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The Role of Multilingualism in the Brain's Developmental Life Span

Julia Gainski

Abstract

Multilingualism provides individuals with the intrinsic ability to learn new languages more efficiently than monolinguals. Bilingual and multilingual individuals have obtained skills during early childhood development that translate to other areas in their lives such as the enhancement of processing information presented in their external environment and a greater attention to detail. Through the use of fMRI, researchers are able to pinpoint specific brain regions that exhibit differences between monolinguals and multilinguals such that multilinguals display a greater tissue density in certain brain regions in comparison to monolinguals. Alongside these visual variations, this article places emphasis on the role of multilingualism throughout an individual's life span as it depicts the neurological benefits such as the maintenance of a cognitive reserve and the protection from age-related decline predominantly seen in dementia and Alzheimer's disease.

Multilingualism is defined as the use of several languages while bilingualism is the use of two languages. Researchers have primarily focused on exploring the differences between bilingualism and multilingualism by analyzing their advantageous roles in the developmental life span of the brain. Multilingualism has the potential to bolster the economy by creating a more diversified and advanced workforce. Research fellow Gabrielle Hogan-Brunab, at the University of Bristol made connections between data that models the relationship between economic growth and linguistic diversity (Hardach, 2018). Switzerland exemplifies this research, as its multilingual heritage makes up 10% of its GDP, indicating that languages have the potential to create new trade relations (Hardach, 2018). Beyond its immediate economic benefits and financial rewards, multilingualism has shown to have remarkable impacts on brain health through improved concentration, and information processing. Moreover, it can impede the onset of dementia (Hardach, 2018). In a similar manner, multilingualism holds a prominent role in early childhood development as it has shown to improve learning and adaptability to external environments (Marian and Shook, 2012). This paper will explore the differences in cognitive functioning between monolingual, bilingual, and multilingual brains, and the benefits of bilingualism in early childhood development as well as in old age.

The two main cortical regions that are associated with language are the Broca's area (BA) and the Wernicke's area (WA) (Mohades et al., 2012). The BA is located within the left inferior frontal gyrus and is known for its function in language output (Dronkers et al., 2007; Mohades et al., 2012). On the other hand, the WA is a left posterior temporal area that is known for its function in language input or language comprehension (Mohades et al., 2012). The connection between the white matter tracts and the gray matter subdivisions of the WA establishes a dorsal link (Mohades et al., 2012). Moreover, the dorsal white matter connections that are located between the temporal and inferior frontal language cortices follow both the arcuate fasciculus (AF) and the superior longitudinal fasciculus (SLF) pathways (Catani and Mesulam, 2008, Catani et al., 2002, Catani et al., 2005, Crosby et al., 1962, Nieuwenhuys et al., 1988; Mohades et al., 2012). According to Angela D. Friederici, Director and Scientific Member of the Max Planck Institute of Cognitive Neuroscience, it is extremely difficult to distinguish between these two pathways given how unreliable and deficient the spatial resolution limitations of the current DTI methods are (Friederici, 2009; Mohades et al., 2012). As a result, the two pathways are regarded as a single dorsal language pathway that can be noted as: AF/SLF (Mohades et al., 2012).

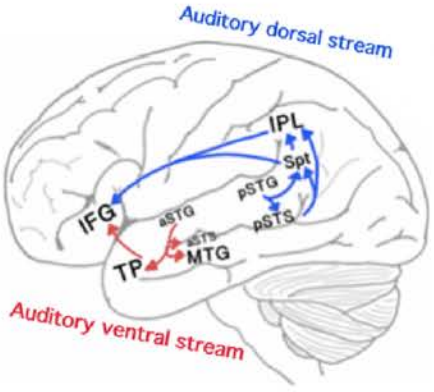

Figure 1. The 2 pathways in the brain that are responsible for language processing are: the auditory ventral stream and the auditory dorsal stream. The auditory ventral is responsible for sound recognition while the auditory dorsal stream is used for sound localization and the articulation of speech (KarinaCor, 2016).

Neurological processing and structure in bilingual and multilingual brains greatly vary from monolingual brains, so it is important to first interpret the general differences between individuals who are monolingual, bilingual, and multilingual. Data from fMRI and MEG studies have presented significant differences in bilingual and monolingual brain functioning during tasks encompassing linguistic and non-linguistic processing (Mohades et al., 2012). Bilingualism studies in general focus on aspects such as language interference and the ability to switch between two languages (Crinion et al., 2006; Marian et al., 2003; Mondt et al., 2009; Rodriguez-Fornells et al., 2002; Mohades et al., 2012). Common examples of nonlinguistic cognitive tasks would include testing speed processing, auditory working memory, and

attentional control. In contrast, linguistic cognitive activities would encompass selective attention, grammatical judgment, and social language skills. Ultimately, these studies have found that bilinguals engage with both common and specific cortical areas in order to use their two languages (Crinion et al., 2006; Marian et al., 2003; Mondt et al., 2009; Rodriguez-Fornells et al., 2002; Mohades et al., 2012). Other studies have presented findings regarding the structural plasticity of bilingual brains by analyzing differences in density of gray and white matter in bilingual and monolingual individuals. Results from these studies suggest that bilingual individuals have more gray matter density in the left inferior partial cortex than monolingual individuals but no differences in cortical white matter (Mechelli et al., 2004; Mohades et al., 2012).

Another study reveals that there are noticeable structural differences in white matter tracts between bilingual and monolingual children. The results of this study ultimately determined this by analyzing the fractional anisotropy (FA) and by tracking the left inferior occipitofrontal fasciculus (IIFOF) which connects the anterior regions of the frontal lobe with the posterior regions of the temporal and occipital lobes (Mohades et al., 2012). The FA is a quantity that measures the direction that the water diffuses through, which signifies white matter anisotropy as well as fiber organization (Mohades et al., 2012). The FA value is dependent on the number, density, and size of the axons along with the extent of their myelination (Basser et al., 2000; Schmithorst et al., 2005). The study's goal was to use the FA to compare the white matter microstructure of the four language pathways which encompassed the four bundles of fibers - namely the lIFOF, left AF/SLF, AC-OL, and AMB-PMC. Through tracking the IIFOF, researchers found that the mean FA value of the IIFOF in simultaneous bilinguals was 0.548 ± 0.019, the sequential bilinguals demonstrated a mean FA value of 0.526 ± 0.025, and the monolinguals depicted a mean FA value of 0.516 ± 0.025 (Mohades et al., 2012). These values represent the mean FA for each bundle and their respective standard deviations (Mohades et al., 2012). All of these values present an unequivocal trend of there being a higher mean FA value in simultaneous bilinguals than monolinguals and sequential bilinguals (Mohades et al., 2012).

The results of this study were significant and illustrated a higher anisotropy of white matter over the IIFOF in simultaneous bilinguals which further proved that they are faster in semantic processing and transmission of semantic information than in monolinguals (Mohades et al., 2012). Additionally, language researchers have found a lot of variability in certain tracts within the corpus callosum. These differences indicate that there is variation in bundles between bilinguals and monolinguals in a wide range of linguistic traits such as verbal fluency, reading, writing, and dyslexia (Beaton, 1997, Castro-Caldas et al., 1999, Gazzaniga, 2000, Hines et al., 1992, Hynd et al., 1995, Nosarti et al., 2004a; Mohades et al., 2012).

Bilingual individuals experience many advantages in regards to executive functioning. Executive functions control one's

Researchers have used functional magnetic resonance imaging (fMRI) to identify which brain regions are active when bilingual individuals are performing tasks and switch languages in the midst of those tasks (Marian and Shook, 2012). The studies concluded that the participants have shown an activation in the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate cortex (ACC), bilateral supramarginal gyri, and left inferior frontal gyrus (left-IFG) (Marian and Shook, 2012). These structures are associated with executive function. The left-IFG specifically, is known as the language production center of the brain and has roles in both the non-linguistic and linguistic cognitive control (Marian and Shook, 2012). Similarly, the DLPFC is responsible for attention and inhibition (Marian and Shook, 2012). The left inferior occipitofrontal fasciculus's role is to connect to the inferolateral and dorsolateral frontal cortex in conjunction with the posterior temporal and occipital lobe, which extends below the insula and along the inferolateral edge of the claustrum (Catani et al., 2002, Jellison et al., 2004; Mohades et al., 2012). This large bundle is consequently responsible for language semantic processing (Duffau et al., 2005, Leclercq et al., 2010, Mandonnet et al., 2007, Rodrigo et al., 2008; Mohades et al., 2012).

Researchers have deduced that playing simple speech sounds for monolingual and bilingual adolescents in the presence of background noise sheds light on their sensory processing capabilities. They uncovered that bilingual adolescents were able to express a large neural response which ultimately translated to a higher efficiency in encoding the sound's fundamental frequency and pitch perception (Marian and Shook, 2012). Blood flow in the brain is considered an accurate measure of neuronal activity. The aforementioned study also discovered that bilingual individuals had more blood flow in the brain stem in response to sound, proving that they exhibit a larger neural response. Therefore, bilingual speakers have an enhanced auditory attention which can be owed to their advanced ability to encode sound. In essence, bilingual speakers have more efficient cognitive control mechanisms and sensory processing capabilities (Marian and Shook, 2012). Being bilingual or multilingual can serve an individual with a multitude of benefits that can last them a lifetime. In particular, these benefits and differences are initiated during early childhood development. In bilinguals, the construction of brain circuitry occurs earlier in life and the pathways that promote the learning of the first language are well-developed (Berken et al., 2017).

