main epileptic zones of the epilepsy brains, it was found that there were a lower PV density and number than the rest of the brain and compared to non-epileptic brain tissue (Figure 1).

Discussion

These data indicate that multiple types of interneurons are affected by epileptic conditions within the brain. Both SST and NPY cell densities are lowered in the epileptic brain compared to the non-epileptic brain. These two types of cells have been shown to previously be important in the generation of brain network activity and seizure-like activity.[8] The reduction of SST and NPY cells may indicate damage to these cells in the brains of epileptic patients. PV cells were reduced in the epileptic zones but maintained in the rest of the brain within patients with epilepsy. PV-containing neurons have the function of inhibiting, or slowing the activity, of other neurons.[9,10,11] The lowered intensity of inhibiting neurons in epileptic patients corresponds with the high activity of neurons during seizures. Other studies have found that the intensity of PV cells was also reduced in certain sections, including both frontal and middle, of the epileptic brain.[12] Because of the relationship between PV cells and epilepsy, these data indicate that PV cells are a good target for epilepsy treatment. Targeting PV cells should provide more information on the mechanism of damage to PV cells due to seizures. Future studies should focus on ways to restore PV-containing neurons as a possible treatment for recurrent seizures and epilepsy or create formulas to prevent PV loss during future seizures.

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Gene therapy for epilepsy

Snowball et. al have potentially discovered a way to bypass invasive surgeries commonly used to treat epilepsy, often risking a patient's ability to function normally. This new method utilizes a less invasive gene therapy approach targeting the KCNA1 potassium channel gene, in turn decreasing the disorder's effect.

Jessica Faraca

An article published by Snowball et al. in *The Journal of Neuroscience* investigated potential ways of treating refractory focal epilepsy using gene therapy techniques[1]. Refractory focal epilepsy is a form of epilepsy which is resistant to antiepileptic medications[2]. Currently, treatments for epilepsy mainly consist of medication or surgery. Of those diagnosed with epilepsy, 30-40% do not respond to treatment with medication[3]. This leaves a large percentage of the epileptic population considering surgery, and not every patient will be a viable surgical candidate. Surgical interventions can also pose risks to function and may result in severe side effects[4]. Researchers found when implanting a gene encoding modified voltage-gated potassium channels via a viral vector there was a significant reduction in seizures in a rat model of focal neocortical epilepsy[1]. These findings suggest gene therapy techniques are a potential noninvasive option for treating refractory focal epilepsy.

Snowball et al. began by engineering the Kv1.1 voltage-gated potassium channel gene, KCNA1, to accelerate the recovery time following inactivation[1]. By increasing the recovery time of voltage-gated potassium channels the idea is hyperpolarization will be reached more successfully. This would then decrease the cells' likelihood of firing again in rapid succession as seen in epilepsy [5]. In order to incorporate the engineered potassium channel gene, the team of researchers utilized a nonintegrating lentiviral vector. Lentiviral vectors are a form of viral vector derived from human immunodeficiency virus (HIV)[6]. These viral vectors allow for genetic information to be incorporated into the host cell. Snowball et al. employed a non-integrating lentiviral vector, the non-integrating vector does not incorporate into the DNA of the individual, making it a safer option. The viral vector was under the control of a cell type-specific CAMK2A promoter[1]. The animal model used in this research was a male rat for focal neocortical epilepsy. In order to quantify and analyze seizure reduction, continuous electrocorticography (ECoG) readings were taken. ECoG recordings measure the cortical potentials, it is often used to determine the location of epileptic tissues and predicted a surgery's effectiveness in treating epilepsy[7]. Seizure activity was then compared to baseline in order to determine the gene therapy's effectiveness.

The team of researchers ended up finding a significant reduction in seizure activity compared to baseline in a male rat model of neocortical epilepsy. Following the lentiviruses injection, there was a decrease in seizure activity compared to control. The results of this preclinical trial suggest the engineered KCNA1 gene administered by a non-integrating lentiviral vector is a promising treatment for refractory focal epilepsy and further research should be done assessing its efficacy.

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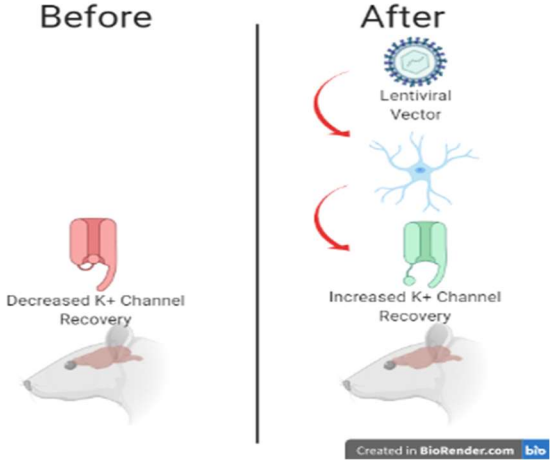

Figure 1. Figure depicting voltage-gated potassium activation/recovery before versus after genetically engineered len,tiviral vector administration to male rat neocortical epilepsy model.

One thing to consider when considering the results of this research is the use of an animal model for refractory focal epilepsy. In humans there can be many different causes of epilepsy. And although mutated potassium channels are of great interest, there are many variations and phenotypes that may be possible[8]. This study focused on the Kv1.1 voltage-gated potassium channel and engineering of the KCNA1 gene. However, more studies will need to be completed in order to truly determine this specific gene therapy's effectiveness. With that being said, the work performed by Snowball et al. is promising for the future of refractory focal epilepsy treatment and has the potential for clinical applications.

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Therapeutic potential of taurine supplementation for multiple sclerosis

Beyer et al. found taurine, in combination with drug-induced remyelination, to significantly enhance oligodendrocyte differentiation, making it a potential supplement for patients with demyelinating diseases.

Maribel Garcia-Igueldo

The biological mechanisms associated with oligodendrocyte progenitor cell (OPC) differentiation and function and additional treatment strategies for demyelinating diseases are not well understood. In an article published in Nature Chemical Biology, Beyer et al. used global metabolomics to identify endogenous metabolites that are altered during OPC differentiation and serve to directly impact cell fate. The research team found that exogenous taurine, in combination with drug-induced remyelination, significantly enhanced oligodendrocyte differentiation by increasing available serine pools. Serine pools serve as a primary building block for glycosphingolipid (GSL) biosynthesis. GSL makes up 31% of the lipid components of myelin, which serves to insulate nerve fibers and increase the speed at which signals are propagated.[1] Therefore, taurine supplementation enhances oligodendrocyte (OL) maturation by directly enabling GSL biosynthesis, making it a potential treatment for demyelinating diseases such as multiple sclerosis (MS).

Multiple sclerosis affects nearly one million people in the US, making it the most common demyelinating disease.[2] MS is an autoimmune disease of the central nervous system characterized by inflammation, demyelination, and axonal loss.[3] Typically, MS follows a variable relapsing and remitting course with clinical features not limited to weakness, pain, vision problems, balance problems, and cognitive difficulties.[4] Existing immunomodulatory and immunosuppressive drugs have limited efficacy in preventing the progression of MS in patients, therefore it is important to identify additional treatment strategies.[5] Resident OPCs in the adult brain differentiate into mature OLs which then remyelinate axons, however these cell's ability to restore myelin in MS patients diminishes as the disease progresses.[6] Beyer et al. were interested in the biological system by which OLs remyelinate axons and potential strategies to enhance OPC differentiation.

Several methods were used in the study conducted by Beyer et al. in 2017. The in vitro OL maturation analysis was done using neurons isolated from the cortical and hippocampal tissue of mice. In the study, researchers highlighted the utility of global metabolomic analysis for identifying endogenous metabolites that modulate cellular characteristics.[1] Metabolomics is a comprehensive assessment of endogenous metabolites from a biological sample. Endogenous metabolites include lipids, amino acids, peptides, nucleic acids, and other low molecular weight molecules which makes it an ideal method for discovery-based research.[7] In the 2017 study, researchers integrated the analytical platforms high-pressure liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC). The two analytical platforms are used for legal, pharmaceutical, and medical purposes to detect metabolites in biological samples.[8] Specifically, Beyer and his research team identified 22 upregulated metabolites in mature OPCs

treated with a drug known to induce OPC differentiation named triiodothyronine (T3). The research team identified the metabolites involved in the taurine pathway as the most upregulated in OPC differentiation.

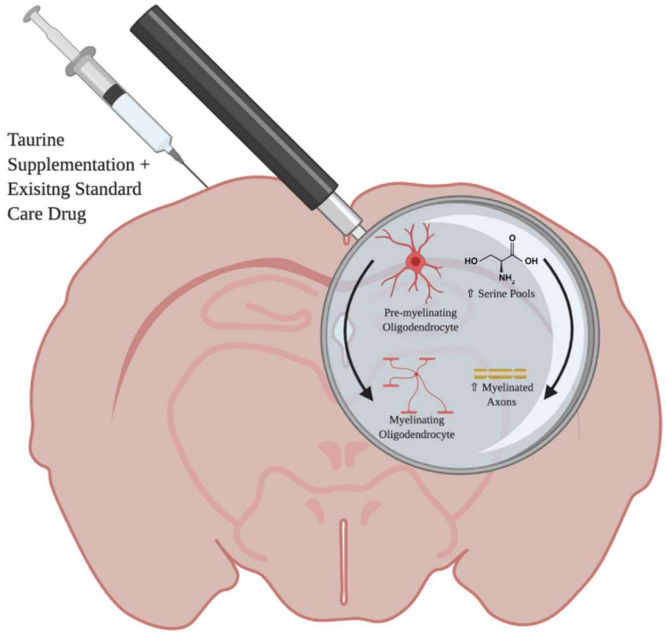

Figure 1. Beyer et al. found that taurine supplementation, in combination with drug-induced remyelination, facilitates maturation of pre-myelinating oligodendrocytes. The research team also found that taurine increased available serine pools which serve as a building block in glycosphingolipid biosynthesis. Glycosphingolipid is an essential lipid component of myelin.[1]

According to Beyer et al., the taurine pathway is the most altered event of OPC differentiation, making taurine the metabolite of interest in their study. In addition to T3, benzotropine and miconazole were used to induce OPC differentiation. Benzoptropin is used to treat Parkinson's disease by blocking an endogenous molecule in the brain called acetylcholine.[9] Miconazole is an antifungal agent that functions in OPCs through mitogen-activated protein kinase (MAPK) signaling.[10] The study found that OPC differentiation and myelin basic protein (MBP), a protein important in the process of myelination, expression significantly increased when exogenous taurine was added to each drug condition.[11] The same researchers became interested in determining the amount and stage of OPC differentiation at which taurine optimized MBP expression. In separate experiments, they found that 2 mM of taurine added between differentiation day one and day three optimized the positive effect of taurine on OPC differentiation. In addition, 2 mM of taurine supplementation, in combination with either benzotropin or miconazole, significantly increased MBP colocalization with axons in cultures compared to the base condition and drug treatment alone. Beyer et al. also conducted an experiment to identify metabolite pools impacted by exogenous taurine supplementation. Specifically, the researchers found an increase in serine after three days of taurine supplementation combined with T3 compared to observed quantities after six days of the same treatment.[1]

The article "Metabolomics-based discovery of a metabolite that enhances oligodendrocyte maturation" published in Nature Chemical Biology by Beyer et al. identified a novel potential treatment for demyelinating diseases, such as MS. However, the study failed to address how the mechanism by which taurine enhances OL differentiation may be influenced by MS. Future research should focus on identifying if MS prevents taurine from enhancing OPC differentiation using experimental mice models of demyelination. According to the study, 2 mM of taurine supplementation, combined with drug-induced differentiation, increased MBP colocalization with axons which suggests that taurine enhances remyelination.[1] Myelin destruction is the driver for the presentation of signs and symptoms in MS patients, making taurine supplementation a potential treatment that restores myelin. The researchers also found that taurine added between differentiation day one and day three optimized MBP expression. The timing of peak MBP expression suggests that taurine mediates maturation of premyelinating OLs.[1] There was also a greater amount of available serine when exogenous taurine, in combination with T3, was added

on day three than day six, which suggests that serine pools derived from taurine are taken up by other pathways in mature OLs.[1] In conclusion, Beyer et al. provided evidence for the therapeutic potential of taurine supplementation for multiple sclerosis.

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Brevican: perineuronal net protein captures new insights on interneuron activity

Study finds that brevican, a protein found in perineuronal nets and parvalbumin interneurons, is needed for short term memory. This is because brevican regulates the firing properties and type of inputs onto parvalbumin interneurons.

Abby Gligor

Introduction

In a fascinating paper published in Neuron, Emilia Favuzzi and her team identify the molecular mechanism by which the perineuronal net protein, brevican, influences the cellular and synaptic response of inhibitory neurons to the changing environment.[1] The inhibitory neurons they specifically look into are the parvalbumin interneurons located in a region called the hippocampus, which is responsible for learning and memory. The study was conducted because the molecular mechanism behind the adaptability of pavalbumin interneurons to sensory experience is mostly unknown. In addition it also provides more information on how perineuronal nets, a dense net-like structure that surrounds some neurons, influence cellular function. The findings in this study show that brevican is regulated by cellular activity and it controls the excitability of the cell. Even moreso, the study shows that brevican is required for spatial working and short-term memories. Overall, this study provides more information behind the ability of parvalbumin interneurons to adapt to their environment, which is relevant because parvalbumin interneuron dysfunction is linked to various psychiatric disorders.[2]

Background

First, it is important to understand the complexities behind all the components the study explores and their rationale. For instance, sensory perception, cognition, and behavior are all the result of neural circuits adapting to a continuously changing environment by experience-dependent plasticity. The exact maintenance of these different components is mediated by the balanced interaction of excitation and inhibition.[3] In fact, parvalbumin interneurons provide the inhibitory balance in the circuits, which dynamically sustains neural circuit balance.[4] Parvalbumin interneurons are able to adapt their intrinsic properties and their outputs during sensory experience.[5] Unfortunately, the exact molecular mechanism behind these abilities is largely unknown.

Furthermore, the occurrence of plasticity in the brain relies heavily on the complex interactions of neurons and the extracellular matrix that surrounds them. For parvalbumin interneurons, this matrix is known as perineuronal nets. These nets are largely known to stabilize synapses as well as prevent any new synapses from forming.[6] Thus, perineuronal nets block plasticity. Previous studies have shown that degradation of some of the components of perineuronal nets reactivates plasticity and enhances learning.[7] However, the exact mechanism behind this is unknown.

Perineuronal nets are made up of chondroitin sulfate proteoglycans.[8] One such proteoglycan is brevican. This proteoglycan may be involved in regulating experience-dependent plasticity

because it is a component of perineuronal nets, which surround parvalbumin interneurons; it is present in cell membranes and synapse areas; and it is required for long-term potentiation or long-term changes of neuronal synapses.[9,10,11] However, brevican's exact function in parvalbumin interneuron plasticity is unknown.

This study conducted by Favuzzi et al. (2017) bridges the gap of knowledge of the complex interaction between perineuronal nets and parvalbumin interneurons. The knowledge found in this study shows how perineuronal nets and parvalbumin interneurons work together in order to create changes in the neuronal circuitry as a response to sensory experiences.

Results

In order to conduct the study, Favuzzi et al. used various types of mice and techniques to perform various small experiments that comprise the study. They used male mice in each of their various experiments. Some mice genetically expressed brevican while others lacked brevican. The authors sacrificed the animals and stained for brevican, parvalbumin, different sides of the synapse, and other cells using fluorescent tags. Favuzzi et al. also examined the electrical properties of neurons in the absence and presence of brevican. In addition, the authors looked at the protein levels and intensities of various ion channels. Finally, the authors nicely examined the effect of brevican at the single protein level to the behavioral level. They did this by using various mazes such as the T maze and the Y maze. The T maze looks at spatial working memory by using a reward at either end of the T shaped maze and closing off different sides. The animal then has to orientate themselves properly to find the reward. Another maze used was the Y maze. This maze was used to test short term memory by seeing if the animal would like to explore a novel part of the maze after training. Another test was also looking at short term memory by seeing how much time mice spend exploring a new object instead of a familiar object.

Favuzzi et al. found several fascinating results regarding brevican after completing their study. First, they found that brevican is expressed in some glia, but most importantly in parvalbumin interneurons. Brevican is mostly concentrated at the synapse. Another finding was that parvalbumin interneurons have more excitatory inputs onto them when they have brevican compared to cells without brevican. This finding is illustrated in figure 1. In relation to this finding, the authors found that cells that express brevican are less excitable. As noted in another finding, this occurance is because brevican controls the localization and amounts of excitatory receptors and certain ion channels that affect the threshold of cellular activity. The authors also found that brevican decreases with high activity and increases with low activity. Lastly, the authors found that brevican is needed for spatial working and short-term memories.

Significance

The study conducted by Favuzzi et al. provides an in-depth evaluation of the perineuronal net protein, brevican, in the adaptable properties of parvalbumin interneurons. In other words, the authors find a molecular mechanism behind the ability of parbalbumin interneurons to change in response to sensory experience using a perineuronal net protein. This finding is important because dysfunctions of parvalbumin interneurons are shown in many psychiatric disorders.[2] This work provides a basis of understanding of the role of perineuronal nets in modulating parvalbumin interneuron adaptability.

Personally, I enjoyed the paper and its implications, but I had some skepticism. The study was not clear whether or not parvalbumin cells themselves also expressed brevican or if the brevican is only from the perineuronal nets themselves in the experiments done. Other than

that, the paper was a well written and thorough investigation of the role of brevican in parvalbumin interneuron function.

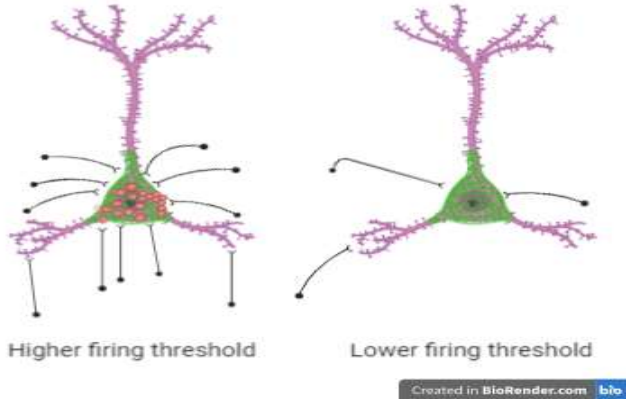

Figure 1: Parvalbumin interneurons that have brevican have a higher amount of excitatory inputs onto them. The presence of brevican also increases the minimum threshold of activity needed to cause the cell to fire action potentials.

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What do cocktail parties and your reaction to a thunderclap have in common?

A new study finds that dopaminergic projections to the medial prefrontal cortex (mPFC) are heavily involved in reactions to aversive or dangerous stimuli.

Gavin Harvey

Both phenomena relate to your brain and how dopamine (yes that feel-good chemical in the brain) influences your ability to allow a signal to go through and cause a reaction to a something scary or bad. This is kind of like how you can be in an impressively busy and noisy cocktail party yet, still manage to focus on the person you want to. This filtering is done by screening what is important among the noise. This is, more or less, what happens in the brain too. In a paper recently published in the journal Nature by Weele et. al. on the influence of dopamine and its effect on the medial prefrontal cortex (mPFC: an area in the front of your brain) in changing behaviour. Specifically, behaviour in respect to things we avoid or aversive stimuli. This helps us understand more about the intricate ways dopamine plays with our behaviour and reactions. It also gives us evidence to continue pursuing methods to specifically administer dopamine to targeted areas of the brain instead of all over. The current treatments that involve dopamine distribute it indiscriminately in the brain and can have unintended effects in how we operate. This research helps further this point.

The idea that dopamine modulates behaviour in some way is a long-held notion and has been studied in a variety of situations such as addiction, positive and negative association behavioural mechanisms, visual information discrimination, and working memory.[5,6,7,8,9,10,11] It has also been implicated to modulate the mPFC and its activity.[1] This is through an area of particularly dense dopaminergic cells called the Ventral Tegmental Area (VTA).[2] These dopaminergic cells are cells that secrete dopamine and have further specific characteristics. Before, it had been predicted that dopamine underlays a variety of functions in the mPFC with a role in signal-to-noise ratio.[5] A signal-to-noise ratio (SNR) is something like how a radio just plays static if not tuned in properly to a station, or if the signal is not strong enough. However, when a signal is strong enough a sound can be heard on the radio. The same principle applies to the brain. It is constitutively active, especially in processing centres like the mPFC. The result of this is a kind of “static” noise. This would be the noise in the SNR acronym. The signal is when there is some signal from the rest of the body, or other part of the brain, that comes in with information. Now as the mPFC has a lot of areas it effects, it is important to know that it has connections to an area known as the periaqueductal grey (PAG). This is an area of the brain that has been shown to be involved in defensive social behaviours.[2,3]

In the research conducted by Weele et. al. a variety of methods were used. These involved many surgeries on rats, dissecting and imaging their brains, while they were alive, as well as when they were dead. The scientists used viruses to implant changes to the nerves they were looking at. Well, more than just the nerves in all honesty. It involved the use of fluorescent proteins (proteins that give off light!) and special channel proteins, called rhodopsin channels (these are channels that can be controlled by flashing a specific light). They also used an

imaging technique called Fast-scan cyclic voltammetry. This was used to analyse samples after they cut them from the rats' brains. It is basically, a machine that quickly changes the voltage of a sample, then uses the information gathered to determine the concentration of dopamine. The researchers also performed experiments on the behavior of their rats when conditioned with a sound and pairing it with either a shock or with something delicious. By combining these techniques with some impressive imaging hardware and software the researchers were able to identify some very interesting things.

Among these interesting things was a further link between the VTA and the specific connections between the mPFC and the PAG. The information that this connection codes for is the reaction to an adverse stimulus. What was determined was that this information could just be filtered out as "noise" to a degree if no dopamine was released by the VTA. In order to determine that other areas weren't changed in their activity; further testing was performed on the mPFC and its connection to an area known as the nucleus accumbens (NA). The NA is an area of the brain that is associated with reward-related processes. The opposite of the PAG in respect to behaviour conditioning. What was found was that even though there were dopamine receptors on the connection between the mPFC and the NA, it appeared the VTA (the specific dopaminergic cells studied) did not change the activity in the NA. What was also surprising was that directly puffing dopamine on the mPFC connection with the PAG didn't change the SNR. What did change the SNR however, was stimulating the VTA dopaminergic cells. The conclusion to draw here is that the VTA is somehow influencing the signals coming into the mPFC and not only the mPFC itself.

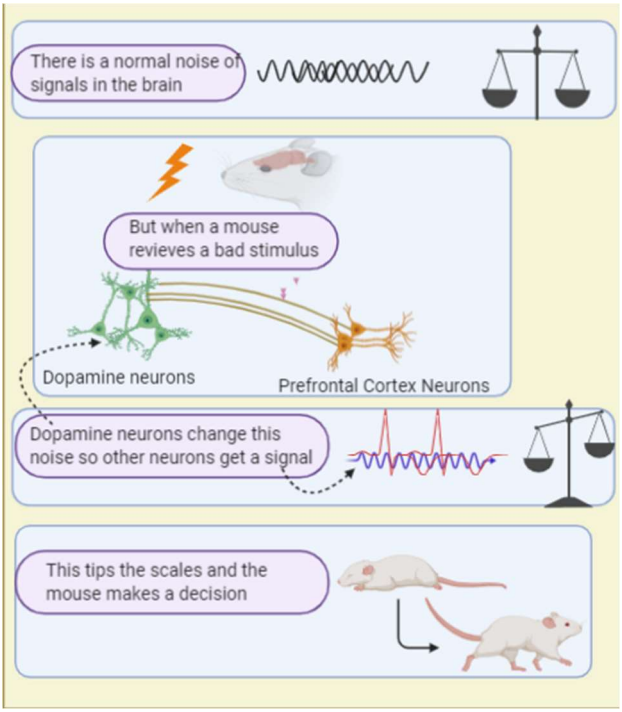

Figure 1: Mouse decision with dopamine in the VTA-mPFC

The type of action that would be produced by this connection is also intriguing. Through behavioral tests mice were seen to show a stronger adverse reaction to sounds that signaled the bad stimulus. An electrical shock, in their case, was the bad stimulus. They froze up longer than those mice who did not have their VTA cells stimulated. They also were slower to react when they heard the sound that meant they would get a treat. What can be gleaned is, that dopamine has a specific function that helps us react to things we know are bad or dangerous (see Figure 1), like a thunderclap. When we hear it, we turn to it and freeze, then we act further to preserve our safety.

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Sensing acid in the brain

A study performed by Cakir et al. finds that acid-sensing ion channels, including calcium, sodium, lead, and zinc have varying effects on the excitability of stellate cells in the cochlear nucleus.