While it is difficult to pinpoint the specific cognitive processes in bilinguals that give them an advantage over monolinguals, there is research that supports that young bilinguals are able to perform better than monolinguals on tasks requiring high executive functioning. These tasks require participants to select the best response from a set of options, and discard other unimportant information (Kuzyk et al., 2020). These tasks, when administered as early as infancy to 6- and 7-month-olds that have had exposure to bilingual input specifically, show that bilingual children have more efficiency

in attention than monolinguals (Kovács & Mehler, 2009; Singh et al., 2015; Kuzyk et al., 2020). This study used the Stroop task which entails individuals to see a word and then being asked to say the color of the font presented on the word (Marian and Shook, 2012). Inhibitory control was explored in the study and is defined as the suppression of behavioral responses and stimuli that is irrelevant to its goal (Tiego et al., 2018). The study ultimately demonstrated that 24-month-old bilingual toddlers performed better than monolinguals on inhibitory control as they disregarded unnecessary more efficiently information which was measured by a modified Stroop task (Carlson & Meltzoff, 2008; Poulin-Dubois, Blaye, Coutya, & Bialystok, 2011; Kuzyk et al., 2020).

Despite these research findings, it is important to be mindful of the fact that these discrepancies illustrate that there are variances in the language pairs that the bilinguals speak, the age of acquisition of their languages, and the proficiency of their spoken languages (Kuzyk et al., 2020). With that in mind, some researchers have proposed that individuals with a more balanced proficiency in their spoken languages are more likely to have an advantage in inhibitory control (Prior, Goldwasser, Ravet-Hirsh, & Schwartz, 2016; Kuzyk et al., 2020). Executive function can be divided into three connected abilities: cognitive flexibility (which involves the ability to shift between mental sets), examining working memory representations, and impeding responses or distracting stimuli (Miyake, Friedman, Rettinger, Shah, & Hegarty, 2001; Kuzyk et al., 2020). Abundant evidence suggests that bilinguals have cognitive advantages during conflicting tasks and older bilingual adults are efficient in managing conflicting attentional demands through interference suppression (Bialystok, Craik, Klein, & Viswanathan, 2004; Kuzyk et al., 2020). However, there is also evidence of cognitive benefits seen in children performing conflict tasks (Poulin-Dubois et al., 2011; Kuzyk et al., 2020). In essence, bilinguals demonstrate a higher efficiency in suppressing inferences than monolinguals (Kuzyk et al., 2020)

Individuals who are multilingual or bilingual exhibit various learning improvements specifically pertaining to cognitive and sensory processing (Marian and Shook, 2012). These enhancements allow for bilinguals to have a keen ability to process information in their external environment leading to an enhanced ability to learn new things. This also explains why bilingual adults are able to learn a third language better than monolingual adults striving to learn a second language (Marian and Shook, 2012). Another plausible explanation is that bilinguals may have an advantage in learning that stems from their ability to solely focus on the information presented in the new language while simultaneously minimizing the interference of information from their previously learned languages (Marian and Shook, 2012). Bilinguals consequently have a better vocabulary due to their capabilities of comfortably recalling and accessing newly learned words (Marian and Shook, 2012). Peal and Lambert conducted studies on children in Montreal who were either French-speaking monolinguals or English-French bilinguals and measured their performances on a battery of tests (Marian and Shook, 2012). Peal and Lambert predicted lower scores in the bilingual group but instead observed that the bilingual children scored the highest on most of these tests, particularly on tests that involved symbol manipulation and reorganization (Bialystok et al., 2012). These conspicuous differences between monolingual and bilingual children led to further analysis of this phenomenon in studies. These studies demonstrated the remarkable advantages that bilingual children had in their abilities to solve linguistic problems through the use of metalinguistic awareness and nonverbal problems (Bialystok et al., 2012). In essence, many researchers have shown that the advantages of knowing multiple languages go beyond the linguistic benefits that can last a lifetime.

The neurological benefits seen in multilingual and bilingual infants, children, and young adults, continue to extend past early childhood development and into old age. One study focused on lifelong bilinguals in their old age, the maintenance of their cognitive functioning, as well as the delay of the onset of dementia symptoms (Khan, 2011). Within this study, there were 228 patients and 51% of them were bilingual and had a wide array of differences concerning cognitive impairment that were closely monitored in a memory clinic (Khan, 2011). The study concluded that the monolinguals presented symptoms of dementia about 4 years earlier than the bilinguals. The other components of cognitive tests remained the same (Khan, 2011). Another study by Howard (2010) was conducted in Montreal, Canada. This study examined multilingual immigrants who were suffering from Alzheimer's dementia as well as bilingual non-immigrants who grew up speaking French and English in Canada (Khan, 2011). The study consequently reported that participants who could speak two or more languages had a delay in the onset of dementia for an average of 5 years (Khan, 2011). Overall, these findings illustrate the point that bilinguals and multilinguals have a substantial delay of the onset of dementia of about 4-5 years and further proves how multilingualism has the ability to protect against age-related decline.

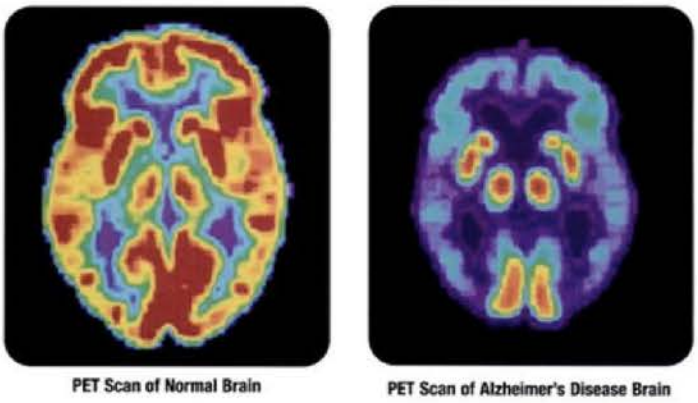

Figure 2. These PET scans depict the differences in brain composition and size between a healthy individual as shown on the left and an individual with Alzheimer's Disease, as shown on the right (Health and Human Services Department, National Institutes of Health, National Institute on Aging, 2013).

Another cognitive benefit of bilingualism is its ability to avert the diminishing of cognitive function and maintain a "cognitive reserve" (Marian and Shook, 2012). A cognitive reserve encapsulates the effective use of brain networks, which augments brain function during the aging process (Marian and Shook, 2012). Bilingualism can contribute to the reserve through the maintenance of cognitive mechanisms as well as the involvement of alternate brain networks. These processes indemnify those circuits that are damaged with age (Marian and Shook, 2012). Older bilingual individuals also exhibited improved memory and executive control in comparison to monolingual individuals (Marian and Shook, 2012). Alongside cognitive benefits, studies that consisted of older and younger individuals, investigated neuroplastic changes in bilinguals and monolinguals. The researchers consequently discovered language group differences in grey matter regions of the brain which are associated with executive function and the control of language (Duncan et al., 2018). Amongst these differences, the older bilinguals exhibited a higher amount of brain matter in comparison to the monolinguals specifically in the left anterior inferior temporal gyrus (Abutalebi et al., 2014) as well as the left and right inferior parietal lobe (Abutalebi et al., 2015a), and both hemispheres of the anterior cingulate cortex (Abutalebi et al., 2015b; Duncan et al., 2018). While on the other hand, the younger adults depicted increased amounts of brain matter in the left inferior frontal gyrus (Klein et al., 2014), the left Heschl's gyrus (Ressel et al., 2012), the left putamen (Abutalebi et al., 2013), the right and left supramarginal gyri (Grogan et al., 2012), and the left and right cerebellum (Pliatsikas et al., 2014; Duncan et al., 2018). In effect, these findings suggest that bilingualism is linked to greater tissue density and thicker cortex than presented in the monolinguals of the study (Duncan et al., 2018).

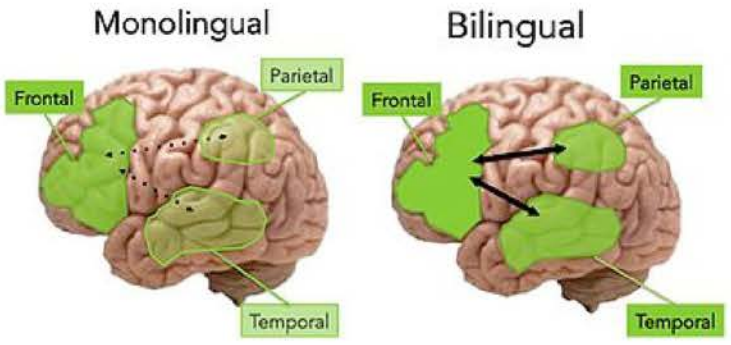

Figure 3. This image encapsulates the differences between the monolingual and bilingual aging brain as the bilingual aging brain presents more connectivity between frontal and posterior areas which form a cognitive reserve. The monolingual aging brain demonstrates a link to a heavier reliance on the frontal regions.

Multilingual and bilingual individuals possess a multitude of benefits such as an enhanced and sharpened cognitive ability which persist throughout their lifespan. Visual representations in fMRI scans demonstrate these specific differences in brain regions between monolinguals and multilinguals. The advantages of being multilingual during early childhood development include improvements in executive function, auditory attention, and the coordination of cognitive tasks in daily life. In older adults, studies have demonstrated the neurological benefits of being multilingual and bilingual and its potential to setback onset symptoms of dementia and Alzeheimer's Disease. Given these exceptional impacts seen throughout an individual's life span, one should strongly consider teaching themselves or their children a second or third language as the benefits can reap rewarding impacts on one's well-being and pave the path for an innate ability to pick an additional language.

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The Effect of Weight Training on Brain Function

Khushi Agarwal

Abstract

The benefits of weight training on physical health are widely known, but this can also affect the structure and function of the brain. From small-scale studies done on rats, monkeys, and humans, there have been results showing lower levels of atrophy of cortical white matter, an increase in grey matter density in the cerebellum, an increase in neural plasticity, and an increase in hippocampal volume BDNF expression - all of which result in higher cognitive function. In rats, this resulted in those with mild cognitive impairment to perform even better on a cognitive test than those without any impairment. Further research could strengthen the preventative potential (in some cases, interventional potential) of resistance training for dementia, depression, and other neurodegenerative diseases.

Resistance training has been emphasized as part of healthy living for many years not only for its bodily benefits, but also prevention of disease and mood enhancement. However, the exact neurobiology and mechanics that take place which lead to these benefits, or any relationship between muscular strength and the brain, have not been explained as extensively. Studies thus far have unanimously shown that weight lifting has a positive effect on both cognitive function and memory through the preservation of white matter, the increase in the density of grey matter, an increased ability to make new neural connections, and an increase in hippocampal volume. Further research could also point towards weight training as a preventative method against aging of the brain, neurodegenerative disorders, and mood disorders such as depression.