Briana Mason

Introduction

Calcium toxicity is currently the most promising cause of ischemic brain injuries, and the resulting acidity may be the method of damage. Acid-sensing ion channels (ASICs) can be permeable to calcium, and when blocked can prevent ischemic brain injuries.[1] Stellate cells have been discovered to have several isoforms of ASICs, but their properties are still largely unknown. In a paper published last year by the Journal of Comparative Physiology, researchers Cakir, Yildirim, Buran, Onalan and Bal look at the excitability of stellate neurons due to the acid-sensing ion channels (and their ionic permeability) in the cochlear nucleus.[10] The cochlear nucleus is the relay station between the auditory nerve and the higher auditory processing centers of the brain.[2] Stellate cells have been identified as important to speech comprehension and sound localization.[2]

Background

Stellate cells–which can be subdivided into T and D type stellate cells– are one of three main cells found in the cochlear nucleus, and they are one of six pathways to the inferior colliculus.[3,4] Acid sensing ion channels (ASIC) are voltage independent and proton dependent, and have been found in stellate cells.[5] Acid sensing ion channels are channels that create propagations based on the surrounding pH, and these channels were previously associated with taste, nociception, etc. ASICs are cation specific, therefore it is important to understand which cations it responds to for further determining function of the stellate cell.[5] Out of the three main cells in the cochlear nucleus, stellate cells are the only ones who respond to repeated signals in order to create its own signal. Acid sensing ion channels have different subunits and depending on their makeup, they have different functions and signals that it responds to.

Methods

This study took DNA from the cochlear nucleus and using a process called Real Time Polymerase Chain Reaction, they were able to increase the amount of DNA so that it was easier to manipulate. Then they took the DNA strands that make up the acid sensing channel subunits so that they would bind to the place on the DNA that was taken from the cochlear nucleus to see if there was a match to confirm the presence of the acid sensing ion channels. After they confirmed the presence of those subunits, they moved to see how these subunits worked in the brain by using a patch clamp technique. The patch clamp technique is when the researcher identifies a neuron in the living brain (in this case a mouse) and they use a small pipette to give small doses of ions or electricity to that neuron to see how it reacts and whether or not it sends a signal.

Results

The paper looked at various ions to see whether or not their presence would increase or decrease the amplitude of the signals that the stellate cells produce in response to the ions. Some of these acid sensing ion channels were found to respond to sodium molecules as a signal while others respond to calcium, while others only respond to a mixture of sodium and calcium. Most of the subunits activated around the pH of 7 and continued as the pH became more acidic. Throughout development, they are found to be present and don't appear to go away, which seems to mean that they aren't important to a certain developmental time period. Lead and zinc were also looked at. Lead decreased the signaling of stellate cells while zinc increased it when there was a high concentration of zinc, but when there was only a small amount it tended to decrease the amount of signaling.[8]

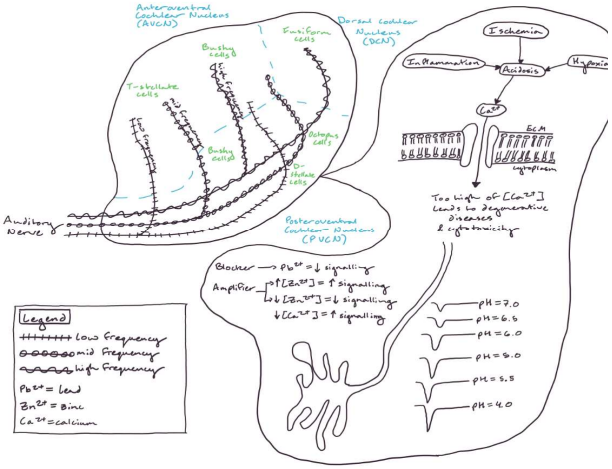

Figure 1. Role of acid sensing ion channels on excitability of stellate cells found in the cochlear nucleus.[6,7]

Significance

The importance of these findings is in related to diseases such as ischemic stroke, epilepsy and multiple sclerosis that tend to be correlated with acidic pH. A change in pH is often associated with inflammation or by proinflammatory factors. If there is a low pH chronically, then stellate cells would continue to fire and this overactive signaling could lead to toxicity. In animals without acid sensing ion channels, there is a lowered fear response to predators, and therefore increase numbers of ASICs may be involved in anxiety disorders.[9] As far as hearing loss, stellate cells weren't correlated with direct hearing loss but with the sensitivity of hearing. This paper identifies acid sensing ion channels and their diversity in form and cationic permeability. Future research should look at the correlation between the proportions of these acid sensing ion channels found within stellate cells and the role of stellate cells in converting primary auditory firing to chopper patterns, which appears to be another large area of research.[3]

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Too much excitement can create painful hallucinations

Researchers found that excess glutamate can result in cortical spreading depression, and this can promote neuroplasticity in the primary motor cortex of the brain and potentially induce migraine with aura (Conte et al, 2010).

Caleb Man

Research in the area of migraine often makes a distinction between migraine with aura and migraine without aura. Put simply, aura is a broad term encompassing many symptoms that present themselves during a migraine attack. Some research articles acknowledge the differences between migraine with aura and migraine without aura, but many seem to lack an explanation behind the neurophysiology involved in migraine with aura. Therefore, the purpose of this paper review is to describe the neurophysiological processes involved in creating aura for some migraine patients, as this can be beneficial in formulating a more effective treatment for them. Research conducted by Conte et al. has found that the migraine aura may be linked to a more restless brain.[1] Furthermore, they found that excess glutamate (a neurotransmitter, or messenger in the brain) may be responsible for this restlessness.[1]

Before diving into what it means to have a restless brain, it is important to understand what migraine is, what aura is, and what neurological changes result in the manifestation of aura. First off, migraine is a severe and painful type of headache that is usually localized on one side of the head, and the pain is often described as being pulsating/throbbing.[2] This severe pain can also present with other signs and symptoms, such as nausea, vomiting, and an increased sensitivity to light and sound.[2] Things get more interesting, however, when aura is present. Aura is essentially an umbrella term that encompasses a variety of symptoms experienced during, or sometimes right before, a migraine. Aura commonly refers to visual illusions or hallucinations, and other cortical disturbances, but the umbrella has also been expanded to include other symptoms, as well.[3] In terms of visual disturbances, the most common types include flashes of light, blurry vision, blind spots (scotomas), or the presence of jagged/zigzagging lines that interfere with vision.[4,5]

Additionally, symptoms can also include difficulties with motor function (dyspraxia), sensory function (tingling or numbness), and the ability to effectively speak or process one's thoughts.[4,6,7] Olfactory hallucinations and problems with memory can also occur, with the latter being more common.[6] In terms of what produces the aura in the first place, one common belief is that migraine aura results from cortical spreading depression (CSD).[8] Several more studies support that CSD is the mechanism behind migraine aura, and it is described as a slow, self-propagating wave of neuronal depolarization that moves throughout the brain.[9,10,11,12] All that being said, what exactly causes CSD is not well understood at this time, but one possibility will be discussed below.[12]

When an excessive amount of glutamate is released into a synapse, especially when not properly removed afterwards, it binds to the NMDA receptors in the brain and produces too

much excitatory stimulation, resulting in a hyper-excited brain. This hyper-excitability can trigger CSD, which is likely the cause of migraine aura, as mentioned earlier.[13] Interestingly, a study found that migraine patients with aura have a more "restless" primary motor cortex (M1) than those without aura. This is likely due to the difference in glutamate dependent short-term M1 synaptic potentiation between migraine patients with aura and those without it.[13] This study sheds some light on the possibility that synaptic plasticity, specifically short-term potentiation, can be a mechanism behind migraine aura.

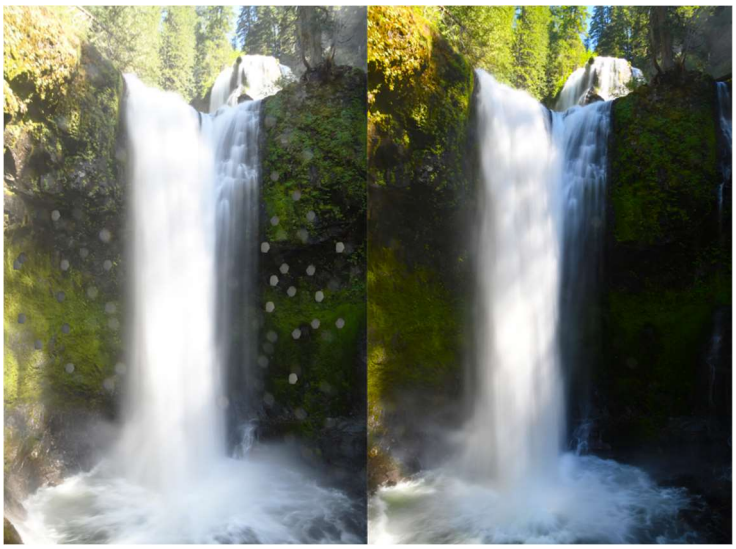

Figure 1: The figure above shows what it is like to see the environment during migraine aura (left) and without migraine aura (right). Additionally, the text below shows what it is like to see words during migraine aura (left) and without migraine aura (right). This is just one example of an aura related visual hallucination, called scotomas (blind spots), but other visual hallucinations may occur as well.

In order to arrive at this conclusion, researchers had to gather a sample of migraine patients with aura (MA), migraine without aura (MwoA) , and also patients without migraines (to serve as a control). All the patients had brain MRIs done on them, and the scans were normal. Before starting the experiment, it was also noted that the participants were not on any medication three days prior to the study, and this was important in terms of reducing a potentially significant confounding variable. Participants then underwent repetitive transcranial magnetic stimulation (rTMS), with the muscle between their thumb and index finger, called the first dorsal interosseous (FDI) muscle, being stimulated and the TMS train (a series of electrodes) being placed on the head. Stimulation was done on their right hands. All patients were studied at rest and when they contracted their FDI muscle for several seconds, and electromyogram (EMG) recordings were collected by measuring activity from electrodes placed on the left hemisphere of the brain. The two rTMS recordings that were measured via EMG were peak-to-peak size of motor evoked potentials (MEPs) and cortical silent period (CSP) duration evoked by each stimulus. All patients were studied when they were not having headaches, with the exception of three patients who had a spontaneous migraine attack (they were studied after their visual aura ended).[1]

While understanding the reason behind using MEP size and CSP duration as measurements is not necessarily important for this paper review, it was found that first MEP size and first CSP size were similar for all three groups (MA, MwoA, and control). That being said, it was also found that rTMS stimulation over the primary motor cortex (M1) resulted in easier glutamate dependent short-term synaptic potentiation in MA patients than MwoA patients or the control patients.[1] The results may be a bit confusing to understand, but essentially, all this means is that the main problem in patients with MA is that they have defects in the "tuning" of their cortical circuits. The rTMS stimulation resulted in hyperactivity of the M1 because of this problem/deficit. Ultimately, the research in this paper supports the hypothesis that glutamatergic transmission differs in patients with MA and patients with MwoA. The patients with MA have an excess of glutamate, which results in an increase in CSD, and this is believed to result in the symptoms

associated with aura. It is also thought that the previously mentioned defects in tuning could be altering how cortical neurons in the M1 interact with other cortical neurons, such as those in the visual cortex, and this could be an important mechanism behind the causes of visual auras.[1] That being said, further studies need to be looked at to better determine how strong the relationship between cortico-cortical projects from the M1 are to the visual cortex, and how this could tie into experiencing visual auras.

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Degeneration of cells stimulated by *a-synuclein*

The brain is not the only organ involved in the development of Parkinson's disease. Parkinson's disease spotted in the gut en route to the brain.

Elisei Cosovan

A neuronal pathologic protein, namely a-synuclein, spreads from the gastrointestinal tract to the brain via the vagus nerve – eliciting similar features to those seen in Parkinson's Disease (PD). This protein specifically stimulates the degeneration of the cells principal to PD-like motor and non-motor symptoms. These cells are known as dopamine neurons. Surgical removal of part of the vagus nerve and a lack of the pathologic protein in this region resulted in the prevention of neurodegeneration and down-regulation of behavioral deficits in the PD mice models.[1]

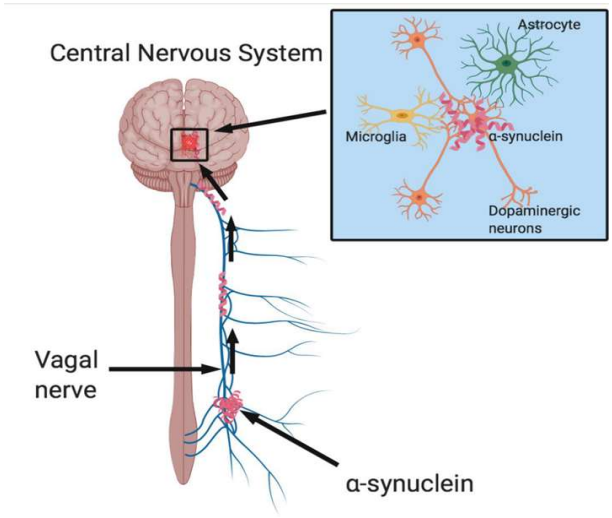

Figure 1 – Pathologic a-synuclein protein spreads from the gastrointestinal tract (part of the enteric nervous system) to the brain (part of the central nervous system) via the vagal nerve. Upon entrance into the brain, the pathologic protein forms clumps between the cells of the respective regions and elicits similar features to those of Parkinson's Disease.

The human gut microbiome has significant indirect effects on the brain. The diversity and health of the microbes in the gut also impact brain health in many ways. The gut microbiome is able to influence REM sleep, memory, mental health, and mood. The gut also has relevance in various disorders. Among these disorders are fibromyalgia, alcoholism, and chronic fatigue syndrome. The bacteria of the gut are able to directly stimulate afferent neurons to send signals to the brain. This occurs through the vagus nerve.

A large nerve that innervates much of the enteric nervous system, which will be referred to as the gut, but one which also reaches all the way up into the central nervous system. Overall, the gut uses this nerve as transportation. The bacteria stimulates a mechanism that travels to the cells in the brain which then sends signals to the rest of the body to perform a specific function.[2] The gut microbes have a say in stress activity and sleep performance as well. In this review, we will focus on the effect a certain pathologic protein has on PD.

Parkinson's Disease affects more than 200,000 people in the United States annually and yet no cure has been discovered for this disease.[3] A study done by Sangjune Kim and a team of scientists, which was recently published in *Neuron,* examined the effects of gut-injected pathologic proteins on the brain in several groups of mice. The results of this study reveal that the pathologic protein, a-synuclein not only causes PD but also develops in the gut and is then transmitted to the brain via the vagus nerve pathway (Fig. 1).[1] It is also discovered that

dopamine neurons – which are lost as PD develops – are degenerated in mouse models containing the pathologic protein transmitted from the gut to the bran. The study reviewed here serves to fast-track research on specific cellular and molecular pathways related to the centripetal trafficking of the pathologic proteins in the route from the gut to the brain. For future research, this study will also aid in testing possible therapeutic interventions that will diminish the risk of PD.

The pathology of this protein follows a similar pattern of transmission through the body as the Lewy bodies observed in dementia in postmortem brains. The Lewy bodies are composed of clumps of the a-syn protein and are transmitted from the dendrite to the axon in a retrograde manner.[4,5,6] Accumulation of these proteins is one of the main characteristics of neurodegenerative diseases.[7] Braak, a German scientist of the 20th century observed a-syn pathology spreading from the gut to the brain through the vagus nerve.[8] This observation is tested in this study through mouse models.

PFF (preformed fibrils) injections were made into the muscularis layer of the pylorus and duodenum – so to mimic the spread of a-syn in PD.[9] The pylorus is the opening of the stomach which leads into the small intestine (also known as the duodenum). Together, these two structures will be termed as the gut throughout this review. The reason behind injecting PFF in this part of the gut is due to the high density of vagus nerve intervention in the area. Once the volume that could be injected between the muscles was determined, a set amount of 2.5 microliters was injected. A pSer129-a-syn immunostaining – which works as a stain that indicates a-syn – was used to observe the advancement of this protein from the gut to the brain. The progression of the protein was observed 1 month, 3 months, 7 months, then 10 months after the injection.

One month after the injection, the stained protein was detected in the medulla oblongata and the pons. These are two structures located in the upper and lower regions of the brainstem, respectively. The stain was also seen in the gut (specifically, the opening of the stomach and the small intestine). The protein was further observed accumulating in the brainstem 3 months after injection. Additionally, the staining indicated protein aggregation in the amygdala and in the ventral midbrain. The amygdala is a region of the brain located just above the brainstem, towards the center of the brain. Small amounts of protein were also seen in the hypothalamus and prefrontal cortex; two brain regions located deeper in the brain than the amygdala. Seven months after the injection the protein was observed more extensively in all regions mentioned earlier, plus in the hippocampus and the striatum. These two regions are deeper in the brain than the regions mentioned earlier. Finally, 10 months after injection, a-syn increased in the olfactory bulb, the hippocampus, the prefrontal cortex, the substantia nigra pars compacta, and the striatum. A decrease in protein accumulation was detected in the amygdala, the medulla oblongata, and the ventral midbrain. It was also concluded that there was a significant loss of dopamine neurons 3 months after the gut injection in the mice, then more drastically 7 months after injection. To test whether or not the vagus nerve is required for the transmission of the pathologic protein from the gut to the brain, part of the nerve was surgically removed. 7 months after the surgery, a 65% decrease in the amount of positive cholinergic neurons – which function to transmit messages – was observed. The pathologic proteins were still observed in some regions of the brain 7 months after the PFF injection, and partial removal of the nerve. When the vagus nerve was entirely removed, the spread of the pathologic protein was not observed. Upon behavioral analysis, it was concluded that the PFF-injected mice (with vagus nerve intact) had significantly decreased latency to fall by the rotarod test, and significantly increased the amount of time on the pole test. These tests display the key features observed in PD.

The results of this study support the Braak hypothesis and the notion that the pathologic protein, a-syn efficiently spreads from the gut to the brain via the vagus nerve. These results are consistent with Braak's hypothesis because they offer a probable mechanism for the induction of a-syn pathology in the enteric nervous system.[10,11,12] Additionally, these results support the idea that Parkinson's Disease begins in the gut and transmits to the brain because the a-syn protein was seen in brain regions that are anatomically connected to each other. Although the results of this study imply that the transmission of pathological a-syn follows interneuronal transmission patterns, the Lewy body pathology was not observed in all affected brain regions.[5] Further studies need to be done to differentiate which aspects of transmission contribute to the selective susceptibility of the given neuronal systems to the Lewy body pathology. Overall, I believe this study did a wonderful job of testing the Braak hypothesis and providing sufficient evidence to support the idea that PD begins in the gut and is then transmitted to the brain via the vagus nerve.

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No lonelier reverse transcriptase inhibitor

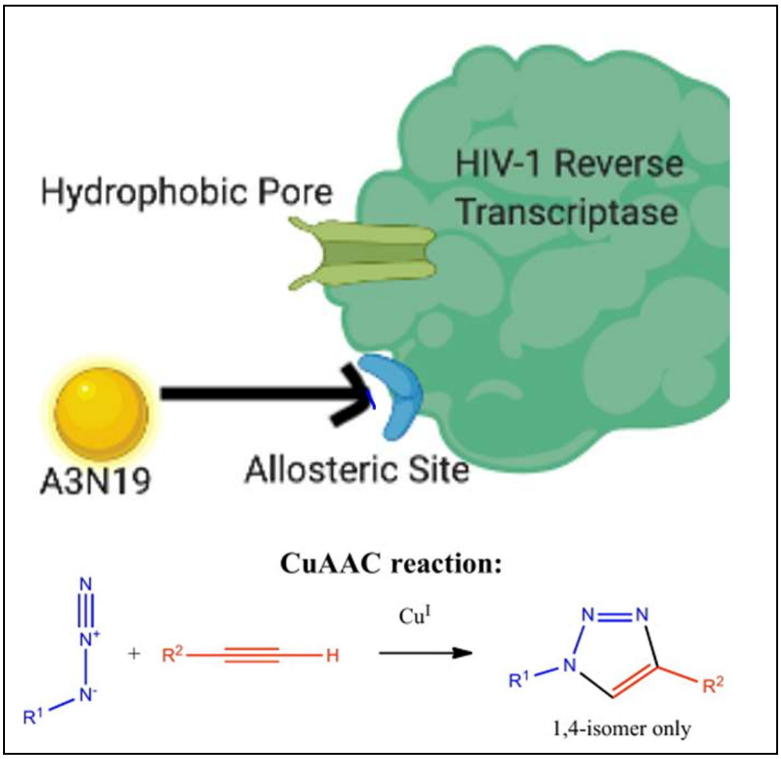
In a paper written by Kang, et al., a host of newly discovered azide compounds have been implicated in the treatment of HIV/AIDS in humans. The compounds are demonstrated to interact with a hydrophobic channel as well as the non-nucleoside inhibitory binding pocket (NNIBP) which is also known at the allosteric site of the channel.

Micah Black

The Human immunodeficiency virus has infected a total of more than 37.9 million people worldwide in 2018, including more than 1.7 million newly infected people in 2018.[1] It is still considered a pandemic, and the United Nations has created a joint program in an attempt to combat the disease (UNAIDS). Kang et al. have published a research paper regarding the topic of compounds that are designed to treat the virus.[2] They are referred to as antiretroviral drugs and a large portion of this research study was devoted to drugs that inhibit the reverse transcriptase protein from reproducing the virus in living organisms.

One of the more promising avenues of research is the study of the HIV-1 reverse transcriptase protein. Many anti-HIV drugs seek to block the channel pore or to allosterically hinder access to the pore in order to reduce the possibility for the virus to reproduce. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a class of compounds that are used to treat HIV, and some are implicated in their ability to specifically target the HIV-1 reverse transcriptase site.[3,4] This class of compounds is referred to as HIV-1 NNRTIs. UNAIDS has stated that “62% of people living with HIV were receiving antiretroviral treatment in 2018” and approximately 50% of the compounds used in this treatment falls under the category of HIV-1 NNRTI.[1] They bind themselves to the non-nucleoside inhibitory binding pocket (NNIBP) and serve to allosterically hinder the reverse transcriptase site, by inducing a conformational change in the protein and therefore preventing the DNA polymerase from functioning in such a way that it can proliferate the virus.[5,6]

Drug research often involves the creation of a ‘library’ of compounds that can all be used in a similar fashion to achieve a certain result. In the case of this study, a library of diverse azide substituents was created and they were labeled N1-N39. Three types of alkynes, labeled A1-A6 were combined with the azide substituents. An example of an NNRTI created in this study would be A1N1, followed by A1N2, and so on. The alkyne compounds and azide substituents were coupled together by employing a method of click chemistry derived from the use of copper in a process called copper(I)-catalyzed azide-alkyne (3+2) dipolar cycloaddition (CuAAC). This method boasts fast reaction time, high yields, little or no need for purification steps as well as nearcomplete chemoselectivity.[7,8,9] CuAAC has undergone some recent developments in the field of chemistry and is rapidly becoming a primary method for generating a bioactive molecules library for research purposes. Following the testing of the synthesized NNRTI compounds, A3N19 has been considered one of the more promising compounds for the treatment of HIV.



The CuAAC reaction represents a prototypical example of click chemistry. It is a highly stereospecific reaction that produces a single product and therefore does not require separation by chromatography. Click chemistry is a term given to a form of chemistry that is high yielding, takes place in a benign solution, and is wide in scope such as an alkyne-azide coupling mechanism. In the case of the HIV-1 NNRTI referred to as A3N19, the intention was to perform the alkyne-azide coupling reaction under physiological conditions. The reaction will create only the 1,4-isomer and no other products will form. Once formed, the HIV-1 NNRTI is able to bind specifically to the NNIBP of the reverse transcriptase protein.

A list of each compound can be found in the research article, along with some of the reaction steps required to create alkyne starting materials A1-A6 along with their subsequent reactions with the azide starting materials. Click chemistry is a method through which a scientist can perform chemical reactions in physiological environments to demonstrate the function of organelles and other biological markers. There are many avenues that a researcher can take in order to study how chemicals can eradicate a virus and prevent it from spreading. This team's avenue of research was to study how a triazole molecule can work to prevent a channel pore on the protein that is directly responsible for its replication may be the most viable method. Many years of research were required in order to understand the 3-dimensional conformation of the channel pore in question, as well as its conformational shape under a variety of physiological conditions.

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Stimulation frequency contributes to reward and aversion

New findings in the continued effort to understand the neuropsychiatry of addiction and depression suggest stimulation patterns in neuronal reward pathways contribute to reward and aversion behaviors.

Trent Pratt

There are many challenges one must face in the world, and we must adapt our behaviors to receive rewards in order to benefit from opportunities and to avoid punishments and cope with potential disappointments. There are many common clinical conditions such as depression and addiction that have raised questions regarding potential treatments and solutions to improve the quality of life in those that may struggle with these issues. Several regions in the brain are involved in someone's ability to process reward and aversive behaviors in response to a given situation. Deficits in the brain's ability to process reward and aversion can lead to the onset of several psychiatric disorders, i.e. depression and addiction. The prevalence of these conditions has raised questions about what physiological dysfunction may be present in the nervous system, and more importantly, how to solve it. It is important to attempt to answer some of these questions and understand how the patterns of specific nervous system cells differ in rewarding and aversive tasks in order to better assess how the two subpopulations of cells work together to ultimately generate behavior.