Because the brain ages with the person, an outward presentation of forgetfulness, slowed processing of information, or reduced attention capacity are all occurring as we grow older (Filley, 2005). Inwardly, this aging is the result of the loss of white matter - the area in the brain consisting of axons, wrapped in a fatty layer of insulation called myelin. Myelin speeds up signal transmission, so a myelinated axon will carry information much quicker than an unmyelinated axon. In other words, decreased myelination will result in lower cognitive performance and can lead to neurodegenerative disorders and dementia. Studies have shown that resistance training specifically can slow the atrophy of this white matter as the brain ages. One study looked at older adults, in which one group was assigned resistance training and the other was assigned balance and toning (Herold, 2019). After fifty two weeks, the group that performed resistance exercises had a lower level of white matter atrophy and lesions in comparison to the group that performed toning exercises. This suggests that the repetitive motor movement requiring maximal force is more effective in slowing aging of the brain and induces different changes in the brain in comparison to other forms of exercise. In addition, older adults with mild cognitive impairment that performed resistance training twice a week for 26 weeks exhibited increased cortical thickness of grey matter in the posterior cingulate gyrus and better cognitive performance (Herold, 2019). These results are significant as a form of late intervention for MCI and the diseases associated with it, especially in the older population and over a period of only 6 months. Mild cognitive impairment (MCI) is associated with aging and presents itself through memory problems. MCI is also a marker for Alzheimer's, a disease characterized by the decline of grey matter. The results in this study of improved cognitive performance and an increase in grey matter show promise in the prevention of neurodegenerative diseases such as Alzheimer's just through resistance training.

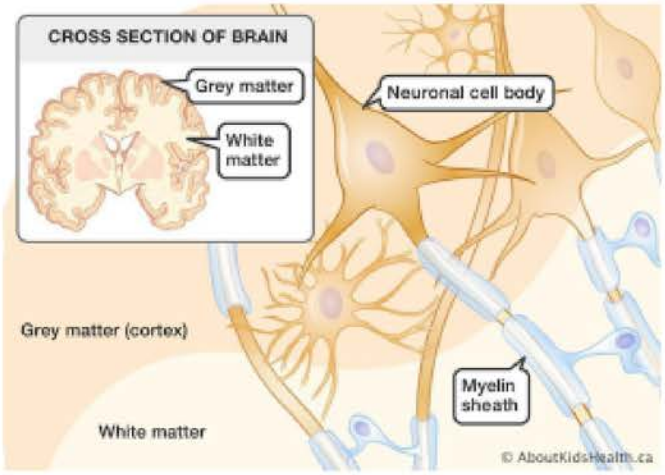

Figure 1: A schematic of the white and grey matter in the brain, composed of partly axons wrapped in myelin and neuronal cell bodies respectively.

Prevention is only possible in the time before diagnosis; after diagnosis, it's often much more difficult to slow, stop, or show improvements from many neurological diseases such as forms of dementia. However, one study has shown promise for improved cognitive function in patients with dementia through resistance training (Kelty, 2019). A group of rats were given an injection to induce inflammation in their brains, similar to dementia in the human brain. Half of those rats began a form of strength training with a progressive increase of weights. When placed into a maze as a cognitive test, the rats with the inflammation performed significantly worse than the rats without the inflammation. However, after six weeks, the rats with the inflammation on the strength training regime

improved and performed equal or better than the rats without the inflammation. In the brain tissues of these rats, there were increased signaling proteins and genetic markers like IGF-1, which is an insulin-like growth factor. IGF-1 acts as a signaling molecule to activate the conical protein kinase B and ERK1/2 pathways which leads to increased signaling of the downstream protein, AKT. Resistance training induced IGF-1 activation of AKT signaling is associated with increased neuronal proliferation and survival. This indicates the creation of new neurons and greater persistence of the neurons, suggesting that the resistance training allowed the rats' brains to remodel themselves and make new connections - significantly increasing cognitive function. The creation of new neurons and new learning connections exemplifies the increased neural and synaptic plasticity of the brain, which shows promise in reversing the effects of the inflammation. Neuroplasticity is the brain's ability to form or reorganize neural connections and therefore learn new skills or store memories. In this case, it may allow the brain to relearn previously forgotten information in early-stage Alzheimer's disease (Hill, 2011). Although neurodegenerative changes like hippocampal atrophy occur in this early stage, cognitive plasticity can still be maintained; however, the neurobiological mechanisms of these plasticity-related events are unclear at this time. With further research, this can be paralleled in humans with hopes of possibly reversing the effects or improving cognitive function in various forms of dementia.

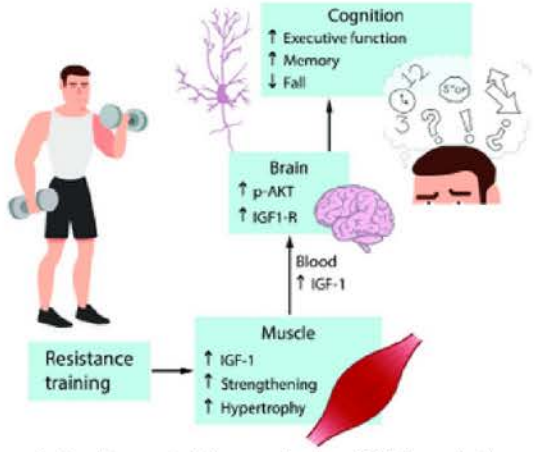

Figure 2: Resistance training can induce IGF-1 production, which acts as a signaling molecule to activate the conical protein kinase B and ERK1/2 pathways. This leads to increased signaling of the downstream protein, AKT, which is associated with greater neuronal proliferation and survival.

Thus far, it's been shown that resistance training is far more effective in preserving both white and grey matter and improving cognitive function than other forms of exercise. However, there is some ambiguity in what variable or mechanism associated with resistance training is responsible for this neural relationship. One study, done on female macaque monkeys, suggests that lifting weights results in changes in the brain before there is any increase in strength or muscle mass. From 50 trials of the monkeys pulling a weighted handle with progressive overload over a period of 9 weeks, it was concluded that the monkeys' brains experienced neural adaptations at the cortical level weeks before their muscles showed signs of hypertrophy (Glover, 2020). This suggests that the effects of strength training on brain function may not come directly from the muscle mass gained, but from the physical training regime itself. Another study supports the theory that these neural adaptations are associated with muscular strength: a group of young, healthy adult humans were studied, in which half of them followed a strength training regime. After four weeks, the individuals who gained the most strength, as measured by the increase in their maximum voluntary isometric contraction, also showed larger increases in white matter (Palmer, 2013). Because the participants' strength and white matter density showed correlation, it can be concluded that the intensity, consistency, or the duration of exercise is correlated with neural changes, since muscular strength can be a confounding variable (which also has a positive correlation with the intensity and consistency of the weight training).

The improvement in cognitive function and prevention of neurodegenerative diseases from physical exercise is partially due to the hippocampus. The hippocampus is a brain structure that resides in the medial temporal lobe and is responsible for important functions such as learning and memory, but is also highly susceptible to damage by an array of stimuli such as stress and aging. It is also part of the limbic system, which regulates emotion and motivation. The atrophy of the hippocampus is associated with many conditions such as Alzheimer's disease, major depressive disorder, schizophrenia, PTSD, and epilepsy. Fortunately, its atrophy is preventable through strength training, largely because of its induction of neurogenesis. One study done on 120 older adults over one year found that hippocampal volume increased by 2% (compared to the 1-2% annual shrinkage of the hippocampus) and increased hippocampal blood flow.

It was also found that BDNF, a neurotrophic factor, could be the link between exercise and increased hippocampal volume (Erickson, 2011). Exercise upregulates BDNF gene expression and therefore increases BDNF serum concentration, which also promotes synaptic plasticity. This translates to the prevention of the discussed diseases associated with the atrophy of the hippocampus, improved overall cognitive function, and increased or conserved functions of the structure such as spatial memory, decision-making, character judgements, and empathy (Rubin, 2014). One study also found that BDNF can have antidepressant-like effects for those with major depressive disorder. Using the Western Blot, the study found that the hippocampi of suicidal patients with depression had lower levels of BDNF compared to non-suicidal controls (Karege, 2005). Therefore, exercise inducing BDNF expression shows promise to decrease depressive symptoms. Depression is common and on the rise, impacting roughly 40 million adults in the US, and is associated with mild cognitive impairment and an acceleration of brain aging, which translates to accelerated cognitive decline. The evidence showing the relationship between exercise, BDNF expression, hippocampal volume, and depression is present and significant, but is limited. Further research could strengthen the preventative potential

depression, as well as exercise-induced BDNF as a form of an antidepressant.

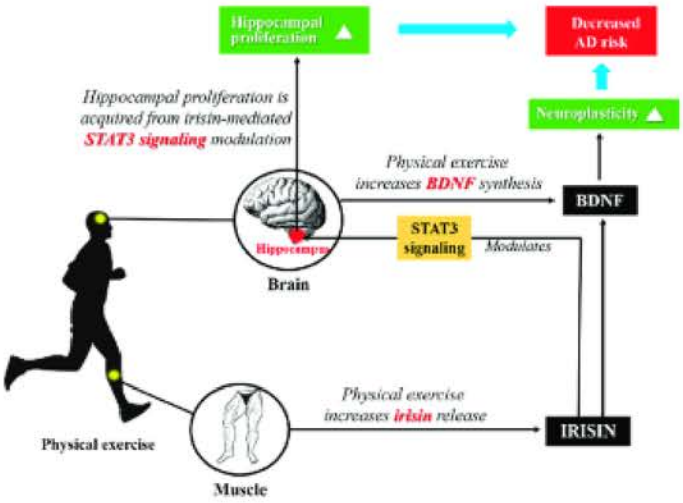

Figure 3: Exercise upregulates BDNF gene expression and therefore increases BDNF serum concentration, which also promotes synaptic plasticity. This translates to the prevention of the diseases associated with the atrophy of the hippocampus, such as AD, and improved overall cognitive function.

Although the studies available right now are small-scale and limited, weight training could be a form of preventative and mitigative medicine for neurodegenerative and neuroinflammatory diseases with further research. This is important even for a population with no cognitive impairment, for which regular resistance training could prevent cognitive decline and improve brain function. Many of the studies on this topic are done on a population with some form of mild cognitive impairment, so further study can be done on populations with no cognitive impairment to see if there are still effects on brain and cognitive function. Further research can also be done on the aspect of weight lifting correlated with improved cognitive function, such as the upper or lower limbs since these deteriorate at different rates, and whether it is the increased muscle mass or repeated motor movement requiring significant force which produces the results. Resistance training is a fundamental facet of healthy living, but also has strong preventative potential for an array of conditions, such as but not limited to: depression, other mood disorders, Alzheimer's disease, other forms of dementia, mild cognitive impairment, and forms of neurodegenerative disorders. With further research, it is possible for resistance training to possibly even have interventional potential in a clinical setting.

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The Science Behind the Guilty Brain and the Future of Neuroprediction

Violet Park

Abstract

As artificial intelligence and neuroimaging advance, society must consider the extent to which predictive algorithms should be applied to everyday life, such as within the legal system. By measuring how "guilty" you feel according to your guilt-related brain signature (GRBS), neuroprediction in court can be utilized for the benefit of humanity and permanently alter the way the justice system runs. Despite the advanced technology we've been able to formulate thus far, we run into obstacles when it comes to bias, privacy concerns, and consent. As society progresses toward the future, it's crucial not to overlook the evident concerns and reflect on how far we're willing to let technology take us.

Introduction

Whether it's lying to someone or secretly stealing a cookie from the cookie jar, we've all felt guilt. Guilt often haunts us and can sometimes push us to confess the truth. But what if there was a way to measure guilt? Knowing whether someone is guilty or not could help us decide whether those accused of a crime truly did commit it. We would be able to ensure those who are guilty pay for their crimes and those who are innocent are proven so. To know how to measure guilt, we first have to define what guilt even looks like.

What Does A "Guilty" Brain Look Like?

Before examining how neuroprediction and other methods of predictive algorithm work, we need to understand how to measure guilt and the science behind it. Scientists have identified what is known as a guilt-related brain signature, or GRBS, that is expressed when one feels responsible for the harm of another (Hongbo et al., 2020). GRBS serves as a key biomarker, which is some measure that indicates a condition like a disease or infection. GRBS is present in conditions of physical pain and emotional memories, which means it has generalizability and can be applied to not only a single sample but in a wide spread of studies. In addition to GRBS, self-reported guilt has often been associated with activation of the anterior cingulate cortex (ACC). ACC was activated whenever the individual was perceiving another's suffering or had the knowledge that their actions were causing the suffering of others. Although there is belief that guilt may be found in more than a single voxel of the brain, associations between the ACC and guilt can be a great starting point for understanding what parts of our brain are encoded by guilt.

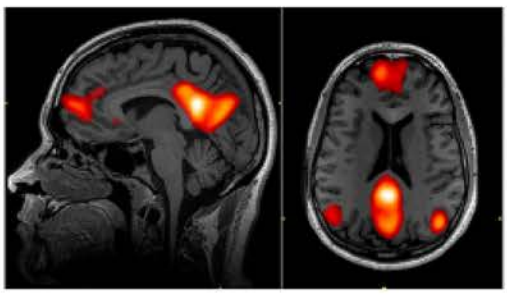

Figure 1. Displays how guilt-related brain signatures are expressed in the brain via electroencephalography (EEG) signals

Current Methods of Neuroprediction

Neuroprediction is the ability to predict human behavior by utilizing neurocognitive data. It can be applied to anticipating recidivism, which is the tendency of a convicted criminal to reoffend (Tortora et al., 2020). The only consistent methods of neuroprediction today are derived from fMRI scans and neuroimaging with AI. These fMRI scans can analyze activity of the ACC,which is in charge of impulse control and error processing. Based on ACC activity, scientists have found that the probability that offenders with low ACC activity would be arrested was approximately double compared to offenders with high activity. Previous fMRI data has shown to be useful in predicting the completion of substance abuse treatment within a prison inmate population using event-related potentials (ERPs) and functional network connectivity (FNS), which identified "neural fingerprints" that predicted cocaine abstinence during treatment (Elliott et al., 2020). However, there are drawbacks to measuring ACC activity alone. There is a likelihood that guilt is stored in more than one part of the brain resulting in an inaccurate measure of guilt. Essentially, neuroimaging identifies potential neurocognitive markers and combines it with statistical machine learning methods to create a multi-voxel pattern analysis (MVPA). MVPA has been readily used in healthcare for years to determine differences between healthy and diseased brains, but it has also been used to measure other factors such as the intention to perform one task over another, sequential stages of task preparation, and lie detection. Unlike examining ACC activity, which compares experimental conditions to identify which brain regions are activated by particular tasks, MVPA looks at patterns of brain activity to decide what subjects are looking at or thinking about like a mind-reading or brain-reading technique. In addition, MVPA has the ability to read the brain in the domain of visual perception by looking at how experiences are encoded in the brain. This is done by training a deep neural network to perform visual image reconstruction from the brain and decode visual content of dreams.

Ethical Issues and the Legal System

Neuroprediction has been a rising topic in the legal system as it could be key in deciding criminal sentences, parole, use of the death penalty, and discharge. The risk assessment

analyzes characteristics about the individual from criminal history, drug use, job history, childhood abuse, and more, to determine their risk of recidivism. Although there are high hopes for utilizing neuroprediction in court, current risk assessment displays poor to moderate accuracy with more than half of individuals targeted as high-risk being misidentified. Ethical issues associated with the use of neuroprediction include bias, privacy, and consent and coercion. In the past decade, there have been numerous cases in which race or gender has often played a role in misidentification, for example, Amazon Rekognition software incorrectly matched members of Congress with people who had been charged with a crime. The facial recognition software disproportionally wrongly identified African American and Latino members of Congress. Similar algorithms such as Predpol in 2016 unfairly targeted certain neighborhoods with a high proportion of people from racial minorities regardless of effective true crime rates. Regardless of the algorithm, those against neuroprediction argue that identifying guilt will always be as biased as the people who use it. For instance, AI trained on data such as criminal files may reflect biases on part of police officers, prosecutors, or judges.

Another pressing issue involves privacy. Researchers have found that neurodata can be used to screen job applications or lead to the commercialization of medical records. If data collected by neuroprediction falls into the wrong hands, privacy of individuals can become easily breached and spread to major corporations or to the general public. In the courtroom, use of machine learning methods can often lead to discussion about consent and coercion. Although there are arguments that algorithms designed to identify high-risk and low-risk offenders could be utilized for legal decisions, performing cognitive violations by forcing people to undergo scans without consent for sentencing becomes a concern. In addition, neuroimaging can exert a "seductive allure" that makes jury and judges overestimate accuracy of neuroscientific images making it misleading and create cognitive biases in the evaluation of evidence. With the endless concerns regarding the use of neurodata, we must evaluate whether the benefits of neuroprediction overpower the costs

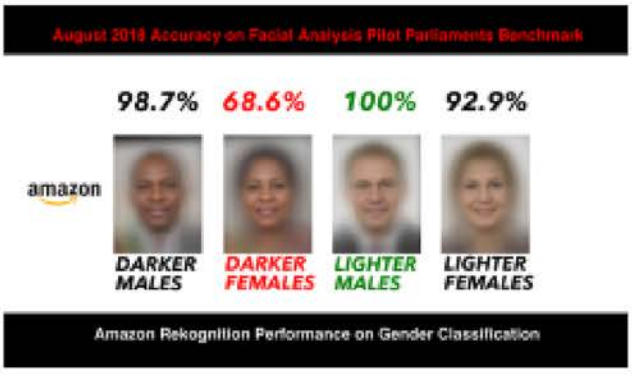

Figure 2. Analysis of Amazon Rekognition software's accuracy presents gender and ethnic bias

Conclusion

As researchers continue to advance the art of neuroprediction, we must take into consideration the negatives that come with it. The use of neurodata is a powerful tool that would allow us to measure "guilt" in the brain and analyze what one is likely thinking. A program like this would give us the opportunity to prevent crimes before they occur, as well as assess the risk of a criminal to help determine their sentence. Despite these benefits, concerns regarding bias, privacy, and consent and coercion as well as the current inaccuracy of neuroprediction bring into light whether we should even continue to work on improving neuroprediction. If implemented into our society, how could we ensure that racial and gender bias are not factors in determining guilt and what can we do to eliminate coercion of offenders to agree to the programs? Before we fully develop neuroprediction, let's first consider as a society how far we're willing to take technology.

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Stem Cell Therapy for Traumatic Brain Injury

Neha Bashir

Abstract

Traumatic brain injury is a prevalent issue in the world, and recent development of stem cell therapy has led to advancements in the treatment of this disease. Using endogenous neural progenitor cells, as well as transplantation of exogenous stem cell therapy can assist in the promotion of cognitive and motor skills by increasing neural stem cell proliferation. Exogenous therapy can be used to further induce endogenous therapy, all while repairing the damaged tissue and maintaining the homeostatic balance of the cells within the body. Molecules like curcumin-loaded niosome nanoparticles can assist in the promotion of the neural stem cells and will further improve the regeneration of injured cells in the brain. Pediatric brain injury differs from adult traumatic brain injury, with differences in the treatment and overall process of the incorporation of the neural stem cells, but using exogenous and endogenous therapy in similar ways can yield proliferation and development in neurons, preserving the cognitive functions of the child.

Introduction

After an object violently hits the head or pierces the skull, entering the tissue, immediate impact on the brain can cause traumatic brain injury. Traumatic brain injury occurs when a sudden trauma causes damage to the brain. Depending on the severity of the traumatic experience, mild traumatic brain injury can occur, which affects the brain cells temporarily. However, more severe brain injury can result in long-term complications and death.