A paper recently published in Molecular Psychiatry by Carina Soares-Cunha and colleagues investigated the roles of medium spiny neuron (MSN) subtypes in the nucleus accumbens (NAc) of the ventral striatum and their respective roles in reward and aversion.[1] MSNs can be subtyped by the types of dopamine receptors they contain: D1-MSNs and D2-MSNs.[2] MSNs bind dopamine using D1 receptors (makes them more active) while some bind dopamine by expressing D2 receptors (decreases their activity). A complication in understanding the connectivity of the striatum arises because MSNs are inhibitory neurons and reducing their activity increases the activity of the cells they inhibit. There are numerous studies attempting to understand the relationship between these two subpopulations and suggest they play a major role in reward and aversive behaviors.[3,4,5] This study sought to further investigate the relationship between these two striatal neuron subtypes by measuring differences in stimulation frequencies and the resulting effects on reward and aversion.

The primary findings of the study were that D1- and D2-MSNs can influence reward and aversion. Stimulation patterns of NAc D1- or D2-MSNs at the cell nucleus, the soma, and at the terminal, the synapse, induced reward or aversion, depending on the characteristics of MSN frequency of stimulation. Positive reinforcement, i.e. reward, was induced by briefly stimulating the neurons. In contrast, aversion was induced by prolonged stimulation of both MSN subtypes. The difference in stimulation patterns resulted in divergent downstream pathways in the VTA and the VP, referring to their impact on either reward or aversive behaviors.

It is important for the brain to determine which environmental situations and stimuli are rewarding and those that are not. The manner in which the brain processes reward and motivational situations is by encoding reward and aversion, where positive reinforcement occurs with reward, and negative reinforcement with aversion. The brain processes information through distinct pathways, in this case, the mesolimbic reward pathway, which is crucial in understanding psychiatric disorders such as depression and addiction.[6] The striatum plays a crucial role in this circuitry, particularly the NAc.[7] The NAc is a key player in the brain's ability to process and respond to rewarding and aversive behaviors. There are a number of factors that can affect this region's typical function, such as chronic stress and gene expression.[2] When dysfunction does occur, an individual may be vulnerable to depressive and addictive behaviors, so it is important to understand the relationship between neurons in this region. MSNs in this region different receptors for dopamine, expressing either D1 dopamine receptors, which induce facilitation or D2 dopamine receptors, which induce inhibition.[8] The relationship between the activity of these two striatal subtypes determines the effects on reward-related behavior. The implications of this research are important in the continued effort to understanding how the brain processes rewards and their effects on behavior. The resulting behaviors are crucial for the maintenance of an individual's success that could determine the quality of life of a given individual, or even the survival of an individual or an organism.

There is a bit of controversy attempting to explain the roles of D1- and D2- MSNs and the effects on striatal function and ultimately reward-related behaviors. This study is an attempt to answer some of the questions and proposes that the opposition that has existed in the field should be further explored. In the striatum, the majority of neurons, upward of 95%, are MSNs.[9] Both subtypes of MSNs are GABAergic, meaning they are inhibitory and release the neurotransmitter GABA. There are numerous neurotransmitters, i.e. dopamine, that bind to a single receptor, in this case, either D1- or D2- MSNs, and vary in their effects on reward and aversion.[10] MSNs receive excitatory input via glutamate on the heads of the numerous spine synapses on the ends of neurons, dendrites, and dopamine inputs are received at the necks of these dendritic spiny synapses.[11] The combined activity from both glutamatergic and important neurotransmitters ultimately determines the activity of a given MSN, which by extension, would determine an individual's behavioral response.

In this study, mice were optogenetically modified to express a light-activated channel which allowed the researchers to stimulate specific sets of inputs onto MSNs using blue light. This optogenetic approach injected mice with halorhodopsin (activated under blue light), which fluxes Cl^- which allows light to hyperpolarize and inhibit neurons; channelrhodopsin (activated under amber light), which fluxes Na+ which depolarizes cells to excite them; and eYFP for the control group. Historically, there has been some controversy regarding which NAc MSN subpopulation encodes reward and aversion, so this study used optogenetic manipulation to target NAc D1- or D2-MSN activity using this approach to allow for greater specificity of MSN activity. It involved placing an electrode to stimulate the neurons with a fiber optic patch in the region being studied, in this case, the NAc. Stimulation of individual neurons were then recorded and statistically analyzed. This study found that brief stimulation resulted in increased ventral tegmental area VTA dopaminergic activity either directly, for D1-MSNs alone, or indirectly via the VP for both D1- and D2-MSNs. In contrast, the study also found that prolonged stimulation of the two MSN subpopulations resulted in varying effects on activity downstream, indicating that D1- and D2-MSNs' stimulation patterns are implicated in both reward and aversive behaviors. Additionally, optogenetic inhibition of D1-MSNs induced aversion and similar results were found in D2-MSN optical inhibition.

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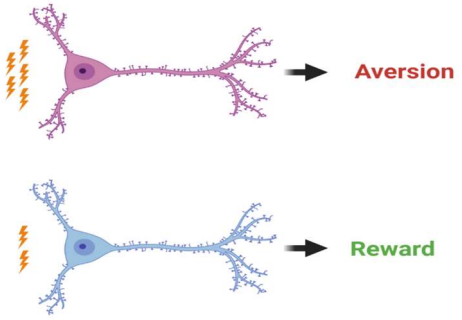

Figure 1. Prolonged stimulation (60s) of D1- and D2-MSNs resulted in aversive behaviors in mice. Brief stimulation (1s) of D1- and D2-MSNs resulted in rewarding behaviors in mice.

The hypothesis of this study was to determine the relationship D1- and D2-MSN activity has on reward and aversive behaviors by changing activation patterns of these two striatal neuron types, as well as changes in regions receiving input from projecting MSNs, the VTA (D1-MSNs only) and VP (both). Because this study finds that the two subtypes of MSNs have distinct and varying effects on reward-related behaviors, it suggests there is a complex relationship that warrants more research in the field. In doing so, the search for answers in treating psychiatric disorders such as depression and addiction could potentially find a better direction. This study sheds light on what appears to be a controversial area of research, suggesting that both subtypes can drive reward and aversion, determined by their pattern of stimulation. The methodology via optogenetic manipulation allowed for specific neurons to be targeted, versus stimulation the local region of other methods, which does not allow for as much specificity. In doing so, it provided some important findings in the relationship between the activities of striatal MSNs. The major takeaway from this research is that striatum is organized in a more complex manner than was previously thought, and additional research can help shed more light on the matter, which could lead to more potential solutions in addressing psychiatric disorders due to striatal dysfunction.

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Schizophrenia with depression: not just a black and white matter

A recent study challenges the classical model for schizophrenia with depression, finding evidence for a unique brain mechanism that is unseen in schizophrenia or depression alone.

Sydney Wolfe

In an article published by *Frontiers in Neuroscience,* a team of Chinese researchers investigates the differences between the individual pathways for depression and schizophrenia and the pathways for schizophrenia and depression combined.[1] The researchers were able to create experimental groups for depression and schizophrenia using male mice. They found that the group of mice who were administered drugs subsequently for schizophrenia and depression had severe behavioral and neurological impairments. These impairments were especially true when compared to mice who were administered the drugs in the opposite order. If the classical theory for schizophrenia-depression comorbidity was accurate, the order of induction of the disorders should not matter, as the individual pathways for depression and schizophrenia would be additive. Additionally, the administration of antidepressants and antipsychotics to mice with schizophrenia and depression failed to mitigate any adverse psychological effects.[1] These findings suggest a possible new pathway for patients diagnosed with schizophrenia, who subsequently experience depression.

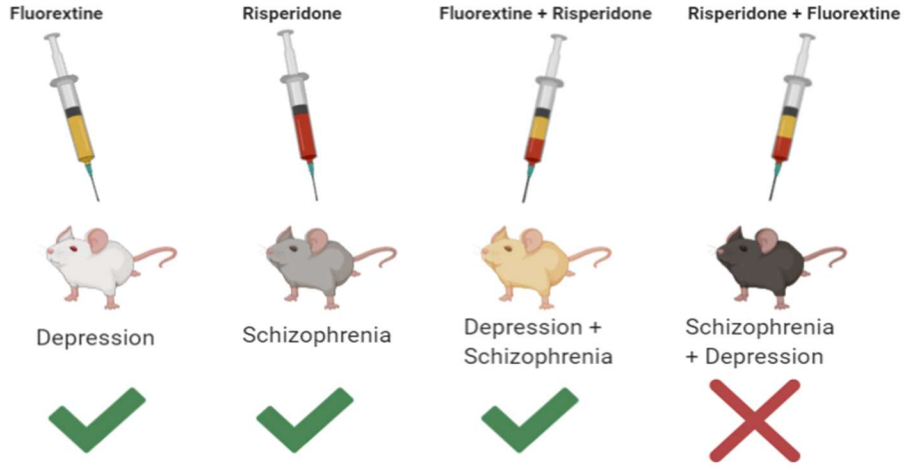
A complex disorder

Schizophrenia is a bewildering mental disease, with multiple theories surrounding its causation. Scientists believe schizophrenia stems from physical, genetic, psychological, and environmental factors.[2] The current theories surrounding schizophrenia include brain inflammation, NMDA-receptor dysfunction (especially in inhibitory neurons), and redox dysregulation.[4,5] Studies have also observed abnormal sulfide production in schizophrenia studies.[6,7] The causes for depression are equally elusive, with the primary theories revolving around neuroinflammation, neurogenesis, and the monoamine hypothesis.[8,9] Curiously, schizophrenia is often accompanied by other psychiatric ailments, with 50% of schizophrenic patients being diagnosed with depression.[3] In the medical and scientific world, schizophrenia and depression are often treated as distinct entities, with distinct pathways.[10] However, clinical trials have revealed overlap in the symptoms and effects of comorbid schizophrenia and depression, challenging this classical model.

The experiment

The experimenters used male mice and split them up into five different groups: control, schizophrenia, depression, depression + schizophrenia (subsequently), and schizophrenia + depression (subsequently). The schizophrenia mice were injected with a drug called MK801, also known as dizocilpine, which causes cognitive distortions.[1] The researchers used chronic unpredicted mild stress (CUMS) to create a depression model.[1] The stressors used included wet bedding, forced swimming, cage tilting, physical restraint, and sleep deprivation.[1] The mice performed a variety of behavioral tests, including the forced swimming test and the sucrose

preference test. To observe activity in the prefrontal cortex, the researchers measured calcium levels in the live mice. After sacrificing the mice, the researchers observed visual-evoked brain activity and electrophysiological activity in medium spiny neurons (MSNs). These neurons were chosen because behavioral deficits in schizophrenia and depression have been associated with impaired calcium activity in the thalamic nuclei. MSNs, which are heavily localized in the striatum, are thought to initiate or suppress movement, making them a viable target for studying mouse behavior.



Created using bioRender: Displays the experimental groups (minus control), including the CUMS depression model, the MK-801 schizophrenia model, the CUMS+MK-801 comorbidity model, and the MK-801+CUMS comorbidity model. Administration of the appropriate antipsychotic/antidepressant drugs (Fluorextine and/or Risperone) for each model leads to the mitigation of adverse symptoms for all groups apart from the MK-801+CUMS model.

Curious findings

The schizophrenia + depression group of mice had the longest immobility time and the lowest preference for sucrose.[1] This group demonstrated higher auditory deficits when compared to the other mouse groups.[1] Additionally, the schizophrenia + depression group presented the lowest amount of calcium signaling out of all the groups, after antipsychotic and antidepressant administration.[1] Paradoxically, visual and behavioral responses were made worse by the drugs. Interestingly, antipsychotic and antidepressant administration mitigated adverse symptoms in the depression + schizophrenia group. This shows possible mechanistic differences between schizophrenia and depression comorbidity and the other treatment groups. Differences in the neural circuit for schizophrenia-depression may explain why classical treatments, such as antipsychotics or antidepressants, are ineffective in treating cognitive/emotional deficits.

Further avenues

This study provides promising evidence for the mechanistic differences between the combined disorder and depression or schizophrenia alone. The two pathways are not simply additive, as previously thought, as drug treatments were ineffectual in the schizophrenia + depression group. Behavioral, auditory, and physical deficits were also exacerbated in this group. Further investigations into the mechanism of schizophrenia + depression are needed to specify which regions of the brain, and what specific neural circuits, might be involved. The use of human trials to image the brains of patients with schizophrenia, who later developed depression, might allow for a more in-depth examination of mouse models by nailing down key brain regions

involved in the disorder's pathophysiology. Gaining insight into these puzzling mental illnesses is crucial to improving drug treatments, and as a result, the quality of life for sufferers.

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A new drug approach increases cognition in rats

In a new study the α4β2 nicotinic acetylcholine receptors, a subtype of nicotinic acetylcholine receptors was studied in relation to their role in attention and cognition. This research presents a novel approach using a positive allosteric modulator on α4β2 nicotinic acetylcholine receptors to increase cognition in rats. This approach can be used in cognitive deficit disorders such as Alzheimer's Disease and schizophrenia, which show a reduction of α4β2 nicotinic acetylcholine receptors in several parts of the brain.

Petru Buracioc

In a paper recently published in *Pharmacological Reports*, Agnieszka Nikiforuk and her colleagues investigated the cognitive effects of desformylflustrabromine (dFBr), a positive allosteric modulator of a α4β2-containing nicotinic acetylcholine receptors. The study found that this modulator increased procognitive activity, improved recognition memory, and rescued drug-induced cognitive deficits in rats. These results indicated that positive allosteric modulator could have pro-cognitive activity effect on α4β2-containing nicotinic acetylcholine receptors which could potentially be implemented to treat cognitive deficits associated with schizophrenia and Alzheimer's disease.[1] Allosteric modulators are substances that bind to the receptors of cells to alter the receptor's response to the stimulus. Among the various allosteric modulators are positive, negative, and neutral modulators. Positive allosteric modulators such as the dFBr presented in this study increase the response of the receptor by increasing the probability that the receptor will be activated.[2] By increasing the affinity and efficacy of agonists, positive allosteric modulators can modulate the effect agonists have on the targeted cells.[3]

The involvement of α4β2-containing nicotinic acetylcholine receptors in cognition has been determined by mounting evidence. Postmortem studies indicated reduction in these receptors in Alzheimer's Disease (AD) and schizophrenia.[4] Additionally, studies have indicated that beta-amyloid accumulation, one of the hallmarks of AD significantly increased in the brains as α4β2 nicotinic receptors significantly decreased, establishing a connection between the two factors.[5] Regions in the brain showing the reduction of these receptors included the hippocampus, amygdala, and the neocortex as well. These regions of the brain are also areas affected the most in AD.[1] Nicotinic acetylcholine receptors are found in all layers of the neocortex regulating the excitatory activity by inhibiting excitatory signals. In layers II and III, pyramidal neurons are inhibited by nAChRs stimulation, and pyramidal neurons in cortical layer V are activated by nAChRs stimulation.[6] Thus, each cortical layer is modulated differently and that is in part due to the different types of nAChRs present in each layer. Furthermore, pyramidal neurons in layer V of the neocortex are modulated by inhibitory interneurons which are mediated by the dis-inhibitory effect of α4β2 nAChRs, which increase GABA release onto interneurons.[7] In other words, these receptors inhibit interneurons such as PV basket cells that inhibit the activity of pyramidal neurons therefore disinhibiting or removing the inhibitory effect of interneuron.

The effects of dFBr modulators on cognition were determined by two animal tests: the attentional set-shifting task (ASST) and the novel object recognition task (NORT). These tests determine cognitive flexibility and recognition memory, respectively. In the ASST test rats

initially learned a rule and developed an attentional set for the initial rule. This was followed by the extra-dimensional (ED) shift stage where animals switched attention to a previously irrelevant stimulus dimension and differentiating between the odors and not the media covering the bait. This part of the test measures cognitive flexibility. Additionally, the ability to alleviate the cognition deficits induced by a competitive antagonist called by dihydro-β-erythroidine on α4β2-nAChRs was also determined in this study.[1] Agonists are substances that bind to the receptors and activate receptors to produce a response.[3] Antagonists block or reduce the activity of agonists when they bind to receptors. Therefore, agonists and antagonists have opposing effects on receptors. Competitive antagonists bind to the same sites as the agonists, without activating the receptors and by blocking the action of the agonist. On the other, non-competitive antagonists bind to different sites on the receptors to prevent the activation of the receptor.[3]

In the attentional set-shifting task the rats showed cognition improvement with dFBr in a α4β2-dependent manner. To test that the result was in fact because of the allosteric modulator, administration of the antagonist was used to block the effect of the modulator. In the object recognition task, the rats were treated so that they could not discriminate the novel object from the familiar objects – creating a natural forgetting in rats. This forgetting was improved by the administration of the modulator. Similar to the attentional set-shifting task, the antagonist blocked the procognitive effects in the recognition task. In addition to the procognitive effects on object recognition and attentional set-shifting task, the allosteric modulator reversed the ketamine- and scopolamine-induced novel object-recognition deficits in rats. When ketamine or scopolamine were administered, the animal's ability to discriminate novel and familiar objects was eliminated but was revered with the administration of the allosteric modulator. Therefore, ketamine and scopolamine were used to test that the drugs do in fact improve cognition by inducing cognitive deficits in rats. The co-administration of inactive doses of the allosteric modulator and agonist lead to procognitive effects.[1] Another positive allosteric modulator on α7-nAChR, a different type of nicotinic acetylcholine receptors in combination with an agonist showed similar procognitive effects suggesting that conditions such as AD have compromised cholinergic functions.[8] These results show potential potent therapeutic models when positive allosteric modulators are administered together with agonists.

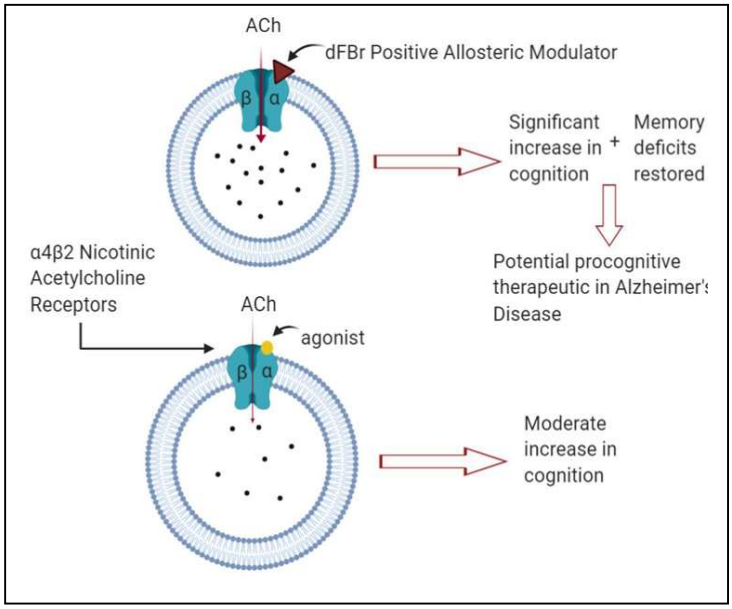

Figure 1: α4β2 nicotinic acetylcholine receptors with administration of agonist vs positive allosteric modulator (dFBr). Positive allosteric modulators increase the probability the receptors being activated having a procognitive effect in compromised cholinergic disorders.

In this article, α4β2 nicotinic acetylcholine receptors have been targeted as a potent target for improving cognitive impairments in disorders such as AD and Schizophrenia. Studies have investigated the role of α4β2 nicotinic acetylcholine receptors in relation to neurodegenerative and cognitive disorders, and potent agonists and antagonists were developed as a result to improve cognitive performance. In this study, the positive allosteric modulator targeting these receptors shows promising precognitive activity which could be implemented in cognitive impairment therapy for disorders such AD and schizophrenia. Further studies should investigate the cognitive effects of

this positive allosteric modulator in AD mouse models in combination with potent nAChRs agonists to observe changes in cognition. Studies have observed a direct relationship between the accumulation of amyloid plaques in AD and the significant reduction in nAChRs such as the interaction of α7-nAChRs and amyloid plaques to play a crucial role in cognitive decline.[9] Further studies should investigate this interaction to further determine the function of flawed cholinergic function in neurodegenerative disorders and how combinations of positive allosteric modulator and agonists on nicotinic acetylcholine receptors can increase cognition.

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How does alcohol affect your brain's signaling?

Excessive alcohol consumption strengthens presynaptic D2R MSN to postsynaptic D1R MSN glutamatergic transmission.

Vicky Nganga

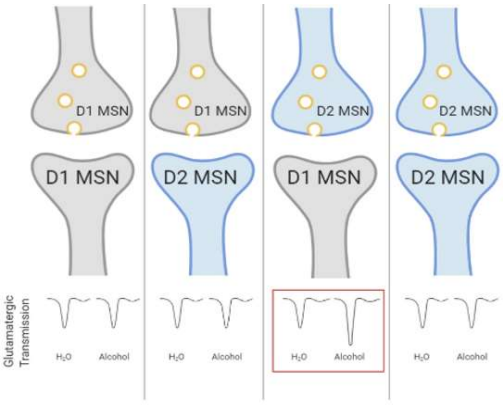

Figure 1. Excessive consumption of alcohol induces longer-lasting glutamatergic transmission in D2 to D1 synapses

The dorsomedial striatum is a brain region essential for goal-directed behaviors and addiction. The neurons located in the DSM are called medium spiny neurons. Neurons are the basic unit of the brain and allow for messages to be sent from one brain area to another. The MSNs that are expressed in the DSM have receptors: dopamine 1 receptors and dopamine 2 receptors. These receptors are able to modulate dopamine; is a chemical messenger that plays a role in pleasure and reward. An article published in Neuropsychopharmacology by Lu and colleagues wanted to observe how addictive substances alter the glutamatergic (related to the excitatory neurotransmitter glutamate) strength of synapses that express these dopamine receptors.[4] They analyzed synaptic activity in presynaptic and postsynaptic dopamine receptor-expressing medium spiny neurons. D1 to D1, D1 to D2, D2 to D1, and D2 to D2 synapses. Since dopamine plays a critical role in reward-driven behaviors and understanding this would allow them to gain information about the mechanism behind brain reward circuitry that is modulated by dopamine.

To do this, the researchers used CRE mice and gave them intermittent access to two bottle choices: 20% alcohol and water for 8 weeks. Fluorescent imaging was used to observe the expression and projections of the medium spiny neurons in the dorsomedial striatum. Electrophysiology is a method used to observe the electrical activity neurons. This method was used to observe the electrical activity at the D1 to D1, D1 to D2, D2 to D1, and D2 to D2 synapses. The data collected were analyzed using a two-tailed t-test, one-way ANOVA, and two-way ANOVA to analyze if there was a significant difference among the groups.

They found that synapses that with D2R on the presynapse and D1R on the postsynapse had stronger glutamatergic connectivity than any of the other tested synapses. In addition to this, mice that drank an excessive amount of alcohol had long-lasting potentiation of glutamatergic transmission. Their final finding was that when D2R inhibition of glutamatergic transmission was controlled by presynaptic and postsynaptic mechanisms. This means that there was something on the sending synapse and the receiving synapse that was controlling the D2Rs that were inhibiting glutamatergic signals. This gave researchers some insight about the alcohol evoked circuit plasticity in the DMS which could potentially be the cause of excessive alcohol consumption. In addition to this, the study gave insight into the role that dopaminergic modulation plays in reward circuitry, specifically in the striatum.

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Want a better memory? More REM sleep could be the solution

Sleep has previously been identified as a critical period for consolidation of memory. A research team spanning labs in both New York and Peking recently identified one mechanism that only occurs during one specific stage of sleep: REM sleep. This mechanism is the pruning of dendritic spines.

Jonah Stickney

Dendritic spines are tiny attachment points on brain cells that help create connections with other brain cells. The more dendritic spines there are on a neuron, the more easily neurons are able to talk to it. Not only have dendritic spines been linked to memory, but researchers have also found that dendritic spine populations change during sleep after learning a new task.[1,2] Furthermore, sleep disruption has been shown to decrease the amount of spines in brain areas associated with memory.[3] The present study published in *Nature Neuroscience* continued this research by specifically targeting the part of sleep associated with dreaming, REM sleep.[4] In their research, the team found that REM sleep that followed new motor skill learning, selectively prunes some dendritic spines while strengthening others. While the mechanism requires further research, the implications for this paper are already translatable to health information: getting sleep is important for memory, but the quality of sleep is just as important.

The brain is a fascinating but unbelievably complicated organ. The process of memory formation has been linked in part to a specific brain region (the hippocampus), but where that memory is stored and how it is converted from short to long-term storage is not fully understood.[5] Part of what is known about memory consolidation is that synapses undergo changes in strength.[6] Synapses are the connections between two brain cells. If you think of the brain as a computer that can rewrite its own circuitry, changing synaptic strength allows the processing of information to become more efficient. Through these changes, multiple sensations, ideas, concepts, and temporal information can be associated: conceptually this is the formation of a memory. Dendritic spines may not all behave the same way, but their change in location and size has been correlated with changes in synaptic strength, which suggests dendritic spines help with memory formation and long-term storage.[7] It is known that dendritic spines change morphology during sleep, but our knowledge has gone deeper thanks to the present study.