Severe brain injury can be categorized into two different types of injury. Primary injury occurs when there is damage to the brain tissue, neurons, glial cells, endothelial cells, and the blood-brain barrier. The harm from the initial impact of an object exclusively causes primary injury. Secondary injury follows primary injury, where the damage causes injured cells to release several toxic, forming a cytotoxic cascade. The formation of cascades the initial, primary brain damage and further increases the risk of lasting neurodegenerative and inflammatory diseases.

Early research has shown that after traumatic brain injury, little can be done to reverse the initial trauma, leading to harmful effects. Arising disabilities that may occur can affect cognitive functions like thinking and memory, sensory processive, communication, and behavior changes including spontaneous outbursts and depression. More uncommon consequences can include unresponsive or vegetative state. Additionally, traumatic brain injury can affect different people differently, depending on the level of brain development and severity of brain injury. Childhood traumatic brain injuries can result in permanent disabilities and reduced quality of life from effects of visuomotor and cognitive impairment.

Recent development in scientific and technological advancements have allowed researchers to explore the therapeutic potential of stem cells following traumatic brain injury on different scales. Experiments have illustrated how intravenous stem cell treatment can enhance functional recovery following damage to parts of the brain. Stem cell therapy illustrates an anti-inflammatory approach to identify injured tissue and repair the damaged cells, speeding up internal recovery and decreasing the likelihood of permanent cognitive disabilities.

Stem Cells

Stem Cells are cells found in the body that are responsible for the specialized function generated by all other cells within the body. To do this, stem cells divide to form daughter cells that can then be specialized for different uses in the body. Stem cells are also incredibly important in providing renewable resources for studying normal development of diseases, and testing drugs and therapies. In addition, stem cells have been found to have potent anti-inflammatory effects. Young stem cells have a regulatory influence on the body and can be utilized in big cell quantity transplantation. They can also lessen the immune reaction that the body is unable to control on its own. Stem cells are able to perform this reaction by regulating how the immune system repressseds pathological responses, but maintains the integrity of fighting off diseases. Rejection of transplantation is then minized because the stem cells are capable of repressing the immune response that prevents the rejection. Many different types of stem cells can be found in the body, including mesenchymal stem cells, found in bone marrow that are important for repairing tissues, such as cartilage, and neural stem cells, cells of the nervous system that make up neurons and glial cells.

Mesenchymal Stem Cells (MSCs) can be vital in differentiating neural tissue and creating neuronal cells. Directional differentiation into mesenchymal and non-mesenchymal tissues is an important capability of mesenchymal stem cells. By differentiating the tissue, they may encourage the repair of injured tissues by reducing inflammation, secreting trophic factors, and enlisting local progenitor cells to replenish missing tissue cells. MSCs focus on only neural tissue that is separated and can increase the maintenance and care of that specific tissue. MSCs can decrease the expression of inflammatory proteins, leading

to anti-inflammation and decreasing edemas and aneurysms. MSCs can also prevent the body from overproducing and using T-cells. T-cells are lymphatic cells vital in fighting infections but can also result in the body attacking its own cells, causing autoimmune disorders. The overproduction of T-cells in the body can signal cancer or other infections the body is trying to fight and the presence of Mesenchymal cells ensure that T-cells are only overproduced when there is a real threat to the body. The impact of MSCs occurs without weakening the patient's natural immune system or making them susceptible to illness, due to a decrease of T-cells. Mesenchymal Stem Cells are important in the regulation of damaged neural tissue.

Neural Stem cells (NSC) are self-renewing stem cells that can develop further into oligodendrocytes, glial cells, and neurons. NSC transplantation could be a successful, long-term therapy for neurological rehabilitation following brain damage. The proliferation, differentiation, and other activities of NSCs might be improved by the transfection of growth-promoting genes into NSCs. Neural stem cells are sensitive to change, and can travel through the nervous system to find different sites of injury and improve recovery.
While Mesenchymal Stem Cells and Neural Stem Cells are the two primary stem cells, many different stem cells exist in the body that exist in smaller quantities. Induced pluripotent stem cells are important in self-renewing cells and are also utilized for differentiation and specialization. They are also useful in restoring brain function immediately following injury. Endothelial Progenitor Cells are recruited to the site of endothelial tissue, and can be critical in endothelial healing following brain trauma. They can help retain the integrity of the white matter, lessen the capillary damage, and control the local angiogenesis, or the process where new blood vessels form from pre-existing vessels.

Endogenous Neural Progenitor Cells Therapy

Endogenous Neural progenitor Cell therapy refers to endogenous restoration of damaged cells via mature neural regeneration. The movement of new neuronal cells to the area of damaged tissue is guided in order to maintain long-term survival of the harmed tissue. Endogenous Cell Therapy in response to traumatic brain injury has shown elevated cell proliferation levels in subventricular zones and in the hippocampus of more severely injured animals. Using rodent models, research concluded that injury-induced endogenous neurogenic stem cell therapy is directly correlated with cognitive functional recovery.

In order to perform endogenous neural progenitor cells therapy different nurophins must be active for support. Metformin is a drug shown to mobilize endogenous progenitors in the hippocampus following traumatic brain injury. Metformin was initially used to manage diabetes by mimicking a binding protein to regulate glucose production. This same binding protein, called CREP, can be mimicked in a kinase pathway responsible for recruiting endogenous neural progenitors in different zones of the brain, called subgranular zone and subventricular zone. Metformin can mimic the CREP binding protein and activate the protein kinase pathway, stimulating endogenous neurogenesis.

One method of endogenous neural progenitor cell therapy is by using oligodendrocyte progenitor cells. These cells can differentiate into oligodendrocytes in the developing and mature brain, and are primarily utilized for the development of myelination and myelin plasticity. Oligodendrocyte progenitor cells are also important in maintaining a homeostatic balance of cells in the brain throughout life. When tissue is damaged, demyelination occurs, resulting in oligodendrocyte progenitor cells becoming reactive to produce oligodendrocytes. The new oligodendrocyte can structurally change, proliferate, and migrate to the site of injury in order to repair and increase the rate of wound closure.

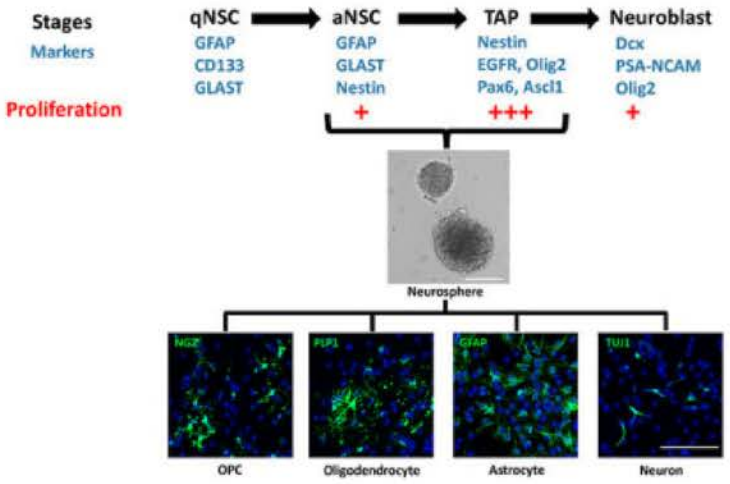

Figure 1. The figure shows the stages of neural stem cells and the level of proliferation. Only activated NSCs and transit-amplifying progenitors grow as neurospheres and differentiate into the oligodendroglial, astroglial, and neuronal lineages in vitro. Endogenous Neural Progenitor Cells Therapy increases the proliferation of the different daughter cells.

Exogenous Stem Cell Therapy

Exogenous stem cell therapy works by migrating exogenous stem cells towards damaged brain tissue, which then participate in the repair of damaged brain tissue by further differentiation to replace damaged cells, while releasing anti-inflammatory factors and growth factors, thereby significantly improving neurological function. Exogenous stem cell transplantation has accelerated immature neuronal development in damaged areas, allowing for potential treatment for post-traumatic brain injury regeneration by increasing development of new neurons that are not inherently damaged. Transplanted cells can replace the damaged neural cells with new, viable cells and also provide neurotrophic support to reestablish and stabilize the damaged brain tissue.

Given the limited supply of endogenous neurogenic stem cells, exogenous stem cell supplementation to the injured brain tissue by neural transplantation is a promising therapy for post-traumatic brain injury regeneration. In addition to being able to replace the destroyed neural cells, the transplanted cells will also provide neurotrophic support in the aim of reestablishing and stabilizing the destroyed brain

tissue. Mesenchymal stem cell transplantation, as well as neurotrophic factors derived from MSCs have driven endogenous neurogenesis. Neural stem cells can also secrete neurotrophic factors that can drive endogenous neurogenesis. There is a greater proliferative cell response with exogenous NSC transplantation. These findings demonstrate how exogenous stem cell therapy can upregulate endogenous stem cell therapy, which further increases rates of repairing injured tissue from traumatic brain injury.

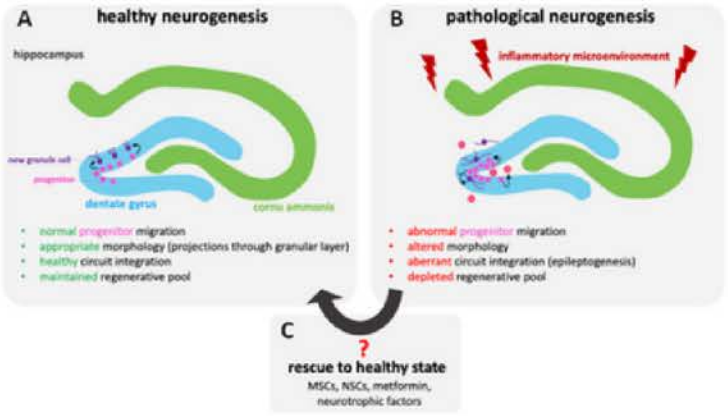

Figure 2. The difference before and after traumatic brain injury, showing healthy and pathological hippocampal neurogenesis. Part A demonstrates hippocampal neurogenesis in healthy tissue. Part B shows injured tissue and the depletion of the regenerative pool, along with abnormal progenitor migration and different structures. Part C illustrates exogenous stem cell therapy through transplantation and how neurogenesis is shown to be restored.

Nanoparticle Therapy

Simply using endogenous and exogenous cell therapy may not be enough to fully restore the functions of the damaged tissue to their full capacity, but combining the neural stem cells with nanoparticles loaded with curcumin noisome can be used to improve brain inflammatory responses associated with traumatic brain injury. Neural stem cells from the brain can be accompanied with curcumin-loaded niosome nanoparticles to improve functional recovery by decreasing the severity of the impact of damage to a pathway in the body referred to as TLR4-NF-kB. The curcumin-loaded niosome nanoparticles show decreases in neuroinflammation after injury because of inhibition of stress oxidatives and down-regulating the pathway.

Pediatric Therapy

Traumatic brain injury (TBI) is prevalent in the pediatric population as it is the leading cause of death and disability in children. Characterizing the activity of neural stem cells and neural progenitors in the immature brain in response to injury can help in the understanding of brain injury pathology and the development of therapeutic targets for pediatric TBI. The location, type, and population density of neural stem cells (NSCs) are developmentally regulated. Adult and pediatric brains display different responses to stem cell therapy. Both neonatal and pediatric traumatic brain injury result in a large increase in proliferating glial fibrillary acidic protein (GFAP)-positive cells, indicating increased astrocytic proliferation and astrogliosis, which in turn leads to inflammation and further damage.

The main focus in pediatric therapy following traumatic brain injury is highlighting the anti-inflammatory effects, while also enhancing the long-term survival and integration of the stem cells. Mesenchymal stromal cells may help in tissue repair and regeneration, are activated by neonatal HI and go to the site of the wounded tissue from the peripheral circulation. Between 3 and 7 days after neonatal HI, stem cell factor expression rises in the periventricular, corpus callosum, and hippocampal regions. In the injured brain, neural stem cells' capacity for self-renewal and natural propensity to differentiate into neurons and glial cells may help to promote regeneration and neurogenesis.

Conclusion

The recent advances in stem cell therapy in managing the treatment of traumatic brain injury have been improved using endogenous neural progenitor cells and exogeneous stem cell transplantation. The development of stem cell therapy increases cognitive and motor skills and directly increases the repairment of cells in damaged tissue. Stem cell therapy has been used to treat adults and children alike, with assistance from different particles to further enhance treatment. More research should be conducted on the effects of stem cell transplantations in different regions of the brain, along with different damage levels of the injury site. Many researchers believe the exploration of stem cell therapy is a growing avenue for the improvement of regeneration and healing of damage sites that were once believed to be unrepairable.

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Exploring Human Memory and Understanding The Human Tendency of Forgetting

Delisha Nair

Abstract

Memory is a vast and complex branch of human cognition that is pivotal to the understanding of human beings. In fact, the topic of memory is so extensive that there are three different types of memory: sensory memory, short-term memory and long-term memory. Each type of memory is different on how much information can be stored and for how long, with sensory memory holding the least amount of information and for the shortest amount of time, and long-term memory holding the most amount of information and for the longest amount of time. However, it does not end there as there are multiple divisions of long-term memory: explicit and implicit, which each branch out into further, more specific types of memories. It should come to no one's surprise that with the brain having to store and remember so many different memories, it will forget some in the process of recalling others.To explain the process of forgetting and recalling, theories, like: Interference Theory, Decay Theory of Forgetting, Retrieval Failure Theory and Cue Dependent Theory. However, when a memory is forgotten, it is not removed from your long term memory; forgetting typically has to do with a failure of memory retrieval. Now, in order to help one's brain remember and recall all of the memories, there are various different kinds of techniques that people can employ to improve their memory. These range all the way from exercising the brain by playing puzzles to changing one's diet by incorporating less sugar in their meals. However, it is important to note that no matter how hard one tries, the brain is still limited on how many memories it can store, even if that limit is extensive.

What is Human Memory?

In a simple definition, memory is one's ability to recall information. There are multiple different types of memory, which can be categorized into three types: sensory memory, short-term memory and long-term memory (Harvard Medical School, n.d).

When information is first entered into the brain, it is sent to the sensory memory. Sensory information holds sensory information for one second or less. However, if a person pays attention to the sensory memory, then it can be moved to short term memory and then possibly long term memory (according to one model of theory). This is the beginning of memory processing. Next in the memory stages comes short term memory, which consists of information that someone needs to recall for a few seconds or minutes. Short term memory is limited in the amount of information that it can store as it includes remembering a string of five to seven words and repeating them back or remembering a phone number whilst getting a pen and paper to jot it down for future reference (the golden rule is 7 +/- 2 items). Lastly, all other information is stored in long-term memory after repetition, which is responsible for storing a range of memories and experiences from one's life. Generally speaking, any information that someone recalls for more than 30 seconds usually ends up being a part of long term memory. Within long term memory there are two different categories in which the memories can be categorized into: explicit and implicit memory. Explicit memory consists of conscious memories of events, facts, and other things that a person learns about. Within explicit memory, there is semantic memory and episodic memory. Semantic memory is concerned with remembering factual information like what is the capital of the United States, whereas episodic memory is the memory of events in an individual's life, like remembering their 4th birthday party or where they were on the day of the 9/11 attack. On the other hand, implicit memories are the opposite of explicit memories, meaning that they are memories that people are not consciously thinking about. Procedural memory, priming and conditioning all go under implicit memory. Procedural memory is the memory that a person has about performing a task, whether that be remembering how to drive or how to make their grandmother's famous thanksgiving pie. Priming is when an individual is more likely/faster to identify a stimulus if they have seen it before. Whereas conditioning can be applied to memory in the sense that a stimulus can be remembered due to the learned association of something (Villines, 2020).

There are different cortical structures that are associated with explicit versus implicit memories. More specifically, there are three structures used for explicit memory: hippocampus, neocortex, and amygdala. Located in the temporal lobe, the hippocampus is where episodic memories are formed and stored for later use. Whereas, the neocortex is a sheet of neural tissue that forms the outside surface of the brain and is the largest structure of the cerebral cortex. The information from memories that are temporarily stored in the hippocampus then gets moved to the neocortex where it functions as general knowledge. Lastly, similar to the hippocampus, the amygdala is a structure in the temporal lobe that is responsible for allowing us to process emotions such as fear. The relation between emotion and memory is that the stronger the emotions that are associated with a memory, the more likely one is to not forget it. Moving on, there are only two cortical structures related to implicit memory: basal ganglia and the cerebellum. The basal ganglia are multiple structures located in the center of the brain that assist with habit formation, movement, learning, reward processing and emotion, among other functions.

When it comes to memory, the basal ganglia help facilitate formation of procedural memories that are needed to perform certain skills. Similarly, the cerebellum, a structure located in the back of the head, is involved in processing procedural memories. (Queensland Brain Institute, n.d).

Human Tendency to Forget

Contrary to popular belief, forgetting does not always indicate memory loss; rather, forgetting can be viewed as altered memory access and can lead to more flexible behavior and decision making. Forgetting allows us to have storage space for creating new memories; without forgetting, our brain would have too many memories that would consequently cause our memory recall to be inefficient (Chawla, 2018). Forgetting happens because, in the spur of the moment, the brain cannot decode what is important during encoding, so it tries to remember everything and gradually forgets any information that is not important. Changes in our ability to recall specific memories are based on environmental feedback and predictability. Humans learn to forget memories when trying to retain memories that are more important and necessary to recall in the future.

When focusing on the biological aspect, memories are stored in groups of neurons called “engram cells” and recall occurs when these cells are successfully reactivated. Forgetting occurs when these engram cells fail to activate. Memories are still stored but until and unless the specific engram cells for that memory are activated, that specific memory cannot be recalled. All the different ways of forgetting have one thing in common: they try to make the engram cells harder to access and activate (Chawla, 2018). For memory-related diseases like Alzheimers, forgetting mechanisms that are responsible for “natural forgetting” are taken over, thus resulting in decreased engram cell accessibility, consequently decreasing activation as well. Scientists have now proposed a new theory that states forgetting occurs due to the circuit remodeling which is a mechanism that causes the engram cells to deactivate from being in an activated/accessible state (Trinity College Dublin, 2022).

There are many theories that propose an explanation for the human tendency to forget. For instance, according to Interference Theory, forgetting occurs as a result of multiple different memories interfering with each other. The more similar the memories, the more likely they are to interfere with each other. The Decay Theory of Forgetting states that the length of time between the encoding of a memory to its retrieval determines what memories and information will be recalled and what will be forgotten. The relationship between these two variables is that the longer the time interval, the decreased probability that someone will recall the memory. Whereas, the Retrieval Failure Theory states that humans forget information because the environmental stimulus failed to enter their long term memory. The last theory is Cue Dependent Theory, in which information that is present in our memory cannot be recalled unless retrieval cues are given (Cherry, 2021).

What Happens To A Memory When It's Forgotten?

Memories are first stored in the hippocampus and then can be stored in the neocortex after some time has passed. Through the process of synaptic plasticity, neurons are continuously producing new proteins that remodel their synapse, which is a gap between the neurons that allow for chemical messengers to be passed through. Consequently, this causes the neurons to strengthen the connections with one another. This forms a network of cells that work together to encode a memory, and the more often a memory is recalled, the stronger the connection becomes. When the frequency of a memory being recalled decreases, the connections between neurons weaken, making it harder to access a memory, thus leading to forgetting. Memories are still in your brain even when they are forgotten as there is no passive decay of memories. Everything isn't gone. A memory is not removed from your long term memory when it is forgotten; moreover, forgetting has to do with a failure of memory retrieval (Gravitz, 2019). In a sea slug experiment, when looking at the gene expression on both sides of the brain, 11 genes were still active on one side of the animal’s brain even though they had apparently forgotten about the shock. The test exemplifies the idea that memories continue to stay in the brain even after they have been forgotten (Chawla, 2018).