Methods

A fascinating part of this study is the fact that it was multi-modal: it used behavioral treatments, but the measures were anatomical on a cellular scale. This links activity (learning a motor task) to specific changes in parts of brain cells (dendritic spines). The genesis of new synapses and dendritic spines occur at the fastest rate early in development, though most of these connections are later pruned. As such, the research team used young mice that would have a fast rate of spine formation. Using a high-powered microscope, the team looked at new and old dendritic spines in the area of the brain controlling conscious muscle control, the motor cortex. They then took a subgroup of those same mice and disrupted the REM portion of sleep, while the other mice had normal sleep. This sleep treatment was followed by a second microscope session to see how many new and old dendritic spines survived. After finding that disturbing REM sleep slowed the rate of

dendritic spine pruning, the researchers formed a second experiment. They took a new group of adolescent mice and had them learn how to balance on a rotating bar. This is known to stimulate the growth of new dendritic spines as they improve at the task. After the mice learned how to balance, they performed a live recording of the mouse REM sleep. They specifically observed calcium concentration in neurons of the motor region to see if calcium-regulated spine pruning and strengthening.

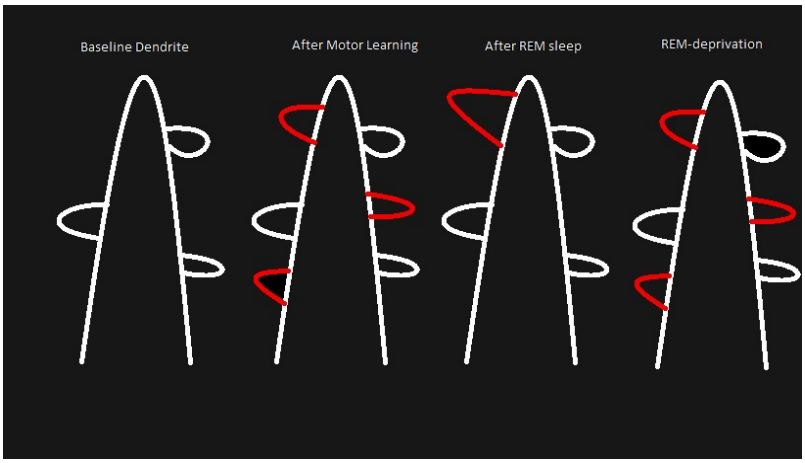

Figure 1: Dendritic spine pruning and selective strengthening occur in REM sleep. The figure differentiates spine populations as generating pre (white) and post (red) a motor learning task. Many new spines are generated after learning. The REM sleep that follows prunes most of these new spines and strengthens the others, pre-existing spines are not affected. If REM sleep is disturbed, this pruning and strengthening simply does not occur.

Results

The primary takeaway from the two experiments in this study was the finding that interrupting REM sleep inhibited dendritic spine pruning (Figure 1). The analysis goes further than this. Firstly, in the adolescent group, only newly formed dendritic spines were affected; dendritic spines in the motor area of the brain that had been established before the motor learning task were not affected by REM disruption. This suggests REM sleep may be involved with learning and consolidating new information, but possibly not involved in the maintenance of stored memories. Another finding from this study was that REM sleep not only prunes newly formed dendritic spines, but it also strengthens the populations of newly formed dendritic spines that remain intact. It's as if learning involves forming many new connections but REM sleep chooses which of those new connections are most beneficial to keep. It was also found in this study that newly formed dendritic spines were most vulnerable to REM modulation during the first 36 hours after formation, after which they were not affected during REM. Another mechanism other than REM must underlie the pruning of more permanent dendritic spines. The final experiment with the live recording of REM sleep found that calcium surges occurred during REM sleep. These calcium transients correlated with the modulation of dendritic spines, both with pruning and strengthening.

Discussion

Many substances are known to inhibit REM sleep, but not many studies have found a relationship between REM and behavioral or cognitive outcomes. Some anti-depressant drugs that are known to disrupt REM sleep were reported in one study to have no effect on memory and in some cases actually improved memory.[8] A case study described REM sleep impairment in a patient who had brain-stem lesions, but again the patient suffered no significant impairment in memory function.[9] This has stoked discussion that centers on sleep's purported function in memory facilitation.[10] This discussion does not remove the importance of the present study's findings, it just suggests a nuanced view may be needed to interpret them. Studies in which REM sleep is impaired but memory is not may suggest that dendritic spines are involved but are not the sole regulator of memory consolidation. This idea of redundant mechanisms seems to be the rule rather than the exception in biological systems, especially for systems as important to an organism's survival as memory. Another interpretation is that dendritic spines regulate a specific type of memory, or rather a specific quality of memory. Perhaps people without REM sleep have seemingly normal memory,

but the ability to relate and compare different memories may be affected. Much more research is needed to be done in the field to truly understand the role of REM sleep, but this research shows that it is doing something. REM sleep exists for a reason, but the question of its utility and function currently remains unanswered.

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spotlight

MICHAEL MORGAN, Ph.D.

Dr. Michael Morgan has been a psychology and neuroscience instructor at Washington State University Vancouver for 27 years! Before teaching at WSU, he earned his Ph.D. in physiological psychology at UCLA in 1989, then pursued post-doctoral research at the University of California, San Francisco.

Morgan's primary research interests revolve around opioid withdrawal and neural pain modulation. Morgan and his team seek to discover how different opioids affect tolerance and resistance to pain in rats. They are also uncovering how chronic pain impacts withdrawal symptoms and are looking to improve their current models for pain assessment. In the last five years, Morgan's lab has utilized a novel method for assessing clinical pain in rats called "home cage wheel running." This method is preferred over other methods of assessing pain, such as evaluating locomotion and reward-seeking behavior, because it involves less stress for the rats.

WSUV students embrace many opportunities from Morgan. Graduate-level lab work gives students the necessary tools to succeed in their careers. Ram Kandasamy, a former grad student who led the development of the wheel-running method, is now an assistant professor of psychology at California State University, East Bay! Morgan has also encouraged undergraduate students to gain practical experience in laboratory work, offering training and academic credit for those that get involved. This hands-on experience allows students to narrow down their interests for future career pursuits. Additionally, undergraduates get the opportunity to be listed as co-workers in published papers! For example, former Morgan Lab students Jonas Calsbeek and Andrea Lee were both co-authors on some of the wheel running papers, and each is now in graduate school. Ameen Baradar, a student at WSUV and former member of the Morgan lab, explains how Morgan has not only taught him how to conduct great science, but how to communicate great science! The Society for Neuroscience conference of 2019 provided Ameen with real experience on how to present research and interact with other scientists from around the country.

Morgan's class is unlike the average lecture, as he utilizes games (including a bit of theater) and active participation to bring the subject to life. Morgan's openmindedness to hear all student perspectives makes his class feel warm and welcoming despite the complex material. Dr. Morgan has an enthusiasm for science that has captivated and will continue to captivate students for years to come. Morgan encourages his students to embrace rhetorical questions; to be okay with not always knowing and to consider several possibilities when seeking an answer. After all, neuroscience comes with a lot of "gray matter"– and that's what makes it fun!

CAPSTONE

Effects of neonatal methamphetamine exposure

Shelby Brock

Introduction

Methamphetamine (MA) is an addictive stimulant which is increasingly abused in 18-25-year-olds in the United States.[1] While it is available pharmaceutically to treat attention-deficit/hyperactivity disorder (Desoxyn), MA is primarily illicitly produced and distributed.[2] Of the 1.9 million users in the US, estimates suggest the prevalence of MA use in pregnancy ranges from 0.7 to 4.8%.[2,3] In 2006, about 24% of pregnant women seeking treatment for substance abuse listed MA as their primary substance.[4] Some studies report conditions seen in MA-exposed neonates including premature birth, low birth weight, decreased arousal, and birth defects such as a facial cleft or gastroschisis, a condition where a hole in the abdominal wall forces the bowel to develop externally.[5,6] While the effects of MA are relatively well-documented (and explored in this review), the mechanisms that facilitate these processes are difficult to study in humans due to confounding variables such as polysubstance abuse, lack of prenatal care, and poverty.[7] Rodent studies provide a solution to this dilemma by allowing control over these variables.

In human adults, MA stimulates the release of monoamine neurotransmitters like dopamine, serotonin, and norepinephrine.[8] With chronic use, MA reduces overall levels of dopamine transporter and causes dysfunction in the striatum and prefrontal cortex of users. As a result of MA exposure in utero, a decrease in striatal dopamine and dendritic spine formation has been observed in rodents, which is consistent with what is known about dopamine dysregulation in humans. In human adults, MA stimulates the release of monoamine neurotransmitters like dopamine, serotonin, and norepinephrine.[8] With chronic use, MA reduces overall levels of dopamine transporter and causes dysfunction in the striatum and prefrontal cortex of users. As a result of MA exposure in utero, a decrease in striatal dopamine and dendritic spine formation has been observed in rodents, which is consistent with what is known about dopamine dysregulation in humans. Studies dating from the 1980s began shifting the focus from prenatal cocaine exposure to MA. For example, cocaine use in pregnancy has been cited as identical to MA exposure, but cocaine has also been shown to cause secondary withdrawals at 4 to 6 weeks of life.[9] Newer studies have aimed to differentiate the effects of MA exposure from other drugs (per self-report), as in Sowell et al.'s alcohol control study and Smith et al.'s longitudinal study of polydrug exposure on development.[5,10]

Infant development, environment, and lifestyle (IDEAL) studies and also correlates

A great deal of human research on MA exposure aims to understand the populations affected and conditions at birth and later in life. The Infant Development, Environment, and Lifestyle (IDEAL) study by Smith et al. is a collection of data from a multi-site longitudinal cohort study which follows up on thousands of mothers and children up to 7.5 years after birth and focuses on how drug abuse affects the life outcomes of exposed children.[5,11,12,13] The data from this study set a baseline for researchers, allowed for future analysis of different aspects of the same data,

and is unique because it screened about 35,000 mother-child pairs and reported the frequency of substance use throughout pregnancy. The original IDEAL study by Smith et al. in 2006 found that the incidence of a child being born as small for gestational age (SGA) was 3.5 times higher in MA-exposed babies.[5] This is concerning because SGA indicates an increased risk for neonatal mortality and morbidity, and one study even suggests that it is associated with lower intelligence, academic difficulties, and behavioral problems.[14] However, it is worth noting that the growth of SGA children typically catches up to their unexposed peers by about 3 years of age.[5] A follow-up analysis by the same IDEAL study team in 2008 found that MA-exposed children were more likely to exhibit decreased arousal and increased central nervous system stress, as determined by the NICU Network Neurobehavioral Scale (NNNS).[11] Not surprisingly, all significant findings from the data were most pronounced in children exposed to MA at least three days per week in utero, which is considered "heavy use." This suggests that the severity of neonatal complications is determined by the amount of exposure to the drug, but the presence of complications is indeed associated with exposure itself.

Participant data from the IDEAL studies are available for other researchers to conduct their own studies. Abar et al. followed up on IDEAL participants for up to 6.5 years after birth to assess cognitive function and early adversity.[13] They found that MA-exposed children experienced more changes in guardianship, extreme poverty, a reduced executive cognitive function such as attention and accuracy, and higher incidence of anxiety and aggression. Aside from identifying demographic information on MA use, this study highlights the real outcomes of the children affected by MA use not only in their own development but from the surrounding environments in which they are raised. As for the populations affected, Good et al. conducted an extensive chart review like the IDEAL studies and found that most MA mothers that presented were white, unemployed women with less than a high school education.[7] Half of the study's MA-exposed pregnancies resulted in preterm delivery and tended to be high risk due to few (typically < 5) or delayed prenatal visits and domestic abuse (stress), on top of substance abuse. Establishing neurobehavioral and environmental outcomes of MA-exposed children allows researchers to delve deeper into the mechanistic underpinnings of exposure.

The most non-invasive way to determine MA involvement in structure / morphological development is imaging studies. Roussotte et al. used functional magnetic resonance imaging (fMRI) to observe activation differences between MA + alcohol-exposed children, alcohol only, and non-exposed controls.[15] Not only did Roussotte's team observe greater inaccuracies in verbal memory tasks, they also found decreased levels of functional activation in the frontal gyrus, caudate nucleus, and putamen of the MA + alcohol-exposed children during these tasks. Another type of imaging, called diffusion tensor imaging (DTI), records the diffusion of water through brain fibers. DTI can indicate the strength of cross-regional brain connections and changes in microstructural integrity by measuring the strength and direction of water flow. However, only one study by Warton et al. has used DTI to examine the effects of MA exposure.[16] DTI revealed lower efficiency of the connections within the striatum (between left and right caudate nuclei), orbitofrontal cortex, and other limbic structures in MA-exposed children compared to nonexposed controls. These imaging studies suggest that MA exerts is effects primarily on the striatum and other limbic structures, which is consistent with the striatal connectivity alterations seen in DT imaging studies of adult MA users.[17]

Rodent studies give insight to human functions

Keep in mind that the effects of MA seen in human applications are often compounded with other substance usage such as tobacco, alcohol, and marijuana. Due to polysubstance abuse in humans, research on the neurological mechanisms that facilitate the external effects of MA is

best suited for a rodent laboratory, as this eliminates discrepancies in self-reports and other confounding variables such as multiple substances or lack of follow up. To understand the effect of MA at critical developmental periods, Schaefer et al. administered MA or saline vehicle during postnatal days (P) 11-15 and 11-20 to groups of rat pups and measured the resulting hippocampal corticosterone and serotonin levels, as it is known that disruptions in these monoamine balances can cause short- and long-term deficits in cognition.[18] In MA-exposed rats, there were increased levels of corticosterone and decreased serotonin expressed on P16 but changes were mostly attenuated by P30. This effectively identifies the short-term effect of MA use on development.

MA exposure induces long-term alterations in function, structure, and receptor and neurotransmitter expression in different brain regions. Crawford et al. found that dopamine content and dopamine receptor expression were detectably reduced in the rat dorsal striatum 70 to 90 days after their last MA exposure.[19] The number of dendritic spines is reduced in the prefrontal cortex and other limbic areas such as the hippocampus and nucleus accumbens under similar conditions.[20] A lack of dendritic spine densities and dopamine content could explain the decrease in functional activation observed in these regions of an MA-exposed child's brain.[15] However, an increase in gene expression of dopamine D3 receptor in the striatum, carboxylesterase 2 in the hippocampus, and glucocorticoid nuclear receptor in the prefrontal cortex of rats has also been observed.[20] An increase in expression has been hypothesized to be a compensation for deficits in other areas. In addition, MA-exposed rats also demonstrate increased compulsive behavior and motivation for reward, which may speak to the behavioral problems observed in human studies.[21] These results add to the growing evidence that MA exerts its effects primarily on dopaminergic projections, limbic structures, and the prefrontal cortex, which are responsible for a variety of things such as impulse control, locomotor function, and memory.

Conclusions

While a great deal of neonatal drug research has focused on the more acutely impactful drugs such as cocaine and opiates, this literature review sheds light on the negative impact of MA use on the developing brain. Human studies have been able to detail not only who is affected by the growing use of MA, but *how* they are affected in development and by their environment. Not only are the affected children burdened by increased adversity, they are also faced with a potential cognitive disadvantage in comparison to their unexposed peers, as they have an increased prevalence of anxiety and issues with concentration. Rodent studies fill the mechanistic gaps in knowledge left by observations of human behavior and outcomes by confirming MA involvement in limbic and executive control regions.

A diffusion tensor imaging (DTI) study of MA-exposed children to observe microstructural changes has been done once, but I think that these imaging techniques could also be beneficial for use in rodent models as well (Warton et al., 2018). If used in a rodent MA model, DTI and even fMRIs could be directly compared with the physical and genetic changes described in the rodent literature of this review. Microstructural changes in the DTI of rodents could serve to validate findings in human subjects, which would bolster the argument that rodent models are applicable to human findings. The reverse can also be shown if protein kinase A (PKA) levels, an enzyme related to dendritic spine development, are able to be observed in humans. Taken together, the studies covered in this review provide a good starting point for further investigations into the role that MA plays in neurodevelopment and behavioral outcomes, especially with regards to the striatum and prefrontal cortex.

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CAPSTONE

Review of Drug Addiction and the Role of Perineuronal Nets

Ashlynn Dean

Introduction

Drug addiction is a disease that is long-lasting and affects not only the physical but mental and psychiatric states of users, families, and communities.[1] Drug addiction is characterized by misuse and dependence of both illicit and prescription drugs, including but not limited to opioids, heroin, controlled prescription drugs, methamphetamines, and cocaine.[2] Since 2011, drug poisoning deaths have been the leading cause of injury-related death in the U.S.[2] In the U.S. alone, there are close to 192 deaths from drug overdose daily.[2] While treatments are available, most programs have a high rate of relapse, making it crucial to find better methods of reducing drug-seeking behaviors.[1]

Many studies on drug addiction focus on the reward circuitry within the brain. This circuitry relates drug-associated cues with the memories of previous drug use.[3,4,5] Because of this association, being in a similar environment as previous drug use will increase drug craving, therefore increasing the probability of continual drug use and relapse.[3,4,5] Because of the direct link between drug memories and addiction, many researchers focus on ways to block or reduce the strength of these memories. Recently, drug researchers have been shifting focus to a structure in the extracellular matrix (ECM), known as perineuronal nets (PNNs). PNNs are a net-like structure composed of proteoglycans that surround fast-spiking GABAergic interneurons throughout the brain.[3,6] These structures were discovered in 1898 by Camillo Golgi but were not intensely researched until about 80 years after.[7] More recently, it was found that PNNs were involved in learning, memory, and drug addiction.[3] Specifically, the stability of PNNs relates to the synaptic plasticity of the surrounding neurons and therefore to the stability of memories, especially drug memories.[6] In this review, I explore the role that PNNs play in drug addiction and relapse and its potential to better drug addiction treatment.

Pnns and their relation to learning and memories

The reward circuitry within the brain is highly studied for its role in addiction. This circuitry and its relation to memories can be simply explained by the original studies done by Ivan Pavlov on dogs.[5] This study involved pairing a bell ring with feeding times. After multiple pairings, the dogs associated the bell with food and began salivating more even if they were not given food immediately after the ring.[5] This same concept goes for other memories as well. The reward circuitry partially controls emotional responses and links them with memories.[8] Due to this association, the brain will recall emotions from previous memories when in the presence of similar stimuli.[9,10] PNNs are highly involved with the formation and maintenance of these memories. A recent study focused on the role of PNNs within the sensory cortex on fear learning and found that PNNs were necessary for the proper formation of fear memories.[11] Reduction of the intensity of PNNs also led to mice more easily forgetting fear memories.[3]

PNNs are regulated by changes in calcium conductance, potassium levels, and AMPA/NMDA receptor activity.[12] The structural integrity of PNNs is directly related to neuronal plasticity within that area of the brain or spinal cord.[3,4,12] While these structures are developing, plasticity of the surrounding neurons is high, allowing for formation and updating of memories.[3,11,12]

Drug-related memories and PNNs

PNNs have an important role in not only fear memories, but drug-related memories as well. Many studies use a cue-pairing and/or self-administration methods in animal models of drug addiction.[5] The cue-pairing method is much like the Pavlovian studies in which an environmental cue is associated with the emotions of a previous memory (in this case the euphoric feeling of the drug). Self-administration is a method in which animals press a lever at a random or fixed number of times to receive a drug reward. Previous studies have shown that knocking out (reducing intensity of) PNNs reduces drug-seeking behaviors in both cue-pairing and self-administration methods.[6,10,12] In the lateral hypothalamus, an important structure in the limbic system, the degradation of PNNs leads to decreased cue-induced reinstatement of cocaine-seeking behaviors.[10] To destroy these nets and increase neuronal plasticity, Chondroitinase-ABC (Ch-ABC) is commonly used. Ch-ABC is an enzyme that cleaves chondroitin sulfate glycosaminoglycan chains, a main structural component of PNNs.[13]

Even without the use of external enzymes, such as Ch-ABC, PNNs change with continual drug exposure. One study found that the intensity of PNNs decreased 2 hours after 1 day of cocaine exposure but increased after 5 days of exposure.[6] This likely reflects the changing of the memories after the first day of exposure and the increased integrity of memories after 5 days of exposure. During nicotine self-administration, PNN intensity in the ventral tegmental area (VTA) and orbitofrontal cortex (OFC) was decreased from 45 minutes to 72 hours after the last exposure, indicating a change in GABAergic interneurons in this area.[4] Prescribed drugs, such as monoamine reuptake inhibitors used for depressive symptoms, were shown to increase PNN-degrading matrix metalloproteinases (MMPs) and lead to a reduction in the integrity of PNNs, indicating that PNNs play a role in drug efficacy.[14]

Pnn-associated proteins and how they affect drug memories

The main proteins involved in the structure of PNNs are hyaluronic acid and chondroitin sulfate proteoglycans, including aggrecan and brevican.[12] PNNs are also co-localized with GABAergic Parvalbumin-positive (PV+) interneurons; around 60-80% of PV cells are surrounded by PNNs.[12] Some of the trends of PNNs are also applicable to its affiliated proteins. A decrease in PNN intensity is normally followed by a decrease in PV expression within cells.[10,12] In aggrecan knockout mice, cells were able to express PV but were unable to form PNNs.[3] Brevican levels were decreased in the mouse hippocampus after environmental enrichment and in brain tissue from patients with epilepsy, indicating that brevican has an influence on the activity of inhibitory neurons.[3] Aggrecan and brevican gene expression increased four hours after drug-conditioning along with intensity and number of PNNs.[3] These data indicate that multiple components of PNNs also play a similar role in regulating drug memories and could be targeted in manipulation studies.

Future directions/conclusion

Together, PNNs are a promising structure to explore when aiming to improve drug addiction treatments and reducing relapse rates. Research has shown a strong relationship between PNN intensity and drug-related memories. With either cocaine or nicotine addiction, the increase in memory strength was followed by an increase in PNN intensity. Knocking out PNNs using Ch-ABC led to a decrease in drug-seeking behavior in rodents. PNN-associated proteins, including brevican, aggrecan, and PV, also play a role in the integrity and intensity of PNNs and therefore affect the formation and updating of memories. Research has shown that multiple components of PNNs can alter memories, making PNNs and PNN-associated proteins promising targets in

possible drug therapy. Studies have shown that trends of PNNs, including increasing/decreasing intensity of PNNs, are followed by PNN-associated proteins (e.g. decrease in PNNs results in a decrease in PV expression). Future studies should focus on targeting and manipulating the expression of these proteins to determine their effect on drug-seeking behavior. Recent studies have also determined the effect of other cellular processes, such as oxidative stress, on the intensity of PNNs, but have failed to fully study how this process affects behavior as well. Due to the relationship between PNNs and drug efficacy, future studies should test the effects of combining prescription drugs, such as antidepressants, with a PNN-altering enzyme to determine if the efficacy changes.

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CAPSTONE

Opioid-induced hyperalgesia and enhanced pain in opioid withdrawal: a comparison of cellular mechanisms

Jonah Stickney

Opioids are clinically invaluable analgesics during acute administration. Chronic use can unfortunately lead to abuse and dependence. Part of the reason why people sustain opioid use is to stave off the unpleasant withdrawal symptoms. A highly supported theory for the mechanism of withdrawal is homeostasis imbalance: chronic use of high-dose opioids causes a compensatory reaction in neurons, and subsequent cessation of drug induces withdrawal. Opioid-induced hyperalgesia (OIH) which increases pain or causes normal touch sensation to be perceived as painful, is a side-effect of chronic opioid use. In therapeutic settings, clinicians may respond to OIH by increasing opioid dosage if pain is the target symptom of treatment. As opioid dosage is a predictor for addiction potential, this increase in prescription could worsen outcomes. Experiments have revealed that OIH correlates with changes in both spinal (upregulation of NMDARs in the central glutamatergic pathway, increase in spinal dynorphin) and supraspinal domains (hyperactivation of neural populations of the rostro-ventral medulla(RVM)). These same changes have been implicated in opioid withdrawal, though withdrawal research has a larger focus on changes in the supraspinal RVM, periaqueductal gray, and locus coeruleus. This review will compare current research describing the mechanisms underlying both pain in opioid withdrawal and opioid-induced-hyperalgesia. If these are symptoms of the same process, it would implicate acute withdrawal as a moderator and target of intervention for the progression from chronic opioid use to abuse. Additionally, treatments uncovered in OIH research could be applied to ease opioid withdrawal symptoms.

Introduction

Opioids are an invaluable family of substances that for many, alleviate otherwise unmanageable pain. Due to their effectiveness, opioid prescription rates increased four-fold between 1999 and 2010. Unfortunately, the rate of deaths due to opioid overdose increased at the same rate.[1] This trend has continued to the present day, in 2018 there were 14,975 reported opioid overdose deaths in the United States.[2] This suggests that many people who end up suffering from opioid abuse started with a legitimate prescription for a surgery or pain problem which develops into an opioid use disorder (OUD). As many people with OUD continue to use in order to avoid withdrawal, the preferred treatment for OUD is to replace short-acting potent opioids such as oxycodone, heroin, and fentanyl with much slower acting opioids such as methadone or buprenorphine, followed by a slow tapering of the long-acting opioid.[1] This strategy is called medication-assisted treatment or MAT. MATs not only prevent withdrawal, but they also stabilize patients' extreme highs and lows which minimize the morbidity caused by opioid abuse. MATs are far from perfect, but they are a viable solution for many struggling with OUD.

MATs are effective at reducing opioid abuse for a large population, but findings are inconsistent regarding MATs effectiveness at treating pain.[3] Some patients are able to manage pain during MAT with use of additional non-opioid painkillers or therapies such as acceptance and commitment therapy, while others find that their continuing opioid use actually exacerbates their pain instead of treating it.[3] This phenomenon is known as opioid-induced

hyperalgesia (OIH). Since OIH was first described in 1975, a broad collection of research teams have investigated possible mechanisms underlying OIH; including dysregulation of the central glutamatergic system in the dorsal horn of the spinal cord, increased systemic concentration of dynorphin, alterations in descending facilitation from the rostro-ventral-medial medulla (RVM) and long-term potentiation of the C-fibers that carry pain information.[4] As this is a shortened list of the theorized mechanisms underlying OIH, it is difficult to ascertain what mechanism is the best to target for the development of novel opioids that do not cause this increase in pain. This is where a cross-sectional analysis of OIH and opioid withdrawal research may be useful.

Occam's razor has led to a robust model of what causes withdrawal: chronic use of a substance leads to cellular adaptations (i.e. downregulation of substance receptors) in order to maintain holistic homeostasis. These cellular changes constitute tolerance, which causes more substance to be taken in order to achieve the same effect. Continued escalation of the dose eventually leads to dependence. When substance use is halted, the shift in homeostasis due to chronic use is what causes the negative symptoms of withdrawal. If this model is true, it implies that the process underlying tolerance in the presence of the substance is the same as the process that causes withdrawal in the absence of substance. Where does OIH fall into this equation, a symptom of withdrawal or tolerance? One of the symptoms of opioid withdrawal is hyperalgesia, possibly caused by changes in the spinal dorsal horn or descending RVM facilitation.[5] However, a study investigating OIH during MAT found a positive correlation between methadone dosage and severity of pain.[3] Methadone is known as an ultra-long lasting opioid with a half-life lasting 36 hours, which means that these patients may have been experiencing OIH while opioid was still in their system.[6] If true, this would imply that hyperalgesia during withdrawal (absence of substance) may function through a different mechanism than OIH (presence of substance). It is very possible this hyperalgesia is due to an interaction of various processes. As such, the following review will seek to describe these processes and if possible, determine if any are exclusive to either OIH or withdrawal-induced hyperalgesia.

Methods and results

A systematic literature search was performed on pubmed, accessed between 2/25/20 and 3/26/20. The search terms utilized were "opioid+induced+hyperalgesia" with "mechanism," and "opioid+withdrawal" with "mechanism." Articles published before 2010 were discarded as well as review articles. 22 OIH and 15 withdrawal papers were coded for 6 domains: model organism, pain treatment for OIH induction, Type of measure (i.e. behavioral, immunohistochemistry, real-time PCR, etc.), specific opioid, the withdrawal time between last opioid and measure, and major findings. A description of the literature analyzed is available in Table 1 post-references. For brevity of report, only the spinal and supraspinal findings will be discussed.

Discussion

In some studies, OIH is defined as an upregulation of C-fiber activity in the spinal cord.[7,8] C-fibers are a class of primary afferents that carry pain information from nociceptors into the spinal cord. The axons of C-fibers are unmyelinated, their somas reside in the dorsal root ganglion, and they synapse onto neurons in lamina I and II of the dorsal horn, which in turn carry pain information to the brain.[9] The mechanisms underlying long term potentiation of this pathway have been investigated presynaptically (increased activity of C-fibers themselves) as well as post-synaptically (neurons downstream of C-fibers have heightened responses). These mechanisms that lead to C-fiber pathway LTP have been detected using immunohistochemistry and RT-PCR to measure densities of proteins associated with LTP, as well as electrophysiology to measure the properties of LTP itself. Within the dorsal root ganglia, morphine injections over 5 days caused an upregulation of both BDNF mRNA and protein, while heroin self-administration

over 12 days increased P2X2 and P2X3 receptor expression.[10,11] Important to note is that P2X2 and P2X3 expression was upregulated after 8 days of spontaneous withdrawal, though thermal hyperalgesia had recovered to baseline at this time-point.[11] Antagonizing BDNF targets in the DRG effectively blocked OIH in rodent studies which suggests BDNF within C-fiber somas is necessary for potentiation.[10] Important to note is this study's use of behavioral rather than electrophysiological measures for OIH: animals may have desensitized to the paw pressure test while still experiencing heightened pain which would go undetected.[10] This underlies the need for new, more sensitive behavioral measures of hyperalgesia.[12]

In contrast to dorsal root ganglia, there is a larger body of evidence investigating changes *within* the spinal cord contributing to OIH. Following 5 days of morphine injections, the density of α2δ-1–GluN1 protein complexes and pre-synaptic NMDAR expression were both increased in the dorsal horn, though post-synaptic NMDAR expression was decreased.[13] This change in expression occurred after only 20 minutes of morphine washout suggesting that this LTP may have been associated with tolerance rather than withdrawal. A single injection of remifentanil increased annexin receptor density in the dorsal horn as well as NMDAR activity: this receptor expression remained elevated for 48 hours of spontaneous withdrawal, though the increased NMDAR evoked current only lasted for 120 minutes post washout.[8,14] This is further evidence of opioid-induced modulation of protein expression persisting after detectable hyperalgesia. Hyperalgesia in two of these studies was reversed through separate administration of α2δ-1 and annexin antagonists. This supports the dorsal horn's involvement in hyperalgesia both with (annexin) and without (α2δ-1) long-term withdrawal. LTP of the C-fiber synapse in the dorsal horn may be also dependent on the rate of opioid cessation, one study found. Following one hour of remifentanil infusion, C-fiber field potentials were increased only with abrupt termination of remifentanil but not a tapered infusion.[7] This is strong evidence that OIH does not occur in the absence of withdrawal. Most of these OIH studies attribute L-fiber LTP to modulation of glutamatergic receptors, but changes in the activity of non-glutamatergic receptors within the spinal cord may also contribute to hyperalgesia. A study using dorsal horn cells *in vitro* found that B2-Adrenergic receptors may modulate hyperalgesia by heterodimerizing with morphine receptors to regulate their internalization.[15] This was followed by a study *in vivo* that found co-administration of morphine with a B2-AR agonist increased behavioral hyperalgesia while co-administration with a B2-AR antagonist reduced hyperalgesia.[15] Another receptor that may be involved spinally is the 5-HT3 receptor. A study found that the hyperalgesia induced with morphine injections over 4 days was reversed with an intrathecal but not intraplantar injection of the 5-HT3R antagonist ondansetron.[16] Although the 5-HT3 may be a useful target for treating hyperalgesia, it might not be involved in the natural process of opioid hyperalgesia.

Changes in protein expression not only occur in the spinal cord to upregulate the effects of C-fibers, they also occur in the brain during hyperalgesia. These changes in brain protein expression are comparatively sparse in opioid withdrawal research. In an intensive rodent study, phosphorylation of ERK2 (*p*-ERK2) was found to correlate with the mechanical hyperalgesia that followed 5 days of spontaneous withdrawal from high-dose fentanyl while *p*-ERK1 did not.[17] The study was thorough as the research team found a systemic signaling pathway of *p*-ERK2 that correlated with hyperalgesia. A MEK inhibitor which prevents ERK2 phosphorylation was injected into the central nucleus of the amygdala and the spinal cord. Astoundingly, both reversed not only behavioral hyperalgesia, but also normalized LTP induction following high-frequency stimulation.[17] In the analyzed opioid withdrawal literature, GluR1 expression in the amygdala, as well as adenyl cyclase and D2R expression in the striatum correlated to different aspects of withdrawal, but none of the analyzed withdrawal papers mentioned ERK2 supraspinally.[17,18,19] This would be a good target for future withdrawal studies. Protein expression in the amygdala may be especially important for OIH as it is not only associated with

hyperalgesia through *p*-ERK2 activity, but in a separate study, rats who maintained self-administration behavior of morphine were more likely to have increased GluA1 expression in the amygdala over rats who ceased self-administration.[18] Another study that implicates the amygdala's role in hyperalgesia consisted of fMRI brain imaging following a single 30-minute administration of remifentanil to humans. Note that any participants with a pre-existing pain condition were excluded from the study which is a clinical population of interest for treating OIH. Self-reports of thermal pain were not altered by this acute remifentanil withdrawal, though fMRI activity during thermal pain was significantly increased in the posterior-insula, the thalamus contralateral to stimulus and bilateral amygdalae. Activity in descending pathways from the rostral-ventral medulla was also increased.[20] The amygdala may be a site of importance for integrating affective and physical pain sensations.

This literature review has concretely shown that opioid-induced hyperalgesia is a symptom of opioid withdrawal, though it is still not certain if OIH may occur in the absence of withdrawal. Although LTP in the dorsal horn was detected after only 20 minutes of washout, another study found that 3 washouts after morphine administration (no time period listed) had a comparable effect to naloxone-induced withdrawal, which may imply this 20-minute washout could have induced acute withdrawal.[13,21] OIH being only a symptom of withdrawal is supported by the fact that only abrupt and not gradual cessation of opioid-caused hyperalgesia.[7] Thus far, opioid-induced hyperalgesia has not been reported with continuous perfusion of opioid, which would definitively classify OIH as a separate process from opioid withdrawal. The body of literature analyzed lacked mechanistic studies of opioid withdrawal in the brain that were correlated with behavioral assays. If research is intended to end with clinical translation, the assays used need to include a measure of the animals' behavior as restoring function is the goal for treating pain, opioid-induced or not. Furthermore, none of the withdrawal studies investigating mechanisms in the brain utilized a non-opioid pain source. This is an important factor in research as many people who suffer from OIH receive opioids for a pre-existing pain that is not associated with opioid use. Non-opioid pain was severely under-represented in the literature analyzed, with only 8 of 37 papers investigating OIH in surgery or inflammatory pain. These proposed directions in research would 1. Ensure that hyperalgesia was detected during opioid withdrawal and 2. Better define the various processes that contribute to opioid withdrawal, with or without a pre-existing pain condition.

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CAPSTONE

The role of the alpha 7 nicotinic acetylcholine receptor subunit in temporal processing, inflammation, and learning

Vicente Chavez

Introduction: alpha 7 subunit role in learning

Learning is a multifaceted mechanism that integrates a breadth of modules from our environments such as touch, vision, and sound. Complex sounds such as speech and music are all processed by different nuclei throughout the auditory pathway, traveling from the periphery where sound is captured to the auditory cortex for interpretation (Figure 1). Disruptions throughout the transmission pathway may result in a broad range of auditory processing disorders, many associated with neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) or Asperger's syndrome.[1] Detection of ASD continues to increase in response to clearer diagnostic technology and procedures.[2] Research suggests a link between these pervasive developmental disorders and auditory processing disorders that impede one's ability to form speech and in turn face communication obstacles.[3] School aged children receive clinical tests that are not able to detect neuropathies such as central auditory processing disorders.[3] Tests include raising one's hand in response to a noise and detecting which ear a noice came out of. These children may experience long term developmental complications in academia and social life.[3] A group significantly affected by these identification gaps include people with ASD. Brain regions implicated in ASD also are involved in auditory processing and memory, both important for auditory learning. Many neurodevelopmental disorders such as ASD have an underlying dysregulation of inflammation.[4] A promising pharmaceutical target for these inflammatory regulation includes the nicotinic acetylcholine receptor because of its ubiquitous roles in the peripheral and central nervous systems as an immunomodulator. Specifically, alpha 7 subunit acetylcholine receptors serve a vital role in our cholinergic anti-inflammatory pathway and can lead to neurodegenerative diseases if immunomodulatory function is chronically disrupted.[5] I emphasize the role of alpha 7 subunit (alpha 7 nAChR) in inflammation, central auditory processing disorders (APDs), and auditory learning. Current research on these subunits and their relationship with inflammation and auditory processing focuses on treatments to reduce inflammation and receptor regulation to increase auditory processing and increase learning outcomes.

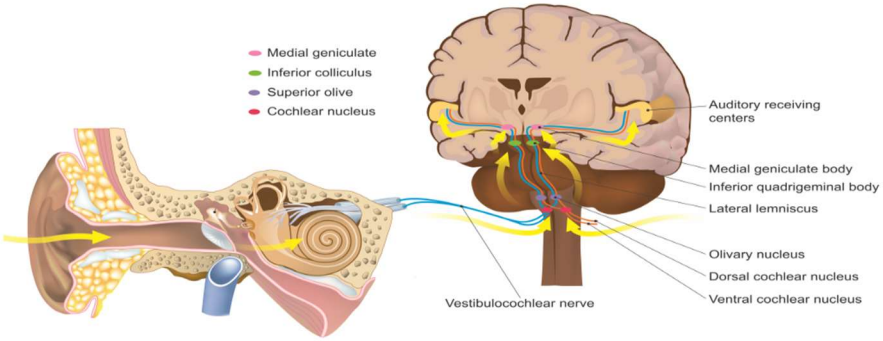

Figure 1: Auditory pathway and nuclei that process information starting in the periphery (ear) and leading into our auditory cortex.[6]

Alpha 7 nAChR knockout decreases auditory processing precision

Auditory processing is complex and neural networks responsible for processing sound begin developing before birth.[7] Processing complex sounds relies heavily on precise timing of sounds in our environment for proper encoding to occur and small millisecond changes can distort perception.[8] Hearing loss in children can lead to disrupted speech production and learning difficulties.[7] The auditory cortex neural projections of normal-hearing children and those born with congenital deafness have distinct observable differences. If children are born with hearing loss or other neurodegenerative disorders that effect auditory processing, then those brain regions affected undergo structural plasticity.[9] However, if auditory processing dysfunction is identified early on before critical periods of development, then treatment such as cochlear implants can be applied in hopes of preserving the auditory cortex neural projections.[9,10]

Nicotinic acetylcholine receptors are abundant throughout the central and peripheral nervous system and important in neurodevelopmental signaling.[11] Specifically, the alpha 7 nAChRs in the central nervous system are of interest because of their role in auditory learning. Research has identified degraded temporal processing of auditory information in the midbrain and brainstem in alpha 7 knockout mice.[12] This degraded signaling can result in social and cognitive challenges for developing children.[13] The effected nuclei include the inferior colliculus (IC), superior paraolivary nucleus (SPON), and ventral nucleus of the lateral lemniscus (VNLL).[13] The IC has an important role in auditory integration encoding semantics of complex sounds. Previous studies have linked alpha 7 KO mice with maturation delays potentially due to high levls of alpha 7 expression in early development in the VNLL which send signals to the IC.[14] The mechanism that inhibits precise timing is unknown, but a clear link between alpha 7 nAChRs and timing precision exists. Precise temporal processing results in proper speech formation and early age disruptions can result in learning impairments.

Alpha 7 mediated cholinergic anti-inflammatory pathway

Our immune system plays a vital role in the background of all biological processes. The body is a war zone and the immune system is the military. The frontline soldiers include macrophages and microglia primed to engage with all internal and external threats with an arsenal of cytokines. Cytokines induce different immune responses such as pain and inflammation. Tight inflammatory regulation exists maintaining homeostasis between pro-inflammatory and anti-inflammatory agents. Inflammation dysregulation in the central and peripheral nervous system is associated with a wide range of neurodegenerative disorders, most notably ASDs, multiple sclerosis, and Alzheimer's Disease.[15,16]

Studies into the inflammatory mechanism behind macrophages have found alpha 7 nAChRs as the key inflammatory mediator.[17] Findings demonstrated mice without alpha 7 receptors failed to inhibit macrophage tumor necrosis factor (TNF) release upon vagal nerve stimulation.[17] Since these findings numerous studies have confirmed the alpha 7 presence in macrophages and has inspired research into alpha 7 agonist as anti-inflammatory agents. Alpha 7 receptors in macrophage serving an immunomodulatory function provided a template for the search of a central nervous system receptor in microglial cells and isolated alpha 7 nAChRs to also mediate similar inflammatory pathways.[5] Further investigations found microgial cells to have the ability to modulate TNF release evoked by lipopolysaccharides through activation of alpha 7 nAChR.[5] Central APDs can be a result of inflammatory lesions due to chronic inflammation.[18]

Binaural fusion occurs at the pons effectively integrating information received from both ear pathways that are encoded at the IC and SPON. This intricate and delicate integration of auditory information has been implicated in spatial detection deficits as a result of brainstem

lesions inducing chronic inflammation.[19] The endogenous mechanisms are not well understood, but alpha 7 nAChRs serve as a promising pharmaceutical target to treat inflammation.

Future treatments and conclusion

Control of the cholinergic anti-inflammatory pathway present in the central and peripheral nervous system is a promising pharmaceutical target for anti-inflammatory control. It is clear that alpha 7 nAChRs are main modulators in the cholinergic anti-inflammatory pathway, but the underlying mechanisms are not well understood.[20] Conflicting studies have results that are inconsistent with nicotinic binding and sometimes contradict what has been observed *in vivo*.[20] For example, ulcerative colitis with nicotine or other nicotinic ligands have mixed results. Also, nicotine has a relatively low selectivity for a variety of nicotinic receptor subtypes confounding contributions and results of alpha 7.[20] This a potential result of nicotine synergistic signaling because nicotine has a relatively low selectively for a variety of nicotinic receptors, a highly selective ligand should be explored to test efficacy.[20]

Similarly, to technology made for heart pacemakers, exploration of vagus nerve stimulators to attenuate macrophage mediated inflammation is warranted.[21] Vagal nerve stimulation has been found to mediate microglia morphological changes that may provide insight into the underlying mechanism by further investigating the signaling cascade that leads to these changes. In conclusion alpha 7 nAChRs serve a central role in auditory processing and the anti-inflammatory pathway in macrophages and microglia. This may result in inflammatory lesions resulting in APDs inhibiting learning. Further research into the underlying mechanism of microglia anti-inflammatory pathway are needed.

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CAPSTONE

The interactive role of Aβ plaques and tau pathology and their effects in Alzheimer's disease

Petru Buracioc

Introduction

Alzheimer's disease (AD) is a neurological disorder that affects nearly 50 million people worldwide.[1] This disease is the leading cause of dementia which is characterized by cognitive impairment and memory loss.[2] Individuals with AD suffer from behavioral changes and inability to recognize familiar faces as a result of neurodegeneration. In the United States, AD affects over 5 million adults and it is expected to increase up to 14 million by 2050 (CDC). The complexity of this disorder has made it difficult for therapeutic drugs to successfully eliminate the symptoms of AD or slow down its progression.

AD is characterized by extracellular amyloid-β (Aβ) plaques and intracellular tau tangles but the relationship between the two pathologies are poorly understood.[3] Aβ plaques develop early during AD development and tau aggregates later in the stages of the AD. The amyloid cascade hypothesis suggests that the deposition of amyloid-β peptide in the brain sets up a series of pathological events that lead to neurodegeneration and Alzheimer's disease. Such series of events include the possibility of Aβ plaques facilitating the aggregation of tau tangles which eventually leads to neuronal loss and cognitive impairment in AD. The amyloid cascade hypothesis has not been proven by studies examining the interaction between the Aβ plaques and tau pathologies using transgenic mice overexpressing human tau.[4] Also, the accumulation of Aβ in the brain is poorly related to cognitive decline in AD patients, therefore the amyloid cascade hypothesis that can facilitate the over phosphorylation of tau should be considered. However, evidence does suggest that genetic mutations in amyloid precursor protein are associated with AD that can trigger other pathologies such as tau tangles and cholinergic dysfunction. A recent study found that Aβ plaques do in fact facilitate an environment that induces the formation of tau aggregates initially detected as tau aggregates in dystrophic neurites followed by the formation of neurofibrillary tangles.[5] This finding is a breakthrough in AD research as it relates the two main contributors of AD by understanding the progression and timeline of AD, early treatments can be developed targeting different stages of AD and potentially slow down the progression of AD and increase the age of symptom onset in AD patients.

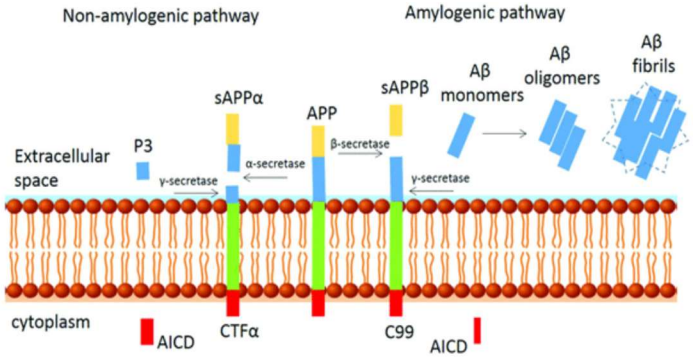

Figure 1: The proteolytic process of APP (amyloid precursor protein) in the production of Aβ by cysteine proteases and secretase activity.

The reduction of Aβ accumulation in the brain is the center and the ultimate target of the amyloid hypothesis. Several antibodies that targeted regulators of APP proteolysis to block the β- or γ-secretase pathways or increase α-secretase activity to reduced Aβ production (figure 1) were tested heavily in clinical trials. These studies failed to improve cognition in AD patients and were terminated. Also, reduction of over phosphorylated tau protein (tau tangles) has been successful with protein capped cadmium nanoparticles.[6,7] Understanding the mechanism of AD such as the interaction between Aβ and tau pathologies can aid in development of drugs that will target early stages of AD.

The amyloid cascade hypothesis and its effects on the brain

The amyloid hypothesis received substantial attention from the academic community and pharmaceutical industries for the past two decades.[1] Mounting evidence indicates that Aβ accumulation plays a key role in AD progression. For instance, the β-secretase enzyme which is also called β-site APP cleaving enzyme I (BACE1) has been linked extensively to AD as it initiates the cleavage of APP generating abnormal Aβ fibrils. β-secretase activity is present throughout the body but significant increase in β-secretase activity was found in neurons of AD patients.[8] BACE1 knockouts studies showed that blocking BACE1 activity in APP-overexpressing transgenic mice diminished amyloid deposition significantly, suggesting that BACE1 is essential to amyloid formation.[9] However, more recent studies found that complete deletion of BACE1 in mice showed adverse effects such as impaired spatial reference and working memories.[10] One BACE1 inhibitor called Verubecestat was tested in AD patients and due to its adverse effects, such as aggravated cognition and was terminated in phase III clinical trials.[11] These studies confirm that complete obliteration of BACE1 and thus complete abolishment of Aβ has adverse effects in AD patients. Additionally, BACE1 knockout mice suggests that normal levels of Aβ is vital to normal functioning of the brain such as memory performance.[12] Another therapeutic that attempted to lower Aβ accumulation was a γ-secretase inhibitor called Semagacestat. This inhibitor reduced amyloid deposition but increased cognitive impairment in AD patients and its use was halted.[13] These results are in conjunction with other studies that showed Aβ is vital to physiological function and may be involved in neuronal growth and memory.[14] The failure to produce potent and efficacious therapeutics by targeting the inhibition of Aβ accumulation in AD suggests that therapeutics can be efficacious only when the Aβ accumulation is reduced to a critical level and not completely eliminated.

Aβ accumulation inducing tau pathology in AD transgenic mouse models

AD is characterized by both extracellular amyloid-β (Aβ) plaques and intracellular tau tangles but the relationship between the two pathologies are poorly understood. Aβ plaques develop early during AD development in the cortex and hippocampus while tau over phosphorylation takes place later in the stages of AD.[5,15] Initially, tau aggregates were found to form in the transentorhinal cortex and further spread to the hippocampal formation and neocortex.[16] Evidence supporting the missing interaction between amyloid-β and tau tangles is the tau mutation in chromosome 17 that causes autosomal dominant frontotemporal lobe dementia which is not accompanied with amyloid-β plaques.[17] However, this claim is insufficient to deny the amyloid cascade hypothesis because studies using transgenic mice models suggest the interaction with Aβ and tau leads to the progression of AD.