Ways to Improve Memory

There are multiple ways in which humans can improve their memory and thus reduce their tendency to forget. Some methods include staying mentally active by engaging in mentally engaging activities like solving puzzles, using a different route when driving, playing an instrument, etc (Mayo Clinic, n.d). These are all methods in which people can exercise their mind to keep it active and improve memory by challenging their mind to complete mentally stimulating activities. Incorporating healthy habits in your day-to-day life like sleeping well, being physically active and eating less added sugar helps your body, which then has a positive impact on your brain. More specifically, sleeping allows the brain to reconsolidate a person’s memory, thus allowing them to better recall that information later. As seen in MRI scans, slow brain waves of deep NREM sleep transported memories from the hippocampus to other permanent sites of storage, according to Matthew Walker, Professor of Neuroscience and Psychology at UC Berkeley (Cappello, 2020). Physical activity leads to an increase in blood flow through the body, especially the brain, which then aids in keeping your memory sharp. When it comes to diet, a high sugar diet has been linked to cognitive decline, poor memory and reduced brain volume, so reducing the amount of sugar present in one’s diet can not only help one’s overall health but also target memory improvement in the brain (Kubala, 2022). Another way to increase the amount of items memorized is by using mnemonics and memory associations can help with making it easier to remember information especially when needing to retain a lot of information. Socializing regularly by talking to loved ones frequently, especially when alone, is shown to

reduce risk of depression and stress, which are both contributors to memory loss (Vogin, n.d).

Conclusion

The human mind and cognition are extremely complex, with memory being one of its most extensive branches. Memory is a significant part of human life, and understanding the science behind storing, recalling and retrieving these memories is so imperative for truly experiencing life. As the brain continues to store more memories, scientists around the world are trying to dig deeper to find answers to questions like what happens when a memory is forgotten? Where does a forgotten memory go? Does a forgotten memory still remain in the human mind? Though there are "surface-level" answers to these questions, there is still so much more that can be learned about this aspect of the human experience.

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Neurodegenerative Diseases & Preventatives

Vyapti Patel

Introduction

For decades, it has been said that the concept of neurogenesis – the process of generating new neurons- occurs during embryonic development only. Though recently through skepticism and experimentation, these doubts have been refuted. The first scientists to make the remarkable discovery were Joseph Altman and his colleagues back in the 1960s. They revealed that adult mammalian species are able to generate new neurons, and that neuron generation does not fully diminish with age. They first started off testing on adult lab rats and then came forth even more scientists who fueled this discovery even further through their own experimentations. This research has opened many doors within the scientific as well as medical community. Unfortunately, combined with the propagation of new neurons comes neurodegenerative diseases in which the opposite occurs, where there is a “gradual loss of different neuronal populations" (Winner, B., & Winkler, J. 1970). It has been shown that “millions of people worldwide” (Hollander et al.) suffer from neurodegenerative diseases as they age. With more pharmacology knowledge and the life expectancy of individuals increasing within the world, it is imperative to find a gateway to help individuals fight these neurodegenerative diseases before it becomes a norm with old-age. With the in-vivo testing methods and the application of already known knowledge with the combination of newly found knowledge on the inner workings of neurodegenerative disorders such as Alzeheimer’s disease as well as Parkinson’s disease, one can prompt prevalent clinical changes to help bring advancements towards a cure for these disorders.

Neurogen. / Neurodegen:

Neurogenesis is a concept that has been widely researched as of recently, especially through the use of adult rats and the inner workings of their hippocampal areas, as well as the olfactory region of their brain, where the creation of new neurons stem from. These new neurons that develop in these areas are what we call, stem cells. Stem cells are what prompted the discovery of adult neurogenesis; these cells are neurons that can ‘divide and differentiate into many types of cells’ (*What is neurogenesis?, 2021*). Their discovery in adult mice has been a realization to the scientific community that there is adult neurogenesis that exists and this may hold the key to fending off neurodegenerative diseases. More in depth mechanisms working in the ‘subgranular zone (SGZ) and subventricular zone (SVZ) of the adult brain’ provide more information on what is occurring within the brain to create new neurons. These sites are major for ‘Cell proliferation’ (Curtis et al. 2013), in which there is an increase in cell numbers due to the division of cells. Proliferating cells in these areas tend to migrate to regions that relate to learning and memory as well as in areas where neurogenesis still occurs in the adult brain, such as the olfactory bulb and in damaged areas due to ‘physical damage or disease’ (Curtis et al. 2013). The SGZ and the Granular Layer help formulate the Dentate Gyrus of the Hippocampus and this has shown that changes in the proliferation of cells in the SGZ can bring about change to an individual's memory and learning.

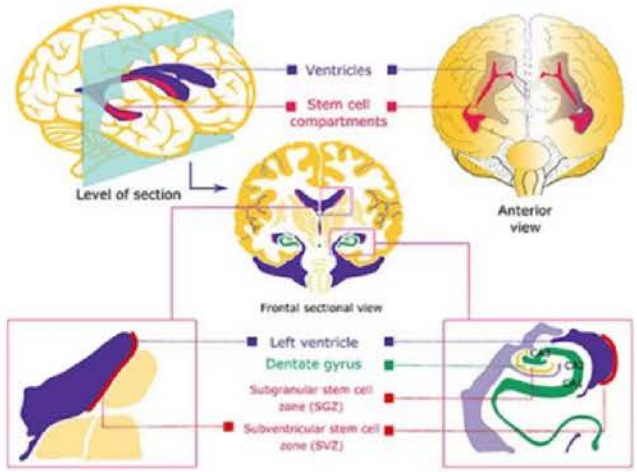

Figure 1. Important Regions Regarding Neurogenesis. In-depth view of where neural stem cells proliferate. (2023). Neural Stem Cell Culture Protocols. https://www.sigmaaldrich.com/US/en/tec hnical-documents/protocol/cell-culture-an d-cell-culture-analysis/stem-cell-culture/n eural-stem-cell-culture-protocols

The human SGZ and SVZ are a bit different than those of rodents, sheep and or other animal models, as humans have “four distinct layers, an ependymal layer (EL) acting as a boundary with the lateral ventricle (layer I), a hypocellular gap region (layer II) where only sparsely scattered cells are seen, followed by an astrocytic layer (layer III) where type A, B and C cells are abundantly located and topographically organized in laminae from superficial to deep” (Curtis et al. 2013). These layers, specifically in healthy adult brain have a cell proliferation “rate of ≈50 PCNA(Proliferating cell nuclear antigen)-positive cells per mm, whereas in the SGZ there were only ≈0.2 PCNA-positive cells per mm” (Curtis et al. 2013), this indicates that any differentiation in this rate can determine whether neurogenesis is occurring at a normal rate or if it is gearing towards a neurodegenerative disease. By being able to examine this, preventative measures can be taken early in order to bring the rate back to its norm, as having a quantitative value can help professionals understand the concept more concretely.

Another factor playing a role in neurogenesis in the adult brain that leads up to neurodegenerative diseases would be the accumulation of the specific proteins termed as proteinopathies. Proteinopathies can originate in the Central Nervous System but are not subjected to just this area as this

can also occur within the peripheral cells, tissues and organs. In principle, these 'proteins change their conformation thereby gaining toxic activity or losing the normal function' (Bayer & T. A., 2013). They can also be characterized by a single aggregate, or by multiple and it is these multiple mixtures that make it hard to come to a 'definite diagnosis' and get 'therapy' for the Proteinopathies. Through understanding the inner workings behind Neurogenesis in the adult brain and its correlation to Neurodegenerative diseases, these advancements can bring about ways to eliminate specific occurrences from happening.

Parkinson's (PD)

Parkinson's Disorder occurs especially in the senior adult population. Parkinson's disease is estimated to affect 'nearly one million people in the United States' (Hollander et al.). The Basal Ganglia plays a huge role in the movements of an individual, and the Indirect and Direct pathways give the ability to do so. The direct pathway is more so related to Parkinson's Disease; as it is a 'positive pathway'(Latifa et al. 2021) allowing for movement to occur. A defect in the direct pathway is what 'inhibits movement' as this results in the Indirect pathway which is a 'negative pathway' to give off stronger signals resulting in the slowing down of movement or a complete stop in movement leading to Parkinson's Disease. These pathways also have excitatory neurons like Glumnate playing a role as well as inhibitory neurons like GABA, in order to balance out movement from the instructions of the release of the different Dopamine groups, but with a defect in one area, results in the abnormal functioning of the movement systems. There often are many pre-disease related symptoms that occur within the individuals such as, "depression, anxiety, cognitive, or olfactory dysfunction, symptoms linked to olfactory or hippocampal function" (Winner, B., & Winkler, J). The symptoms that occur during the stages of Parkinsons can range from Bradykinesia; slow movement, to Hypokinesia; decreased amplitude of movement in which there is an 'abnormally' slow muscle activity, to Akinesia; loss of the ability to move.