A study crossed JNPL3 transgenic mice expressing a mutant tau protein with Tg2576 transgenic mice expressing mutant ß-amyloid precursor protein (APP). When compared to the mice that only expressed mutant tau protein, the double mutant mice showed significant increase in AD-

like neurofibrillary tangles (NFTs) in the limbic system and olfactory cortex.[18] Another study inserted Aß fibrils into JNPL3 transgenic mice expressing a mutant tau protein and determined that Aß induced NFTs formation as fast as 18 days after the injection. Another similar study generated a triple transgenic model of AD (3xTg-AD) harboring three AD mutant genes: APP, preselin 1, and tauP301.[19] In this study amyloid-β deposition was identified prior to the expression of NFTs supporting the amyloid cascade hypothesis that beta amyloid facilitates formation of NFTs and other tau pathologies. A more recent study injected human derived tau fibrils into the hippocampus and the cortex of mice with knock in mutated APP genes.[5] The study found that amyloid-β plaques do in fact facilitate an environment that promotes the development of tau tangles. These tangles initially form as tau aggregates in dystrophic neurites surrounding Aβ plaques which then spread throughout the brain.[5] Tau pathology is considered a downstream event in the amyloid hypothesis suggesting that it is responsible for significant neuronal damage and cognitive impairment.[13]

Conclusion

The amyloid cascade hypothesis is still debatable in AD research in part due to mounting evidence that suggests that amyloid beta is not entirely responsible for the progression of AD. Studies show that some individuals with elevated beta-amyloid accumulation do not suffer from impaired memory and learning. However, it is evident that mutations in the gene that codes for this protein might trigger other pathological events in AD patients that further damage the brain. Also, the inability to generate efficacious and potent drugs that can reduce amyloid accumulation without adverse effects is considered evidence against this hypothesis. Multiple studies using sophisticated mouse models with more than one gene mutation suggest that this hypothesis may induce tau pathology and causes neural loss. On the other hand, evidence suggesting that there is no link between amyloid deposits and tau pathology is insufficient and lacks physiological studies. Expansion of existing research generated from 25 years of amyloid focused research should further investigate the interaction between amyloid deposition and tau pathology utilizing existing successful transgenic mice models. First and foremost, the transgenic mouse models used in studying AD pathologies need to be reexamined as failed clinical trials suggested that AD pathologies represented in humans differ from these mouse models where drugs that seemed to significantly increase cognition in mice did not improve cognition in AD patients. One question that has not been answered by amyloid AD research is where in the when in the stage of AD would the amyloid-β-directed therapeutic be most effective? To answer this question, studies need to investigate when in the stage of AD would the amyloid-β-directed therapeutic be most effective? Besides the interaction of amyloid with tau pathology, studies should investigate the interaction of the amyloid hypothesis with the cholinergic hypothesis as research suggests these two pathologies interact in different nicotinic acetylcholine receptors which causes cognitive impairment. Such evidence include agonists and positive allosteric modulators on nicotinic receptors alleviate cognitive impairments in mouse models.

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Alzheimer's disease: detecting pathophysiological and neuropathological changes with biomarkers

Elisei Cosovan

Neurodegenerative diseases such as Alzheimer's Disease (AD) affect more than 3 million people in the U.S annually. AD is a progressive illness that impairs normal brain function. Therefore, obstructing the formation of new memories and disrupting behavioral and social skills. Symptoms are typically not present at the time of onset, making the disease difficult to diagnose – let alone treat. The only way to currently detect this illness as of now is through histopathological techniques in post-mortem brains. With this limitation in mind, this paper reviews current research on biomarkers and how they can be applied to detecting the onset of AD *in vivo.*

Biomarkers are used to indicate physiological changes. As an example, a blood pressure cuff can be considered a biomarker because it is able to detect changes in blood pressure. So far, the focus in research has been on cerebral imaging biomarkers. More specifically, on amyloid-beta proteins – which form the plaques that are principal to the development of AD – and on phosphorylated tau – a principal component of neurofibrillary tangles.[1,2,3] The reason why these two cerebral spinal fluid (CSF) biomarkers have drawn the most attention is due to their role in predicting cognitive decline in healthy people.

Yet even with the ability for these biomarkers to identify neuropathological changes in AD, there are some downsides to using them in clinical trials, and eventually, in patient care. CSF biomarkers may provide scientists with inaccurate results. In recent studies, CSF biomarkers were able to detect plaque buildup and neurofibrillary tangles, but were not accurate enough to provide the researchers with enough information to indicate whether the disease present was AD or another form of dementia.[4,5] CSF biomarkers are also too invasive to be added to routine clinical use, as they require lumbar puncture. In addition to its invasiveness, CSF biomarkers are just too expensive to be used in the general population screening.[6]

Blood-based biomarkers on the other hand, hold a more promising future for identifying AD as they may be able to detect pathophysiological and neuropathological changes more efficiently than CSF biomarkers. Such biomarkers can be used through epigenetic mechanisms since they modify gene expression, but do not affect the DNA code.[7,8] These biomarkers work similarly to the CSF biomarkers but are less invasive because they are contained in the plasma of the blood. This means that the blood-based biomarkers can be used in screening patients when blood is collected. In a similar manner to how the blood is tested for syphilis upon extraction, the patient would also be tested for AD. Current studies also show that blood-based biomarkers can be used to predict progression in both, disease and prodromal states – making them good candidates for early diagnosis of AD.

One blood-based biomarker that can be used in detecting AD is miRNA. miRNA regulates mRNA translation through complimentary binding, therefore blocking the protein expression.

miRNAs work by dysregulating beta-site amyloid precursor protein-cleaving enzyme 1 and by regulating Hirano bodies. Hirano bodies consist of actin, cofilin, and granulin – proteins which are uniquely seen in the progression of AD. miRNAs are also tissue and cell specific, which means they are disease-specific as well. They are not only found intracellularly, but extracellularly as well. Hence, being located in the blood, these miRNAs can be drawn out of the live body when the blood is screened.

So far, twenty-six studies on mice mimicking AD symptoms have identified at least one miRNA which is expressed significantly differently in AD cases compared to the control cases. Of the 8,098 miRNAs measured, 395 were found to be considerably associated with AD. The most consistent and extensively studied miRNA is miR-107.[8] This miRNA is the strongest candidate as it has been shown to decrease in AD throughout all trials. As miR-107 decreases, the number of proteins associated with AD increase. In a study by Wang and a team of researchers, published in *The Journal of Neuroscience* showed through a one-way ANOVA test that miR-107 significantly decreased in all groups tested, with a p-value of 0.014 (indicating that it significantly decreases in the AD groups compared to the control groups). Particularly, mi-R107 levels decreased significantly in non-demented patients lacking pathology, in comparison to those with mild cognitive impairment. This correlation was represented by a p-value of 0.008. This data suggests that miR-107 is expressed in the earliest stages of AD.[9] From the data in this experiment, and from other such studies, miR-107 can be considered a strong candidate for further testing.

Although blood-based biomarkers hold a promising future for detection of early stages of AD, there are a few limitations to these studies.[10] So far, there are no standardizations between studies. This means that the way each biomarker is tested for effectiveness and efficiency can vary from study to study. Furthermore, Receiver Operating Characteristic curves (ROC curves), which are used by researchers to evaluate how useful certain measures are in predicting binary outcomes (AD cases, control cases) can be biased. ROC curves can be cut and specified to a certain selection of data. The ROC curves must be homogenized across all studies to improve accuracy and reliability of results. For future studies, the multiple testing used by some studies needs to be adjusted. Failure to adjust for multiple testing leads to an increased risk of a type I error. A type I error is considered a report in which an association has not occurred. Additionally, future studies will depend on how accessible large samples will be. Larger samples will allow future studies to obtain more specific and accurate results – increasing the likelihood of discovering a blood-based biomarker that can enter clinical trials.

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CAPSTONE

A literature review of interleukin-6 family cytokines regulation of oligodendrocytes following demyelination

Maribel Garcia-Igueldo

Multiple sclerosis (MS) is the most common of the demyelinating diseases affecting more than 1 million diagnosed patients worldwide.[1] Twice as many women as men are diagnosed with MS, and initial clinical presentation is typically between the ages of 15 to 55. The chronic immune-mediated disease leads to inflammation, demyelination, and eventually axonal loss in the central nervous system (CNS). Presentations of MS are wide-ranging and include weakness, tingling and numbness, vision problems, balance problems, bladder issues, sexual dysfunction, pains and spasms, and cognitive difficulties. MS follows a relapsing and remitting course of action. However, MS can be progressive and the intervals between relapses are inconsistent. The etiology of MS is complex, with interactions between multiple genetic and environmental factors attributed to its development.[2] While there is no cure for MS, it has been proposed that remyelination can protect against axonal loss.

One mechanism implicated in the restoration of remyelination is ciliary neurotrophic factor family cytokine (CNTF) activation of adult oligodendrocyte progenitor cells. Oligodendrocyte cells mature through distinct stages of development via growth factor and cell fate signaling pathways. Upon maturation, adult oligodendrocytes maintain the myelin sheaths that wrap axons.[3] CNTFs have been reported to promote oligodendrocyte survival and differentiation in experimental models of demyelination.[4] Although endogenous factors produce CNS neurons and glia, progenitor cells cannot exclusively replace cells and restore normal functioning in MS patients. Therefore, it is important to identify exogenous factors that increase the progenitor cell's response to inflammatory myelin destruction.[5] Such factors have been studied because of their therapeutic potential for slowing the progression of demyelinating diseases, which can be determined by measuring the time elapsed between exacerbations. In early-onset MS, researchers found the mean duration of the first and second remission to be 71.32 and 58.07 months, respectively.[6] Existing immunomodulatory treatments reduce the interval between relapses by merely 60%.[7] This review explores the signaling pathway by which interleukin (IL)-6 family cytokines regulate oligodendrocytes to protect against the progression of MS.

Activation of jak-dependent stat3 signaling pathway

IL-6 family cytokines include CNTF and leukemia inhibitory factor (LIF).[8] The binding of CNTF or LIF to the IL-6 receptor triggers the association of the IL-6 and signaling subunit glycoprotein 130 (gp130). Stimulation of the receptor complex induces the dimerization of the gp130 subunit which activates associated Janus kinases (JAKs). Activated JAKs phosphorylate tyrosine on the cytoplasmic region of the gp130 protein.[8] Tyrosine-phosphorylation of the gp130 subunit attracts the SH2 domain of signal transducer and activator of transcription 3 (Stat3), which is necessary for receptor association and tyrosine phosphodimer formation.[9] The phosphorylated Stat3 dimer is then imported into the nucleus via importin-α/importin-β1-Ran-mediated active transport.[10] In the nucleus, Stat3 activates gene expression which has been implicated to regulate oligodendrocytes and promote remyelination.[4] Therefore, the JAK-dependent Stat3 signaling

pathway has been studied in experimental models of demyelination. Previous studies used the western blotting technique to determine levels of Stat3 and phosphorylated Stat3 in the developing rodent brain. According to Steelman et al., Stat3 was expressed in the cerebrum of rodents from embryonic day 16 to postnatal day 30, and phosphorylated Stat3 was also expressed starting on postnatal day 13. Expression of myelin basic protein was also evident starting on postnatal day 13, indicating that Stat3 activation correlated with myelinogenesis in the developing rodent brain.[4] Furthermore, postnatal day 14 rodent brain sections immunostained for Stat3 and oligodendrocyte marker CC1 revealed their colocalization in the cingulate gyrus and corpus callosum.[4] The evident colocalization of Stat3 and oligodendrocyte cells, correlated with myelinogenesis, points to the regulation of oligodendrocytes via the Stat3 signaling pathway. In another study, Nobuta et al. wanted to identify the role of reactive astrocytes in the loss of premyelinating oligodendrocytes during development.[11] Their study found that STAT3 was activated in postmortem brains of neonatal white matter injury, which is characterized as loss of premyelinating oligodendrocytes. Nobuta et al. also used an experimental mouse model for neonatal white matter injury to ablate STAT3 in reactive astrocytes and identify molecular changes. The STAT3-deficient astrocytes promoted the production of transforming growth factor beta 1 in microglia, which the study found functions to delay oligodendrocyte maturation, and essentially impedes myelination.[11] In addition, it is important to consider limiting factors of the Stat3 signaling pathway. One endogenous inhibitor of the Stat3 signaling pathway is the suppressor of cytokine signaling 3 (SOCS3).[12] Ablation of SOCS3 enhanced axonal regeneration via the JAK-dependent Stat3 signaling pathway.[12] Based on the literature, reactive astrocytes may modify the STAT3 signaling pathway to promote oligodendrocyte maturation, which is important for myelination during development.

Regulation of oligodendrocyte survival and differentiation following demyelination

It is important to identify exogenous factors that activate the JAK-dependent Stat3 signaling pathway and lead to the regulation of oligodendrocytes survival and differentiation in experimental models of demyelination. Slaets et al., used western blotting analysis to determine the levels of Stat3 and phosphorylated Stat3 following exogenous LIF treatment in mature oligodendrocytes.[13] The expression of phosphorylated Stat3 increased 10-fold in the oligodendrocyte culture that was treated with LIF for 10 min, compared to the culture in the control condition of the study.[13] Another study interested in the relationship between exogenous CNTF treatment and oligodendrocyte viability measured changes in cell count after 2, 4, and 6 days of treatment, as well as the level of phosphorylated Stat3. The results showed that CNTF treatment activated Stat3 and significantly increased the number oligodendrocyte cells in vitro.[4] The effect of LIF treatment on oligodendrocyte viability is consistent with the effect of CNTF treatment. Butzkueven et al. were also interested in the relationship between an exogenous LIF treatment and oligodendrocyte viability.[14] The study found that, compared to the control condition, oligodendrocyte viability was significantly reduced in the induced demyelination condition. However, oligodendroglial survival was potentiated and baseline conditions were restored in the LIF treatment condition following induced inflammatory demyelination.[14] Finally, researchers have shown that LIF treatment enhances myelin protein expression and node of Ranvier formation.[5] One study used an immunostaining assay to assess myelin protein expression in the hippocampal CA3 region of mice under different conditions. The study found that mice in the 12-week demyelinating condition showed a reduction in myelin protein expression compared to the untreated condition. While mice in the 12-week demyelinating condition followed by 6-weeks of LIF treatment showed restoration of baseline myelin conditions.[5] In addition, LIF impacts other cell types, such as astrocytes, to produce factors which stimulate oligodendrocytes.[15] Albrecht et al found that oligodendrocyte progenitor cells

treated with CNTF did not proliferate in greater numbers than cells not treated with CNTF.[15] However, the researchers found that CNTF treated astrocytes released mitogens for oligodendrocyte progenitors which increased their viability compared to the control condition in the study.[15] In overview, experimental models of demyelination treated with either LIF or CNTF increased phosphorylated STAT3 to potentiate oligodendrocyte viability and restore baseline myelin conditions.

Conclusion

Previous studies have shown that IL-6 family cytokines regulate oligodendrocytes following demyelination via the JAK-dependent Stat3 signaling pathway. In experimental models of demyelination, CNTF binds to the low-affinity receptor complex IL-6R/gp130/LIFR.[16] Stimulation of the receptor complex then activates the JAK-dependent Stat3 pathway which changes gene expression in the cell to promote oligodendrocyte survival and differentiation. In demyelinating diseases, such as MS, oligodendrocyte progenitor cells cannot exclusively replace cells and restore normal functioning in patients.[5] Therefore, it is important to identify exogenous factors that increase oligodendrocyte progenitor cells' response to inflammatory myelin destruction. CNTF and LIF treatments in rodent brains have been shown to increase oligodendrocyte viability and restore myelin following demyelination. The mechanism responsible for oligodendrocyte proliferation following changes in gene expression induced by Stat3 has not been studied extensively. Future research should focus on the induced changes in gene expression via the JAK-dependent Stat3 signaling pathway in the CNS of experimental models exhibiting demyelination. In addition, LIF treatment in the CA3 stratum radiatum of the hippocampus has shown to enhance $Na_v1.6$ and Caspr restoration.[5] However, the mechanism by which the sodium channel $Na_v1.6$ and paranodal protein Caspr are restored has not been clearly identified, making it an avenue for future research. Overall, exogenous CNTF and LIF treatments might be of therapeutic interest to promote remyelination in MS patients.[17]

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CAPSTONE

Structural and functional prion malfunction as a catalyst for Alzheimer's disease

Victor Cosovan

Introduction

Alzheimer's disease (AD) is a form of dementia which is described by memory loss and impaired cognitive behavior. Diagnosed by a mini mental status exam (mMSE) and detailed patient history to monitor changes in behavior and memory function.[1] AD is an idiopathic neurological disease which is only diagnosed from an array of signs and symptoms, then a final post-mortem autopsy to confirm the pathology. One study found that in AD autopsy brains, there was a severe and consistent loss of nicotinic receptors.[2] One experimental study found that Amyloid-β binds to α7-nAChRs and modulates their function. Aβ and amyloid plaques indicate that nicotinic receptors play a role in the pathogenesis of AD.[3]

Prions (PrPC) are genetic protein-like structures found in the central nervous system. PrPc manipulate transcription factors and proteins within neurons by conformational conversion of protein structures. PrPc function by activating or deactivating proteins and transcription factors through self-propagating actions.[4] PrPCs function in regular cell maintenance, like Amyloid-β and TIA-1 prions. They affect learning and memory in the hippocampus, "prion-like, self-templating mechanisms also underlie a variety of neurodegenerative disorders, including amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease."[5] PrPC have a rich α-helical structure and small β-sheet content, but the disease-causing isoform (PrPSc) have small α-helical structures and are rich in β-sheet content.[4] Functional prions are likely to have a distinctive regulatable structure. The prion domains are stacked together, exposed, and free to bind mRNA on the surface of the β-sheet. This structure can allow the coordinated translation of the population of interrelated mRNAs required for stabilization of synaptic growth.[6]

Memory storage and synaptic plasticity are regulated by prions assembling into aggregates. These aggregates form into functional prions which regulate memory storage.[7] It is through gene expression that AD is ultimately developed in the hippocampus. Cholinergic dysfunction in the hippocampus, including reductions in nicotinic acetylcholine receptors is a main killer of neurons in AD brains. Using immunohistochemistry, Teaktong found that nAChRs, when malfunctioning, they produce a cytotoxin which leads to astrocytes building up plaque in neurons, "Elevated α7 nAChRs on astrocytes in AD may contribute to alterations in calcium homeostasis and nitric oxide production, which in turn could affect β-amyloid–mediated inflammatory processes in AD."[8]

AD in the hippocampus

Many studies show that AD initially starts in the hippocampus with onset memory impairment.[9] The disease causes damage by disrupting the regular cell function, one degenerating effect is damage to nAChRs. This damage is linked to regulation of nAChRs by functional prions like amyloid-β. Eventually, there is enough damage to trigger apoptotic function through TIA-1 prions activating P53 transcription factors, then neuroinflammation kicks on. The study published in the May, 2019 issue of Science Translational Medicine, found important data that the two leading proteins associated with AD, amyloid beta and tau were seen to act as prions, thus making it a

potential double-prion-initiated disease.[5] The early stages of AD are from the hippocampus and they propose multiple mechanisms to explain potentially why AD may originate from the hippocampus. The two main theories are, dysfunctional neurogenesis could increase neuronal vulnerability to AD and contribute to memory impairment. The second theory is that enhanced neurogenesis is a compensatory response, simply a repair mechanism for the brain.[10] Literature shows that Ad progression starts from the hippocampus. That it is why one early symptom of AD is impaired memory and memory loss. Due to a lack of synaptic plasticity, there is a clear loss of new memory formation and consolidation for other mechanisms of memory formation.

Prion malfunction in the hippocampus

Prions play a critical cellular physiological role, specifically in the hippocampus, PrPc play a role in synaptic plasticity and memory formation. Healthy prion function in the hippocampus is critical for cell physiology. However, when a prion has a folding fault, it can spark a cellular cascade of pathogenesis. There is a study which focused on the genetic mechanisms of prion infection in the hippocampus, specifically CA1 neurons. They found that prion replication results from continual stimulation of a programmed response that is modulated by synaptic NMDA receptor activity that initially promotes cell survival and neurite remodeling.[11] Hippocampal learning and long-term memory retention occur from prion regulation. This study found that PrP^{c} are needed for latent learning and long-term memory retention by knocking out prion genes in mice.[12] Prion disease in the hippocampus was studied and found to attack the mitochondria of the hippocampal cells which decreases synaptic plasticity and regular receptor function.[13] Flies with Orb2 deletion fail to show characteristics of long-term memory tasks and functions.[7]

Conclusion

AD starts in the hippocampus, due to faulty nAChRs. Because of these faulty α7 nAChRs, amyloid β released binds to the receptor and causes a cellular cascade of damage leading cell death. That is why regulatory prions such as TIA-1, activate to start the process of programmed cell death in response to damaged receptors and transcription factors.PrPC like amyoid-beta have faulty conformational changes turning them into PrPSc. This now structurally changed prion, is no longer able to properly alter transcription factors, thus when it binds proteins, the neurocytochemistry is wrong and leads to a halt in synaptic plasticity. It takes only one PrPSc that can change proteins and lead to additional proteins becoming PrPSc through propagating mechanisms. Furthermore, this cascade is what leads to cell death in the hippocampus and ultimately AD. Further studies are needed to investigate the relationship between prion function and malfunction in the hippocampus which is a proposed mechanism of the etiology of AD. Such studies can look specifically at the role of Aβ and amyloid plaques and prion folding faults. There is little known about functional prions in the central nervous system and they can potentially cause many neurodegenerative diseases. Prion malfunctioning and starting disease is theory of neurodegenerative pathology and there is a wide array of research needed to be done in order to further the claims of prionopathophysiology. When more studies can investgate the prionpathic properties of regular functioning prions in the CNS, new treatments and cures of neurodegenerative diseases can be discovered.

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Review on the use of 3D cerebral organoids and the current technical progress in their development

Gavin Harvey

Introduction

An organoid is an artificial mass of cells that operate like an organ. Neural organoids are organoids that model the central nervous system (CNS) and are also known as cerebral organoids. Research into these cerebral organoids is still in its infancy. With less than a decade of history being utilized, however; cerebral organoids are already becoming important for studying the progression of neurological diseases and even developing methods to treat them. This includes a wide range of neurodegenerative disorders like microcephaly, schizophrenia, ZIKA, autism, and Alzheimer's disease to name a few [8, 11, 14, 15, 16, 17]. The basis for this technology lies in the development of human pluripotent stem cells (PSC). PSCs. The modification of these PSCs leads to a variety of neural progenitor cells. From neural progenitor cells, monolayer or neural tubes can be studied in what is known as 2D culture systems. They allow for the relatively easy study of neural systems and very early embryonic modeling, as well as high-throughput screenings for a wide range of genomic testing. These 2D models however have their limitations when studying the longer-term development of diseases [2, 5, 7]. This is where 3D models (neural organoids) are necessary for modeling cell-to-cell and cell-to-extracellular matrix interactions [1, 9, 12]. These systems are currently however lacking important factors necessary for higher development like proper gas and nutrient exchange that are mediated by the vascular system. They also lack the complex glial cell integration necessary for cell guided migration, neuro-chemical control systems, and neurological immune responses. This review will focus on the current organoid design processes, the integration of vascular systems, and the development of glial cell differentiation.

Basics of an organoid

A neural organoid can be seen to have an organization in elaborate progenitor zones like the subventricular zone (SVZ) being split by an inner fiber layer [8]. This separates the SVZ and the outer subventricular zone (OSVZ) where intermediate progenitor and outer radial glia are found. This results in tissue that has clear cortical layer separation [9]. Further, large fluid-filled pockets can begin to form into ventricles and in some cases, retinal tissue forms [8]. Organoids also form distinct cerebellar areas like the forebrain, hindbrain, parietal, and occipital lobe. The cells of the organoids also begin differentiating into the hippocampus and ventral forebrain but more complete structural formation isn't normally attained [8, 9, 17]. Protocols exist for making 3D organoids form human PSCs and proceeding to the production of an embryoid body [8, 9]. These embryoid bodies are then maintained in a suspension fluid until they develop into neural rosettes, where they are then placed in a Matrigel droplet [8,9]. This Matrigel droplet acts like a mesoectoderm, allowing the neural progenitors to have a scaffold [1]. The progenitor cells, that have now matured into embryoid bodies, can use this scaffold to further develop the complex processes of neuronal migration, maintenance of apical-basal polarity, and neuronal stem cell differentiation [8,9]. These Matrigel droplets are then placed in a spinning bioreactor to better facilitate the perfusion of nutrients and gases in the organoids. Due to this perfusion organoids

have been shown to survive for months [18]. This basic model depends on the intrinsic signaling of the hPSCs. Some examples of this would be in the expression of FOXG1, EMX1, and AUSTS2 [8]. These are all molecules that signal the formation of specific regions and these molecules help decide cell phenotype expression. There is also glial cell signaling and metabolic modulation to consider, which aids the development of the organoid [10, 13]. This development continues until the organoid meets with growth inhibition due to the inability of nutrients to perfuse into deeper cell layers. This inability to perfuse nutrients to the core of the organoid results in necrosis of cells therein. However, further research has shown that this protocol can be modified with the addition of further extrinsic growth factors. These can modify the organoid to model specific regions of the brain such as the midbrain, hippocampus, cerebellum, and hypothalamus. These factors include LDN-193189 for the midbrain and A83-01 for the forebrain [7, 17]. In the focus of one article, forebrain organoid formation involved the use of a stem cell medium with Dorsomorphine and A83-01 added, but without FGF-2, for the first 4 days [17]. On days 5-6 this medium was changed to include DMEM:F12, 1X N2 Supplement, Heparin, Penicillin/Streptomycin, Non-essential Amino Acids, Glutamax, WNT-3A, CHIR99021, and SB-431542 and was used to maintain organoids for more than 100 days [17]. Other than developing organoids that model specific brain regions, neural organoid research has focused on modeling degenerative diseases with the goals of learning about disease etiology and developing potential treatments for them. This research has focused on various diseases including, but not limited to Alzheimer's, ZIKA, microcephaly, and the treatment of glioblastoma [8, 11, 14, 16, 17] . In the case of the ZIKA virus, organoids were used to provide evidence for the assertion that the virus had an effect on neuronal development. Some organoids were grown normally to represent the control group and two other groups were infected with 2 different strains of the ZIKA virus. What was determined was that there were specific developmental deficits expressed in the ventricular zone of the infected groups. These deficits were the statistically significant decreases in cortical layer thickness and cell proliferation count in the ZIKA infected organoids when compared to the non-infected organoids [17]. In the use of studying cancer, cultures of a patient's glioblastoma were implanted into neural organoids. In vitro tests then were performed to determine the efficacy of temozolomide and doxorubicin as treatments for the glioblastoma while not harming the neuronal cells [16]. These tests were also conducted when the organoids were surgically implanted in mice. What was determined was that doxorubicin was more effective in reducing the size of the glioblastoma, while also preserving the neural tissues. Something that was mentioned in the discussion was the inability to assess how well the perfusion of the organoids impacted the interaction between the glioblastoma and the drug interaction [16]. The lack of vasculature was said to likely change the behavior of glioblastomas, as they follow vasculature, using it to metastasize. Also, while the drugs used in the study were able to perfuse to the center of the organoid tissue, this process took between 6-12 hours and, had a vascular system been present to aid in the rapid delivery of the drugs, the interaction may have been different between the drugs and the cells.

Vascular integration

Vascularization is an area that is burgeoning and exciting in organoid technology. A few studies have been done by integrating and differentiating epidermal cells (derived from human embryotic cell-lines), which begin to make vascular epithelial tissue that innervated the organoids, followed by the implantation of the organoids in a lesioned mouse cortex. The lesion was performed by excising a small 1X1 mm square of neural tissue from the mice's brains. This has been used as a technique to observe how well-lesioned areas can adapt to implanted neuronal tissue [3]. Organoids have also been implanted into mice without any vascular system preparation, observing how the host's vascular system penetrated the organoid. The degree of integration of the organoids with the host system was reduced and there was a higher rate of rejection when compared to implanted organoids that had a pre-existing vascular structure [7]. It should also be

noted that the mice's microglia infiltrated the organoids, potentially altering the organoid's function. Recently, the incorporation of mesodermal progenitor cells in the Matrigel demonstrated the integration of an *in vitro* model with a more extensive vascular system. More extensive in terms of various cell types beyond endothelial cells, among which were pericytes, smooth muscle cells, a basal lamina, and blood vessel valves. An interesting finding was also that when the organoids were exposed to anoxic environments, around 2% oxygenation between days 1-7 of incubation, there was significantly more vasculature that was identified to have invaded the core of the organoid [19]. These vessels were tagged with CD31 fluorescent protein on αSMA and low-density lipoprotein (which occurs on smooth muscles and endothelial cells respectively) to identify the vasculature structure [19].

Glia

Glial cell formation appears to be inherently occurring in cerebral organoids [13, 18]. Using organoid models can offer insight into how glial cells aid in the development of the cortex [18]. In the past, it had been difficult to identify the degree of glial development in organoids[12,18]. This difficulty is due to the limitation in the development of organoids currently, which is akin to around the first trimester of embryonic development, as glial cells do not mature and differentiate fully. This does allow one the unique opportunity to culture astrocytes that have not yet acquired phenotypic specificity though, which can help study how astrocytes generally function [4, 5]. Difficulty had been previously expressed with proliferating and maturation of microglia in organoids due to dual-SMAD inhibitors. These dual-SMAD inhibitors are used as precursors to proliferating neural progenitor cells, but new research indicates that there is evidence of microglial maturation innately in organoids despite this obstacle [13]. Proper microglial development has also been an issue, as some microglia develop from non-ectodermal cell lineages outside the central nervous system, which has yet to be introduced into organoids [13]. This is important, as glia, in general, are vital for proper neurogenesis, synaptogenesis, metabolic modulation, and differentiation of neurons [13, 18]. Particularly, embryonic microglia in humans have a profound effect on maturation and development of neural tissue, especially in the subventricular zone [10]. Glial cells also have been shown to be important in studies focusing on mechanisms that affect tri-partied synapses in diseases such as Alzheimer's [14]. In a study conducted by Park. et al., it was important to see how glia were affected by Alzheimer's disease, as these reactions can further the processes that result in cell death. These factors included: neuroinflammatory activity that deleteriously affects neurons and astrocytes, neurotoxic activities that result in axonal cleavage, and nitrous oxide release that damages both neurons and astrocytes [14]. It is important to consider glial interactions in development, as proper development is not possible without them, and diseases that target glial cells, like multiple sclerosis, need proper glia cell interface to be accurately modeled. With an accurate model, one can determine mechanisms that lead to impaired function and ultimately develop methods to prevent or fix the problem.

Conclusion

Cerebral organoid development is still in its initial stages. There have already been strides toward more complex and well-developed models with further research in identifying more effective methods for introducing blood vessels and proper glial cell maturation and function, however; an artificial system that can induce blood flow and further glial interaction with the mesoderm, are necessary for further development in organoids. This blood flow, or the flow of the nutrient-rich incubation fluid (which contains glucose, 21 amino acids, fats, and oxygen among other nutrients), through the organoid is possible with the aid of a host animal but this can introduce a variety of variables that may cause complications. Further maturation of the neural organoid is also halted. A method that could overcome this involves integrating a peristaltic pump (in the same manner demonstrated in kidney organoids [6]) with the developing

mesodermal derived vascular tissue in a neural organoid. This would help circulate the incubation fluid in neural organoids and could lead to increases in maturation of the neural tissues. These steps are also necessary for better organoid transplant success as the tissues would undergo more changes that are like those developed *in vivo* [19]. These changes refer to the refining of the vascular system, due to the cellular responses to fluid movement and pressure in the vessels [19]. These organoids would be better developed and as previous research shows; this would aid in transplantation success in hosts [3, 6, 7, 18, 19]. This could aid individuals who have traumatic brain injuries in receiving organoids developed to replace the specific lost tissue. The lack of glia driven research is also something that needs to change in neural organoid research. While glial research has been receiving more attention in recent years, as recognition of their influence on neuronal function and development has become more and more evident, more glial focused research is needed to develop better neural organoids. This should be focused on embryonic microglia, specifically the non-ectodermal cell lineage glia that migrate into the brain before the closure of the blood-brain barrier. Current models lack these cells and organoid development could be improved with their integration. There is also a large lack of research in implementing a blood-brain barrier which requires astrocytes to interact with meningeal tissue. By adding meningeal precursor cells to the matrigel, further research could be done on the interaction of astrocytes and the meninges in the formation of the blood-brain barrier. The interaction between the meninges and astrocytes may have a further developmental role in shaping neural proliferation, which has yet to be researched in organoid models. By better modeling the human brain, neural organoids could become invaluable assets in understanding the biology of human brain development. This could lead to growing tissue replacement therapies, better treatments for debilitating neurological diseases, and may even help with improving neuro-computational interfaces.

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CAPSTONE

The neurological mechanisms of mindfulness meditation

Jessica Faraca

Introduction

Mindfulness meditation is the practice of 'inner listening', concentrating one's attention on the present moment and experiences without judgement. Meditation is an ancient practice that dates back to approximately 5,000 BCE. And yet, only recently has its therapeutic effects been introduced to western medicine. In recent years, meditation has been recommended to individuals suffering from chronic pain, as well as those struggling with mental disorders such as anxiety and depression. Often chronic pain sufferers are diagnosed with comorbid stress and depression. These comorbidities lead to more difficulties for patients, exasperating the pain they experience and decreasing overall quality of life. According to the National Health Interview Survey, approximately 20.4 percent of the U.S. adult population suffered from chronic pain in 2016[1]. In the 4-year period 2005–2008 5.7 percent of the U.S. adult population were using opioids to treat pain[2]. This suggests a large quantity of chronic pain sufferers are currently relying on prescription opioids to help manage their condition. However, opioids are highly addictive and when taken for long periods of time can lead to dependence. Amongst patients with chronic non-cancer pain who were prescribed opioids, addiction rates ranged from 3.2 to 18.9%[3]. Because of opioids addictive nature it is important that we begin to look for alternative ways of managing chronic pain that won't cause patients to rely on medication for relief. Mindfulness meditation has been shown to have analgesic and stress reducing effects but there is still a large amount of skepticism towards the practice's efficacy [4]. This review will summarize the proposed neurological mechanisms of mindfulness meditation, its analgesic effects, and methods of stress reduction.

Analgesic effects of mindfulness meditation

One criticism towards mindfulness meditations therapeutic usage is whether its practice employs unique mechanisms of providing pain relief or if its results are merely a placebo effect. Research utilizing fMRI techniques compared functional activation of brain regions when subjects underwent noxious stimuli produced by a TSA-II device, a computer-controlled device capable of generating and documenting response to repeatable thermal stimuli, paired with either mindfulness meditation, sham mindfulness meditation, or placebo [5]. In the group who practiced mindfulness meditation there was a significant decrease in neural activation in brain regions crucially involved in the facilitation and modulation of nociceptive information. Instead, the mindfulness meditation group displayed greater activation in sensory processing regions as well as its own unique cognitive reappraisal processes [5]. Cognitive reappraisal is the reinterpreting of a stimuli or a situation. Altering the interpretation of stimuli can be achieved through an array of different neurological processes. The neurological processes employed by mindfulness meditation were found to be novel. These findings suggest that the mechanisms employed by mindfulness meditation to provide pain relief are different than those of placebo. In individuals practicing mindfulness meditation while receiving noxious stimuli, greater activation was seen in the anterior cingulate cortex and anterior insula, two brain regions associated with the cognitive regulation of processing pain [6]. There is a significant correlation between pain reduction and activation of the orbitofrontal cortex [6]. It has been proposed that meditation employs a limbic

gating mechanism, which modifies interactions between afferent input and higher order brain regions [6]. These modifications are supported by an inactivation of thalamic brain regions [6]. Cognitive reappraisal processes induced through mindfulness meditation are thought to allow for contextual evaluation of sensory events, altering an individual's subjective experience of pain [7]. A study utilizing naloxone, an endogenous opioid blocker, found a significant reduction in mindfulness meditations analgesic effects compared to those injected with saline [8]. This significance suggests mindfulness meditation employs a mechanism of modulating pain through the release of endogenous opioids, specifically in more experienced meditators [8]. In less experienced meditators it is thought that the influence of placebo was stronger, and that with time and practice opioid release was more persistent against psychological influences [8].

Mechanisms of stress reduction with mindfulness meditation

Mindfulness meditation is often prescribed to help reduce stress and relieve anxiety. Research investigating the neurological mechanisms of mindfulness meditation related anxiety relief found increased activity in the anterior cingulate cortex, ventromedial prefrontal cortex, and the anterior insula [9]. The amygdala is another brain region of interest, that may attribute to mindfulness meditations stress-relieving effects. One study found that individuals who did eight weeks of mindfulness meditation training had decreased activity in the right amygdala, while those who completed compassion meditation had an increase in activity in the right amygdala compared to baseline activity [10]. Unlike mindfulness meditation, compassion meditation involves the repetition of phrases aiming to move from a judgmental mindset towards a more compassionate state. These differences suggest the changes to resting state amygdala activity are process-dependent and enduring [10]. Another study found the resting state of the amygdala is altered via mindfulness meditation. The effect was a decrease in right amygdala-subgenual anterior cingulate cortex (sgACC) activity when under stress [11]. Previous studies found bilateral amygdala-sgACC activity is associated with greater perceived stress [11]. These findings suggest the amygdala-sgACC pathway as a proposed mechanism of stress relief via meditation.

Mindfulness meditation: clinical applications and looking towards the future

Mindfulness meditation is a promising treatment for individuals suffering from chronic pain and comorbid stress/anxiety. There have been several proposed mechanisms to explain mindfulness meditations analgesic effects such as higher activation in brain regions responsible for cognitive reappraisal processes as well as the release of endogenous opioids. It is thought that mindfulness meditation relieves anxiety through the amygdala-subgenual anterior cingulate cortex pathway. However, the literature varies in whether the activity of the right amygdala-sgACC is upregulated or downregulated during meditation. Future studies should investigate the pathway activated by mindfulness meditation to release endogenous opioids and why it may differ between more experienced meditators and inexperienced individuals. Additional research should be conducted comparing the analgesic effects of actively practicing mindfulness meditation in the presence of noxious stimuli as opposed to the effects of a long-term practice. This may provide more insight as to whether or not the neural mechanisms have a lasting effect and can potentially relieve pain without an individual continually meditating. Another pitfall of these studies is the small sample size, future research should work towards increasing the number of participants for more accurate results. The practice of mindfulness meditation should continue to be promoted to chronic pain and anxiety sufferers. It's an attainable skill that is free and can be self-lead making it accessible to the general population with promising results.

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An overview of exercise-induced hippocampal neurogenesis

Trent Pratt

Exercise is widely recognized as an essential component in the maintenance of one's physical health, including clinical benefits in the prevention of obesity and cardiovascular disease. A promising area of developing research is exploring the neurophysiological effects of physical activity on neurogenesis and cognitive enhancement. Research suggests there are a diverse number of potential mechanisms that can explain how exercise is involved in the mediation of neurogenesis and the resulting effects on cortical plasticity and cognitive enhancement.[1] The hippocampus provides an excellent model to study the role of exercise in relation to neurogenesis, as other mammalian species exhibit neurogenesis in the hippocampus throughout adulthood.[2] The bulk of current research seems to conclude that exercise and neurogenesis are intertwined, but that relationship is still poorly understood. The adult hippocampal dentate gyrus is an area where neurogenesis occurs and is sensitive to extrinsic and intrinsic factors, i.e. exercise or genetic disposition.

There are two zones where neurogenesis is known to occur, the olfactory bulb and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG).[3] In the hippocampus, neural progenitor cells undergo division, differentiate, migrate, and integrate into the existing circuitry.[4] The dendrites of these newborn cells migrate to the molecular layer of the dentate gyrus and their axons migrate to the cornus ammonis 3 (CA3) region via the mossy fiber pathway. The addition of new neurons in existing circuitry can aid in synaptogenesis. Research suggests a relationship between important neurotrophins such as brain-derived neurotropic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) and exercise-induced adult neurogenesis.[1] It is likely that interplay between multiple mechanisms contribute to neurogenesis and cortical plasticity. The following review will attempt to provide clarity on the roles of BDNF, IGF-1, and VEGF in relation to exercise-induced hippocampal neurogenesis.

Hippocampal neurogenesis

While there is some controversial research that suggests adult hippocampal neurogenesis ceases throughout adulthood, there is research that suggests otherwise.[5,6] The dentate gyrus (DG) subregion of the hippocampus is associated with memory, pattern recognition and mood regulation.[7] The generation of new neurons in the DG is a form of neural plasticity that occurs throughout life.[8] One study provided evidence of adult neurogenesis in the human brain that came by showing the presence of positive staining for 5-bromo-2′-deoxyuridine (BrdU) in the DG of postmortem brain sections from cancer patients who had received BrdU injections in life.[9] Another study using radiocarbon dating to track cell division in the brain found consistent neural turnover in the hippocampus and estimates approximately 700 new neurons are added to the adult human hippocampus each day.[6] Newly generated neurons in this region are sensitive to synaptic plasticity during their maturation and can account for up to ten percent of the entire granule cell population.[10,11] In the DG, newborn neurons migrate approximately 20 to 25 µm from the subgranular zone (SGZ) to the granule cell layer (GCL), where they are integrated.[3] The newborn hippocampal neurons are believed to aid in the functioning of the hippocampus and may play an integral role in hippocampal-dependent learning, memory, and pattern separation.[12]

In response to these findings, the major point of interest is in addressing how to support neurogenesis in the hippocampus.

Linking exercise to hippocampal neurogenesis

It has been demonstrated that moderate exercise increases the size of the hippocampus in humans.[13] BDNF, IGF-1, and VEGF have been recognized as primary mediators of adult neurogenesis, though there are many potential factors associated with the benefits of exercise on hippocampal neurogenesis.[14,15,16,17] BDNF is vital for many factors contributing to neurogenesis, including proliferation, differentiation, maturation, and survival.[17] One study reported that physical exercise in aging populations effectively aided in the reduction of age-related loss in hippocampal volume in addition to increased levels of BDNF.[13] Another study demonstrated that metabolic derivatives from muscles and endurance factors stimulate BDNF expression in the brain and lead to improved spatial memory in mice.[18] Another factor, IGF-1, appears to be upregulated following physical exercise in rodents.[19] Additionally, transgenic overexpression of IGF-1 during postnatal development promotes neurogenesis and synaptogenesis in the DG, though it is important to note the relationship between IGF-1 and neurogenesis is still poorly understood. VEGF is expressed in the CNS and is shown to be upregulated following acute exercise.[15,20,21] VEGF is associated with an increase of new vasculature tissue in the hippocampus, and it is reasonable to suspect a necessity for increased nourishment via blood flow to support newborn neurons.[20] The interplay between neurotropic factors such as BDNF, IG-1, and VEGF, among others, are vital in the proliferation, maturation, and survival of newborn neurons in the hippocampus.

In humans, exercise-related research demonstrated enhancements in spatial learning, pattern separation, executive function, working memory, and processing speed.[22] Additionally, a meta-analysis study demonstrated that 1 to 12 months of exercise in healthy adults induces behavioral benefits, such as increases in memory, attention, processing speed, and executive function.[23] Midlife exercise intervention has been associated with reduced risks of developing dementia, suggesting that exercise may aid in the prevention of developing age-related cognitive decline. [24]

Future directions

With a prevalence of cognitive decline associated with neurodegenerative diseases among the aging populations, physical exercise, a potent enhancer of adult hippocampal neurogenesis, has emerged as a potential preventative approach to reduce cognitive decline. Research suggests that neurogenesis in the DG plays an important role in hippocampal-dependent learning and memory.[1] Further understanding of this relationship could lead to therapies focused on cognitive preservation of healthy aging individuals and potential therapeutic remedies for those suffering from neurodegenerative disorders, such as Alzheimer's or Parkinson's disease. Several clinical human studies and abundant animal studies have attempted to understand the functional role of adult hippocampal neurogenesis in specific forms of hippocampal-dependent learning and memory, but there is much research to be conducted in humans to assess this relationship. Using cognitive learning assessments after exercise in humans does not point to hippocampal neurogenesis, and so, future directions may find clarity to this dilemma in post-mortem assessments of subjects that have engaged in exercise-related research prior to death. Additionally, there is preliminary research that suggests neurogenesis may also occur in other brain regions, including the amygdala and hypothalamus, which may explain the broad scope of exercise-induced benefits.[25] This promotes the idea that exercise is an essential aspect in the maintenance and physiological success of healthy adults. Exercise is a cost-effective, low-tech method of preserving and increasing cognition and warrants further explanation and clarity to determine where future research could be focused, i.e. via human studies that can help guide

potential therapeutic strategies for cognitive decline. While concrete links between physical exercise, increase adult hippocampal neurogenesis and improved cognition are remain unclear due to the current technical limitations, there is enough evidence to support the notion that exercise is worth investing time in.

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CAPSTONE

Circadian rhythm disruption and PACAP-38 in the pathophysiology of cluster headache

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Cluster headaches (CH) are a rare, severe form of neurovascular headache that tend to occur on one side of the head.[1] Unlike typical headaches and migraines, the pain feels like a burning and stabbing sensation that lasts from 15 to 120 minutes up to 4 times per day.[1] In episodic sufferers, these bouts of headaches tend to occur around the same time each year with a pain-free period spanning one month or more.[2] In chronic sufferers, CHs are recurrent without a prolonged headache-free period. While not life-threatening, these headaches have dramatically decreased the quality of life for all sufferers. Up to 4 in every 1000 people suffer from CH attacks, the majority of them being men.[3] There is no known cause of CHs, although many studies point to structural and functional abnormalities of the hypothalamus. Patients suffering from CH have shown increased hypothalamic activation ipsilateral to the pain during acute attacks.[8] Furthermore, research has pointed to the potential involvement of the suprachiasmatic nucleus within the hippocampus. The suprachiasmatic nucleus is involved in the regulation of circadian rhythm. One study found that in episodic CH patients, a neuropeptide called PACAP-38 was elevated during active CH bouts when compared to headache-free periods.[5] PACAP-38 functions as a regulator of the circadian entrainment to light. Specifically, PACAP-38 release at the retinohypothalamic tract (RHT) leads to an influx of intracellular calcium at the suprachiasmatic nucleus.[4] The involvement of PACAP-38 in light and sleep circadian rhythms and the relationship between PACAP-38 and CH attacks may help to uncover a mechanism for CHs. Current treatments for temporary CH relief include oxygen inhalation and injectable triptans, although these do not prove useful for all patients.[6] In this review, the role of the PACAP-38 neuropeptide and its receptor in chronic and episodic CH will be explored as a novel target for effective long-term treatment.

Hypothalamic structural and functional abnormalities in cluster headache

Abnormalities of the hypothalamus have been a consistent finding in many CH studies, including gray matter volume differences between controls and CH and circuitry dysfunction.[7,8] Using voxel-based morphometry analysis, a study found that the anterior hypothalamus was bilaterally enlarged in the CH group when compared to healthy controls.[7] Hypothalamic gray matter was also increased in chronic and episodic CH sufferers when compared to migraineurs, implicating that there may be physiological differences between migraine and CH.[7] Another study that was conducted on nine CH patients found differences in bilateral hypothalamic gray matter volume during acute headache attacks in comparison to the inter-bout phase.[18] In contrast with the previous study, this volumetric difference was seen in the inferior posterior hypothalamus. This may suggest that CH impacts other areas of the hypothalamus. A structural MRI study also revealed weaker hypothalamic covariance patterns in CH patients when compared to controls. Significant differences were found in the correlation slope between the hypothalamic volume and vertex-by-vertex cortical thickness measurements. [8] Specifically, the hypothalamus showed weaker structural covariance with temporal and frontal areas of the brain when compared to healthy controls. Weaker structural covariance indicates less connectivity between left/right hypothalamus and temporal/frontal brain regions. However, no hypothalamic volume differences

between CH and healthy controls were found. While this could be due to differences in brain imaging methods between the two studies, one might extrapolate that CH is the result of pain circuitry dysfunction as opposed to structural dysfunction. Any structural compromises seen in the studies may have been a byproduct of circuitry dysfunction, but not a direct effect of CH. Despite the discrepancies in whether CH impacts hypothalamic volume or structure, studies implicate the hypothalamus as the origin of CH and an important target for treatment.

Circadian rhythm dysfunction in cluster headache

Circadian rhythmicity, a property of the suprachiasmatic nucleus in the hypothalamus, has also been closely linked to CH. In a study reporting on temporal changes of within-bout CH patients, 86 people (approximately half of the total study population) reported circadian patterns.[9] However, it is unknown whether the other half of CH patients experienced circadian rhythmicity in different bouts as the patients were only monitored during one bout. As the number of lifetime bouts increased, the incident of afternoon and hypnic circadian rhythmicity also increased.[9] Toward the beginning and end of the disease's progression (which varies from patient to patient), nighttime attacks were more frequent, whereas daytime attacks were more frequent during the middle of the disease's progression.[9] A similar study on sleep patterns found that in 80% of CH patients (n=275), a common trigger was sleep.[10] This was especially true for patients reporting diurnal (hours of the day that CH consistently occurs) rhythmicity. About 56% of patients reported yearly fluctuations in CH occurrence, revealing that the most common month for CH worsening was November.[10] This may be due to daylight savings, which ends in November. This abrupt alteration in the body's biological clock may be a trigger for CH. Of these patients, the majority were episodic patients instead of chronic patients. There is no direct linear relationship between CH and time/sleep, as there is wide variance in the reported patterns for CH. Still, based on the surveyed patients, CH tends to manifest itself differently depending on the time of the disease's course and the disease's form (chronic or episodic). The tendency of CH to be linked to circadian rhythmicity, especially as it relates to light and sleep, may implicate the suprachiasmatic nucleus as a key structure in CH.

PACAP-38 and the suprachiasmatic nucleus

In an exploratory study on episodic CH, PACAP-38 was found to be involved in the inter-bout and ictal phases of CH.[5] PACAP-38 is a neuropeptide involved in nociception, neuromodulation, and circadian rhythm regulation.[11,12,14] Animal models involving PACAP manipulation have been shown to exhibit alterations in sleep and in somatic factors, such as breathing, heart rate, and headache.[13] Concerning headache, PACAP-38 has been found to induce vasodilation in rats *ex vivo*.[12] Application of PAC_1 antagonist reversed vasodilation effects, showing promise in alleviating pain caused by primary headaches.[12] Only one human study exists on the relationship between PACAP-38 and CH. Blood plasma was measured in nine age-matched healthy male controls and five male episodic CH sufferers.[5] PACAP-38 concentrations were measured in the CH group during the inter-bout phase (headache-free periods lasting longer than a month) and during the ictal (during bout) phase. There was no significant difference in PACAP-38 concentrations between the healthy controls (mean=30.5 fmol/ml) and the CH ictal phase (28.8 fmol/ml).[5] There was a significant difference in PACAP-38 concentrations between the CH ictal phase (28.8 fmol/ml) and the CH interbout phase (24.4 fmol/ml), suggesting that PACAP-38 may be released upon CH onset.[5] The ability of PACAP-38 to impact circadian phase change has been demonstrated in a few studies. Hannibel et al. found PACAP-38 to be in high concentrations in the retinohypothalamic tract (RHT). At the same time, its receptor PAC_1 was expressed at high levels in the SCN.[15] Binding of PACAP-38 to the PAC_1 receptor causes activation of RHT, which then relays light information to the SCN.[15] The glutamatergic neurotransmission induced by PACAP caused circadian phase-shifting during the day.[15,16] The

existence of glutamatergic neurotransmission via PACAP-38 was confirmed by the application of NMDA antagonists, MK-801 and AP-5, before PACAP application[15] Both antagonists completely blocked circadian phase shift in the presence of PACAP-38.[15] PACAP-38 was also found to have dualistic effects in one study, coding for "light information" in doses lower than 1 nM while coding for "dark information" in doses higher than 10 nM. [17] PACAP-38 is known to selectively target PAC_1, which is tied to several signaling cascades, including ERK and phospholipase C. [14] PACAP-38 has widespread effects that expand beyond circadian regulation and CH. However, its high levels in the SCN, involvement in circadian rhythm shift, and release in CH ictal bouts call for further investigation.

Conclusion

Cluster headache is a painful condition which warrants more attention than it has received. While this disease is rare, the few people that do suffer from these agonizing headaches have expressed depression, anxiety, and suicidality at a higher rate than non-sufferers.[17]
There is no confirmed mechanism for CH. However, emerging studies on the relationship between CH and circadian rhythm, PACAP-38, and the suprachiasmatic nucleus provide a promising avenue for further studies. The current body of research points to the hypothalamus being the origin of cluster headache, circadian rhythm abnormalities being a hallmark in most sufferers, and the release of PACAP-38 being a biomarker in episodic suffers. Still, there are limitations on the number of studies exploring PACAP-38 release *in vivo*. While the exploratory study by Tuka et al. found significant results, the sample size was quite small (n=5), and the study only focused on episodic CH sufferers. It is unknown whether chronic CH, which lacks the pain-free intervals of episodic CH, involves the release PACAP-38. Chronic CH sufferers have also shown less circadian rhythmicity than episodic sufferers, indicating the possibility of a separate mechanism. Future studies should focus on characterizing the distinct differences in the temporal patterns of chronic vs. episodic CH. Additionally, studies must address the role of PACAP-38 in chronic CH. Understanding the pathophysiology of CH is imperative to expand current treatment options for sufferers. Selective targeting of the PAC_1 receptor by PACAP-38 to regulate circadian rhythm may prove to be a promising drug target for CH, although more clinical trials are still needed.[11,12] While no clinical trials have been published, preclinical trials have used PACAP-38 and PAC_1 antibodies in *in vivo* rat studies. Application of the PAC_1 antibody, AMG 301, was able to alleviate nociceptive activity, with an effectiveness rate comparable to triptans.[12] Studies are needed to investigate the side effects of long-term PAC_1 blockade.

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CAPSTONE

Analysis of the functional and neuroanatomical abnormalities in the hippocampus and amygdala of post-traumatic stress disorder patients

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Post-traumatic stress disorder (PTSD) is a mental health condition that is triggered by witnessing or experiencing a traumatic event.[1] The disorder affects 8 million people in a given year.[2] PTSD is often characterized by increased arousal, flashbacks to traumatic events, impaired extinction of fear cues, and overall emotional numbness.[3] The disorder can affect the way that the person engages with the world due to the debilitating nature of symptoms. Neuroimaging is used to observe neuroanatomical and functional abnormalities in brain structures that are implicated in PTSD.[4] Previous research has indicated that the two main brain areas that have been associated with PTSD, including the amygdala and the hippocampus.[4] The function of these brain areas includes memory formation, learning, fear response, and the perception of emotions, including fear and anger. Deficits in these brain areas have been associated with severe PTSD symptoms. The objective of this literature review is to highlight significant functional and neuroanatomical changes in the amygdala and hippocampus to better understand PTSD.

Changes in brain activity in the hippocampus and the amygdala

The hippocampus is responsible for the consolidation of information from short term memory to long term memory, along with spatial navigation. Generalized damage to this region of the brain leads to memory impairments including the inability to make and store new memories. The hippocampus is one of the most plastic brain regions.[5] In animal models, the hippocampus is damaged when it is exposed to stress and glucocorticoids.[5] fMRI scans utilize cerebral blood flow and neuronal activation to observe brain activity. A study looking at memory and learning used a fMRI to observe hippocampal activity while subjects were performing a virtual morris water task. It was found that there was decreased hippocampal activity in PTSD patients during the task when compared to healthy controls.[6] Previous studies have shown that a decrease in hippocampal activity correlates with cognitive decline in Alzheimer patients. The same reduction in hippocampal activity can be seen in PTSD patients that have difficulty recalling traumatic events.[7]

The amygdala is responsible for processing fear and fear reactions. fMRI studies have shown that there is an increase in amygdala activity in PTSD patients in comparison to healthy controls.[8] This hyperactivation of the amygdala is suspected to be engaged with fear circuitry. Fear circuitry a network of brain regions that process fear and activate behavioral responses in response to the fearful stimuli. The recurrence of fear circuitry has been linked to traumatic memories in PTSD that are partially mediated by the amygdala.[9] In animal studies, the hippocampus is involved in inhibiting the amygdala in order for encoding a place as “safe”.[10] If activity in the hippocampus is reduced, this gives way for the amygdala to activate because the hippocampus is no longer strongly inhibiting it. If this occurs then, locations are less likely to be learned as “safe” because the amygdala is no longer being inhibited. This could mean that another network is being activated that could deem the location unsafe due to this inhibition.

Decreased volume in the hippocampus and amygdala

Studies have shown that PTSD patients have significantly smaller hippocampal volumes in comparison to trauma-exposed groups that did not develop PTSD and control groups.[11] However, studies also did find that there was a reduction in hippocampal size in trauma-exposed subjects in comparison to healthy controls. This leads to the conclusion that hippocampal size reduction is linked to trauma exposure. This reduction in hippocampal size can be seen significantly more in PTSD patients than those that are exposed to trauma and do not develop PTSD. can significantly be seen in PTSD patients. Studies have questioned whether the reduction in hippocampal size is due to PTSD or serves as a vulnerability factor for developing PTSD.[12] Smaller hippocampal volumes may create a predisposition to have stronger and more persistent emotional responses – fear, anxiety, and panic – when exposed to trauma.[13] The amygdala is responsible for emotional responses, so if there is a stronger and more persistent emotional response, this could be inherently linked to increased amygdala activity, which has also been implicated in PTSD.

A decrease in amygdala size has been demonstrated in PTSD patients in comparison to healthy controls.[14] This decrease has been linked to deficits in inhibitory control, meaning that patients had a difficult time focusing on the relevant task at hand due to interfering information. However, this decrease is not associated with the length of how long the person has had PTSD or the amount of trauma that they have experienced.[14] Additionally, though there is evidence that supports that individuals suffering from PTSD have smaller amygdala volume, there is no implication that individuals with a smaller amygdala size have a predisposition for developing PTSD. This is interesting, and I think that there should be further research performed to look at whether there is a correlation between amygdala size at birth and the likelihood of getting PTSD.

Predicting PTSD

Glucocorticoid receptors (GR) are glucocorticoid-activated transcription factors that modulate the expression of various neuronal genes.[15] They have been found to be a significant predictor of the development of PTSD symptoms.[16] A study performed looking at the number of GR pre and post deployment of soldiers found that soldiers with increased GR before deployment shower increased amygdala activity after they returned. As mentioned before, PTSD has been linked to an increase in amygdala activity.[16] So, the GRs are predicting PTSD because of their link with increased amygdala activity. Research focuses on looking at the number of these receptors in peripheral blood mononuclear cells.[16] Furthermore, these receptors have shown to mediate the response of neurons in the amygdala.[16] An increase in GR found in this area predicts vulnerability to developing PTSD. The GR could be used as a target for new treatment options for PTSD due to this implication.[16]

Conclusion

The amygdala and hippocampus are primary brain areas implicated in PTSD. These brain areas are the main structures affected in the disorder and, furthermore, could be linked to the progression of the disorder. PTSD patients experience a decrease in hippocampal activity, as well as, a decrease in hippocampal volume. On the other end, patients experience an increase in amygdala activity, as well as decrease in amygdala size. Approximately 8 million people in a given year will experience PTSD. Understanding the role of these brain regions and the abnormalities that are due to the development of PTSD symptoms.

Further research could look at the role of glucocorticoid receptors as a targeted treatment for PTSD. Current research shows a great understanding of the differences between brain activity

abnormalities and neuroanatomical changes. The GR receptor has been implicated in PTSD vulnerability and has been connected to an increase in amygdala activity, along with a decrease in hippocampal volume. Decreasing the number of GR receptors could inherently remove the vulnerability factor of PTSD. Understanding the functionality and mechanism of this receptor could further help us gain some insight into how the receptor could be modified to possibly prevent PTSD or soften the impact of PTSD symptoms.

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CAPSTONE

The role of excitatory/inhibitory imbalance and parvalbumin interneurons in affective disorders

Abby Gligor

Introduction

Anxiety disorders are defined as persistent fears or worries that affect a person's daily life.[1] Over 284 million people worldwide are burdened by this mental health disorder, which makes it the most prevalent mental health disorder in the world.[2] To make matters worse, it is not uncommon for an anxiety disorder to have comorbidity with depression.[3] This other mental health disorder, depression, is defined as a severe, persistent low mood that affects over 264 million people globally.[3,2] In fact, over half of the people diagnosed with depression are also diagnosed with an anxiety disorder.[4] Anxiety disorders develop from a variety of complex causes such as genetics, brain chemistry, and traumatic life events.[4] Surprisingly, not all individuals exposed to a traumatic life event develop an anxiety or depressive disorder.[5] Research conducted regarding behavioral resistance to anxiety and depressive-like disorders indicates that the degree of control an organism has over a stressor modulates the impact of the stressor.[5] Stress that is uncontrollable produces a constellation of biological and behavioral outcomes that do not typically occur when the stress is controllable.[5]

Lab animals like rats provide an opportunity to study stress. Rats exposed to uncontrollable stress express anxiety-and-depressive like behavior.[5] On the other hand, when there is controllable stress, the medial prefrontal cortex (mPFC) perceives the control and activates inhibitory neurons in the dorsal raphe nucleus (DRN), which prevents the anxiety and depressive-like behavior from developing.[6] Failure to inhibit the DRN during uncontrollable stress causes a surge of activity in the serotonergic neurons of the DRN that project to further downstream structures.[7] This activity then induces anxiety and-depressive-like behavior.[7] The mPFC contains fast-spiking inhibitory interneurons that contain the protein parvalbumin. These cells are responsible for regulating the activity of pyramidal cells, which are excitatory neurons.[8] Perineuronal nets surround parvalbumin interneurons and stabilize their connections as well as protect them from oxidative stress. Evidence from other studies shows that anxiety may result from a misbalance between the excitatory and inhibitory components in the prefrontal cortex (PFC).[9] The focus of this review will be to highlight the activity of parvalbumin interneurons in contributing to affective disorders and the effect of their activity on perineuronal nets that surround parvalbumin interneurons in the mPFC.

Over-inhibition in the medial prefrontal cortex: addressing inconsistencies in the literature

First and foremost, it is important to address the fact that the literature reports glutamatergic and GABAergic results on affective disorders that can be contradictory and inconclusive.[10,11] Chronic stress and affective disorders are thought to have dysregulations in the prefrontal excitatory/inhibitory balance. One side of the spectrum explains that the psychopathology behind these disorders is a reduced PV cell activity, which causes an overexcitation of the PFC.

The other side of the spectrum explains that the psychopathology is due to the over-inhibition of the PFC mediated by increased activity of the GABAergic system.[9] The differences in ideas may be due to the time course of the stress effects as well as the type of stress presented. For instance, acute stress increases prefrontal glutamatergic transmission and enhances dendritic complexity and spine density of pyramidal neurons.[10] However, chronic stress causes these same neurons to decrease their dendritic complexity and spine density, which is thought to be a mechanism behind reduced activity in the PFC in affective disorders.[12] For the type of stress presented, it has been found that an increased strength of inhibition happens in the PFC after acute, inescapable stress.[13] More research needs to be done, bridging the role of the glutamatergic and inhibitory systems together in the PFC. Potential studies could include looking at the formation of new synapses over time. Another study could look at what happens to parvalbumin in interneurons as a result of overexcitation. Furthermore, if parvalbumin does change, it would be interesting to see how that change affects the cell and overall excitation of the prefrontal cortex. For the most part, these proposed studies were inspired by the inconsistencies presented in this section. These inconsistencies may be because of the time course of stress and the type of stress presented.

The role of parvalbumin interneurons behind anxiety and depression

Parvalbumin interneurons are fast-spiking inhibitory cells that are present throughout the brain, but most notably in the PFC. They are known to contribute to the brain's excitatory/inhibitory balance.[9] Any imbalance of the excitatory or inhibition circuitry in the brain is a common problem underlying many neuropsychiatric disorders, including affective disorders.[9] For instance, anxiety and depression disorders have been known to have higher inhibition in the brain.[9] In inhibitory interneurons, parvalbumin protein acts as a slow Ca^{2+} buffer that protects parvalbumin interneurons from the deleterious effects of over-firing by preventing facilitation. Thus, parvalbumin promotes neuronal depression. However, repeated firing will saturate parvalbumin and lead to facilitation.[14] In fact, it has been shown in several studies that reduced parvalbumin leads to increased inhibition in the circuit.[13,15,16] High activity of parvalbumin interneurons in affective disorders may be due to their decreased inhibitory inputs. Parvalbumin interneurons are inhibited by somatostatin positive interneurons.[17] Inhibition of somatostatin positive interneurons has been shown to increase anxiety and depressive disorders.[18] In short, parvalbumin interneurons are large players behind affective disorders like anxiety and depression by increasing inhibition.

Effect of high activity on perineuronal nets

Perineuronal nets are net-like structures that surround parvalbumin interneurons and stabilize their connections while also protecting them from oxidative stress. Throughout the literature on stress, perineuronal net intensity has been shown to either increase or decrease.[19,13] These differences may be due to the time course of the experiments. For instance, one experiment looking at perineuronal net intensity after chronic stress found that perineuronal net intensity increases.[19] On the contrary, another experiment that examined perineuronal net intensity after acute stress found that the intensity decreases in rats that had increased strength of inhibition in their PFC.[13] Similarly, another study found that acute stress in juvenile rats also decreased perineuronal net intensity.[20] The reason why perineuronal net intensity increases in chronic stress is possibly due to the fact that perineuronal nets protect neurons against oxidative stress. High firing of neurons may lead to a homeostatic response where perineuronal nets become thicker, which explains the increase of intensity. In summary, perineuronal nets have varying intensities, possibly due to the time course of the stress protocols in experiments. Overall, the varying intensities are important because perineuronal nets stabilize synaptic connections

between parvalbumin interneurons and other cells. Thus, a change in their intensity may mean that new connections between parvalbumin interneurons and other cells can occur. This increased plasticity can contribute to the circuit changes that happen in affective disorders.

Conclusion

Affective disorders like anxiety and depression have underlying neuronal connectivity issues that contribute to aberrant behavior. Such neuronal connectivity issues exist in the imbalance of the excitatory/inhibitory circuitry. Even though the literature has contradictory information regarding which circuitry takes over in anxiety and depression, it appears there is increasing evidence for over-inhibition. The differences seen in the literature may be different underlying mechanisms behind different time courses and stress types used in experiments. There needs to be more studies done bridging glutamatergic and GABAergic evidence together. These studies could potentially look at the synaptic and cellular changes that occur over time during various stressful experiences. Nevertheless, parvalbumin interneurons play a large role behind anxiety and depressive disorders by increasing inhibition in the PFC through the saturation of parvalbumin and the disinhibition of somatostatin positive interneurons. Perineuronal net intensity responds to this high activity by becoming dimmer after acute stress or brighter after chronic stress, possibly due to a homeostatic mechanism. Like some neurological disorders, affective disorders may result from an imbalance in their neuronal circuitry that further contributes to the disorder.

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CAPSTONE

Overview of the biological and psychological factors contributing to anxiety/migraine comorbidity

Caleb Man

Introduction

Migraine is a common and painful neurological condition that many people experience. In fact, it is the third most common neurological disorder worldwide[1,6] and almost a quarter of U.S. households have at least one member that suffers from migraine.[2] When a person experiences a migraine, they will usually describe the pain as being pulsating or throbbing, usually on one side of the head, and this pulsating pain is the most commonly reported symptom, followed by light sensitivity, sound sensitivity, nausea, and other symptoms.[2,8,9] Interestingly, some migraine patients experience unique symptoms that fall under the umbrella term "aura." The most common aura symptoms that patients report having are visual symptoms, as 98% or 99% of patients experience them.[1,11] More specifically, these visual symptoms can include flashes of bright light, blurry vision, or seeing jagged/zigzagging lines that obstruct vision[1]. Other symptoms of aura include problems with motor (dyspraxia), sensory (tingling or numbness), and verbal skills (it can be hard to process thoughts and to speak).[1,12,13]

Interestingly, a correlation between migraine and anxiety has also been established, as many migraine sufferers (migraineurs) experience anxiety more than those who do not have migraines.[4,27] Unfortunately, diagnosing headaches and migraines becomes complicated when psychiatric comorbidities exist. The presence of these comorbidities, such as anxiety and depression, can complicate the treatment of headaches, and proper prognosis for treatment may not be possible without also identifying and treating the underlying comorbidities.[3,30] Additionally, when studies do look at the relationship between migraine and psychiatric comorbidities, they often focus more on depression rather than anxiety.[14] This is surprising, in a way, considering that research has shown that migraineurs are almost twice as likely to suffer from an anxiety disorder than they are from depression.[15] That being said, there also appears to be a genetic link between migraine with aura, anxiety, and depression.[7] This could explain why the three have such a high comorbidity.[15]

While it is possible to have anxiety without having an anxiety disorder, many people suffering from migraines also suffer from generalized anxiety disorder (GAD).[4] In fact, migraineurs, have been found to be four to five times more likely to have GAD.[15] Anxiety occurs when physiological changes occur in an individual that is anticipating or preparing for some sort of threat, but it is classified as a disorder once the anxiety becomes excessive or irrational to a point where it can interfere with daily living.[10] While there are several different diagnosable anxiety disorders, GAD is characterized by excessive worry and significant distress about multiple events or daily activities for the majority of six or more months.[10] Research has found that when patients report having headaches and GAD, the most commonly reported primary headache was migraine.[5]

The purpose of this literature review is to determine if the relationship between migraine and anxiety is causal or correlational. Currently, it seems to be correlational, but some studies suggest that migraines may be causing GAD in some people, as the unpredictability and severity of anticipated future pain may cause significant worry and distress.[4] Additionally, disabilities caused by migraines may also be responsible for causing anxiety and depression.[8]

Biological and Psychological Factors Involved in Migraine and Anxiety

Certain biological factors, such as immune function, genetics, and neurovascular changes have been shown to play a role in migraine.[16,17] There are several immune factors that have been linked to migraine, with mast cells being a target of interest, as they are located relatively close to blood vessels and nociceptors in the meninges.[16] Activation of nociceptors in the meninges has been shown to precede migraine headaches.[28] The reason mast cells are important is because they release proinflammatory cytokines.[16] Cytokines are cellular proteins that are involved in the interaction and communication between cells, and so proinflammatory cytokines would be involved in the increase of inflammation, and therefore pain.[18] Another popular position in the literature is that migraine pain is linked to an increase in vasodilation in the meninges or the cerebral cortex.[19] However, research also shows that while there is a correlation between migraines and vasodilation, it is possible to have a migraine without having vasodilation.[19,20,21] Considering that vasodilation does not cause migraines, researchers have also tried to look at genetic factors. For example, when looking at a group of relatives, researchers found that anxiety/depression was elevated only in those that had migraines.[22] Additionally, while the genetics of migraine are not well understood, a separate study found that participants who had migraine, anxiety, and/or depression also had an increase in the expression of the DRD2 *Nco*I C/C genotype (which deals with dopamine receptors).[7] This same genotype was also shown to affect participants with migraine with aura more than those without aura, or without migraine.[31] The DRD2 gene is of particular interest because using antagonists on the D_2 dopamine receptors that it encodes has shown to be effective in the acute treatment of migraine.[31] As a result, it is likely that the dopaminergic pathway plays a significant role in migraine, particularly when comparing migraine with aura to migraine without aura, as well as in the comorbidity of migraine, anxiety, and depression.[7,31]

Additionally, there are some psychological factors that contribute to headaches and migraines. Research has found that stress (particularly chronic stress), emotional distress, and mental tension contribute to increasing headaches and migraines.[8,24] In fact, one study even found that a migraine would occur if participants were stressed 2-3 days prior to the migraine.[25] It may also be important to note that migraine with aura had a stronger association with psychiatric disorders, such as anxiety, than migraine without aura, even though they both were comorbid with anxiety.[22] It is possible that the symptoms of aura could also be contributing to the anxiety.

Methods for identifying the comorbid relationship between migraine and anxiety

Considering that migraine and anxiety are so closely intertwined with one another, it is important that any existing comorbidities between the two can be identified when patients are seeking treatment for migraine. Research has shown that anxiety predicts long-term migraine persistence, as well as disabilities related to the headache; it's an even better predictor than depression.[3] Several different tests exist to assess migraine and anxiety separately, but they may not be as accurate when testing for comorbidity. That being said, the GAD-7 and GAD-2

are tests that have been developed to effectively screen for GAD in migraineur patients.[3] To create the GAD-7, a set of 13 questions was first compiled—nine questions that reflect GAD criteria from the DSM-IV, and four that are based on preexisting anxiety scales. From there, the GAD-7 became a seven-item questionnaire created by selecting the seven questions with the highest correlation from the 13-item questionnaire.[26] In other words, the 13 original questions were whittled down to the best seven. The GAD-2 is just a simplified version of the GAD-7, and it only uses the first two questions from the GAD-7 (See Figure 1).[3]

Over the last 2 weeks, how often have you been bothered by the following problems?	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3

Figure 1: This figure represents the two questions that are used in the GAD-2, which is a shorter version of the GAD-7.[3,26]

Both tests have been used by many practitioners, and if it is implemented in the treatment for migraine, it may help to identify if there is comorbidity between migraine and anxiety, which will then allow for better patient treatment.[3]

Determining if the relationship between migraine and anxiety is causal or correlational

Understanding that there are genetic factors in play, as well as other biological factors, research leans towards the argument that there is a strong direct correlation between migraine and anxiety. That being said, research also supports that migraine may cause anxiety, even though the mechanisms behind how are not well understood.[23] The relationship may be causal due to how the unpredictability and severity of the migraine pain interferes with the ability to carry out regular household chores, therefore potentially resulting in anxiety.[4] Additionally, anxiety may increase as the pain makes it difficult to complete family or work-related responsibilities.[4] The pain from a migraine results in many limitations and disabilities and the fact that a migraineur never knows when a migraine will occur may induce a state of constant worry and anxiety for the future.[4,8] This is likely why many migraineurs are also diagnosed with GAD.

Future directions

In the future, it would be interesting to compare how the relationship between migraine and anxiety varies for men and women. Women tend to have significantly more migraines than men, and they are also more likely to have GAD.[10,29] In terms of GAD, men and women express similar symptoms, but there is a difference in the comorbid relationship between GAD and other non-migraine related disorders.[10] This literature review did not compare the differences between men and women. This could be a valuable next step when establishing a relationship between migraine and anxiety that holds true for both sexes. Additionally, further research into the relationship between aura and anxiety would also be beneficial. It is possible that the many symptoms associated with migraine, outside of just the pain, can also worsen anxiety. For example, seeing visual hallucinations that occur before a migraine may cause a person to be more anxious about the pain that will soon follow. Over time, multiple experiences like this could eventually lead to GAD.

Conclusion

Taking into account all the biological factors (immune function, genetics, and vasculature) and the psychological factors, it is evident that correlations between migraine and anxiety exist (i.e. increase in vasodilation can result in migraine pain). However, no causal relationship has been established at this time, even though migraine may cause anxiety, including diagnosable anxiety disorders, such as GAD. The severity and unpredictability of migraine pain may cause worry for what the future holds, as the pain threatens to disrupt many different aspects of life.

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