A neuropathological feature of Parkinson's is the build up of the clumped up form of the protein alpha-Synuclein, also known as Lewy Bodies (LB's). LB's "are insoluble, intraneuronal protein inclusions" (Marotta et al, 2021). There appears to be an association between reduced cell proliferation in the Dentate Gyrus in Parkinsons but it is also found through experimentation and manipulation in Mice models that, "impairments of the local trophic support of newly generated DG neurons might play a role in the reduction of neurogenesis in the A53T α-syn transgenic model (Winner, B., & Winkler, J, 1970)". Many cell replacement therapies have undergone trial and error. One of them being, "human dopamine-producing fetal midbrain neurons into the striatum of PD patients was performed (Winner, B., & Winkler, J, 1970)". These transplants of the dopamine producing fetal neurons seemed to be a success in producing dopamine being functionally integrative, as it led to clinical improvement but led to a more severe side-effect of

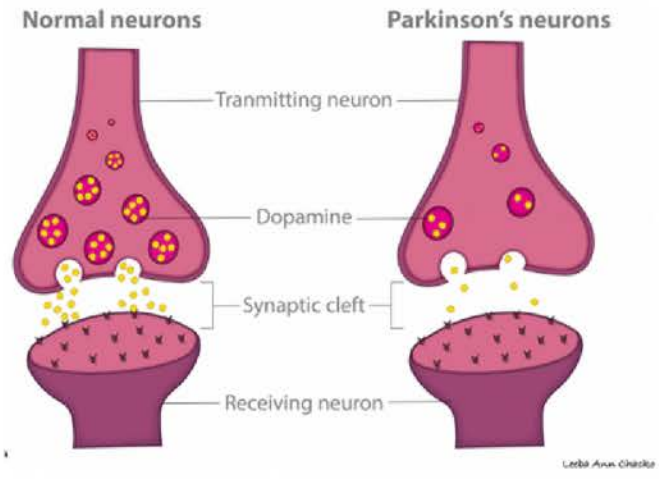

Figure 2. Normal Neurons vs. Parkinson's Neurons
Parkinson's Disease occurs due to the decrease in the release of Dopamine from the Dopaminergic neurons. (2011). My hands are shaking, do I have parkinson's disease? from https://www.scirio.in/shaking-parkinsons-disease/

Alzheimer's (AD)

Alzheimers is a more severe case of dementia that many suffer from, specifically those in their late years. As many as 6.2 million Americans suffer from Alzheimer's Disease as discovered by a report from the Alzheimer's Disease Association. Alzheimers pertains to the diminishing of memory and its 'cognitive and functional deterioration'.This too pertains to the loss of neurons indicating that the diminishing effects of neurogenesis may be in play here. In addition to neuron loss, 'extracellular amyloid plaques and intracellular neurofibrillary tangles' (KATSNELSON et al., 2016) also pertains to the disease.

The amyloid hypothesis 'considers Aβ deposition' –a β-amyloid protein significance to Alzehimers disorder– to be a causative factor of Alzheimer's disease, this hypothesis describes the "sequence of AD etiology" (Bayer & T. A., 2013). The APP; a 'larger amyloid precursor protein' appeared to have an association with Alzehimers, and so in 1999 scientists had mice trials where they "immunized transgenic APP mice with pre-aggregated Aβ1-42, either before the onset of AD-type neuropathologies or after the onset of plaque deposition" (Bayer & T. A, 2013) this immunization shortly proved that this method was effective in reducing 'the extent and progression of these AD-like neuropathologies' implying that the 'immunization with pre-aggregated Aβ1-42' may help prevent and treat AD. In the short-term it was also shown that the vaccination protected 'transgenic mice from the learning and age-related memory deficits'; though when being tested on humans as a form of clinical trials, it was discovered that 'Six percent of AD subjects (18 of 300) developed serious brain inflammation resembling meningoencephalitis' (Bayer & T. A., 2013) and there was no prevention for progressive development of neurodegenerative diseases. Furthermore, the 'Aβ-mediated alters one of the GABAergic neurotransmission and brings an imbalance between hippocampal GABAergic and glutamatergic neurotransmission resulting in an impaired hippocampal neurogenesis' (Winner, B., & Winkler, J, 1970) in AD, which indicates the effect of storing memory within

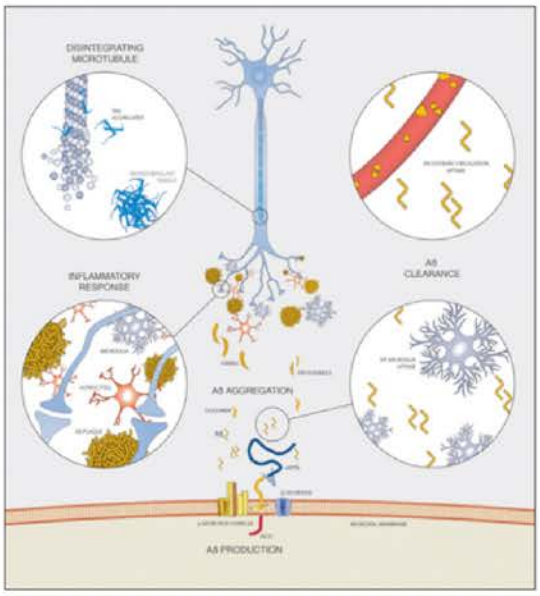

Figure 3. Amyloid-B (AB) cascade hypothesis
The disposition of the amyloid-B peptide and the accumulation of it in the brain is a preliminary step towards the process of the Alzheimer's Disease. (2019). Amyloid-3 immunotherapy for alzheimer disease: Is it now a long shot? doi: 10.1002/ana.25410.Epub

older individuals. As the Hippocampus is one of the places where Neurogenesis still appears to occur even with old age, this blockage is a big factor indicating the progression of AD.

Another major reasoning behind the development of Alzheimer's Disease would be 'presence of inclusions within neurons that are composed of fibrils' which are curated from the 'microtubule-stabilizing protein tau'. The misfolding of the oligomeric and post-translational alterations of the tau molecules is said to partake in the neuron loss and 'cognitive impairments in AD'. Tau is said to be a toxic factor as there is evidence pertaining to the fact that tau oligomers can motivate ' neurodegeneration and memory impairment in the absence of Aβ' (Bayer & T. A., 2013). The exposure that scientists have gotten from the inner mechanisms of these individual proteins that pertain to Alzheimer's Disease can allow us to narrow in on the specifics. So when trying to find a cure, we know the contribution of certain proteins to Alzheimer's disorder allowing us to design a method to turn on or off specific proteins and their functioning.

Preventatives

Many preventative measures have been explored and many have failed as the side effects were too detrimental on their subject of testing, which included mice. By testing on mammals that resemble the behavior and certain organ systems similar to humans, scientists, through trial and error, obtain more knowledge upon these diseases allowing for them to bring advancements towards change. Other than the inner workings of proteins and specific factors within the nervous system, there appears to be a positive association between environmental enrichment as well as physical exercise to neurogenesis. Through testing different stimuli pertaining to these areas of living they appear to have 'positive effects on survival, cognitive performance, as well as reduction of neuronal intranuclear inclusion load'.

Though none of them were able to reduce the effects of neurogenesis, it was shown that environmental enrichment played a role in increasing 'levels of hippocampal neurogenesis to some extent'. Through the experimentation of mice put on trials where mice were placed in an enriched environment which sped up their social interaction and the availability of the 'wheel for exercise' (van Praag et al., 2005) appeared to have inflated the hippocampal neurogenesis versus those mice kept in cages deprived of such enrichment. The voluntary running on a wheel and the physical activity exercised by the mice, has also led to an '[increase] cell proliferation, cell survival and net neurogenesis' (van Praag et al. 1999) even if these mice were not necessarily placed in an enriched environment. If one cannot get rid of neurodegenerative diseases, one can bring upon an opposition that counters the loss of neural cells, through proliferation of these cells.

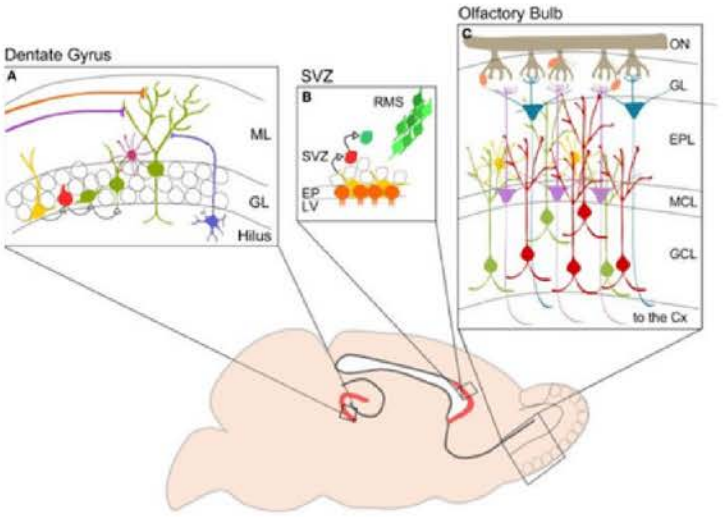

Figure. 4 Neurogenesis in the Adult Mouse brain.
The different processes of the occurrence of Neurogenesis in the neurodegenerative sites, Dentate Gyrus of the Hippocampus, as well as the SVZ and the Olfactory Bulb. (2014). Regulation of adult neurogenesis by GABAergic transmission: signaling beyond GABAA-receptors. Frontiers in cellular neuroscience. 8.166.10.3389/fncel.2014.00166

Antipsychotic drugs appear to also have an effect on neurogenesis within the hippocampus, although it appears to depend on the pharmacology behind it and there is not much evidence pertaining to it. It appears the best approach to combat neurodegenerative diseases consists of treatment for proteinopathies. Multiple vaccines and trials have been conducted, although all appear to have some sort of side effect on other areas of the brain and body that results from the treatment, most common ones to consider would be clinical side effects. Though there appears to be a similarity in what is being the main target when it comes to combating these diseases, new vaccines that were taken on trial initiate to avoid harmful T cell responses, while removing 'harmful Aβ peptides', and targeting plaques. It is discovered that, "Aβ immunization with AN1792 can initiate plaque removal" (Bayer & T. A., 2013) on AD patients, but in order to fully put this to the test, the Neuropathology team of Delphine Boche and James A.R. Nicoll initiated a follow up on the AD patients from the Elan Abeta immunization trial, who were given the vaccination. What they determined from the trial was that the

Aβ load was lowered as well as evidence of 'plaque removal' and 'reduced tau load in neuronal processes, but not in cell bodies' (Bayer & T. A., 2013). The main goal appears to be targeting 'Aβ-amyloid plaques' and there have been multiple vaccines that intended to do so, but they did not get too far in their phases of trial on human trials. In phase II of the vaccine bapineuzumab study, the human participants developed 'vasogenic cerebral edema' (LIEBESKIND & D. A. V. I. D. S., 2002) which is brain swelling that can result in the disruption of the blood brain barrier. Another drug such as the Solanezumab has been tested on participants as well, but it does not show much promise in the upper level phases such as phase III and appears not be very helpful in those individuals suffering from a more intensive case of Alzheimer's Disorder.

However, those struggling from mild levels have shown a bit of an improvement. This form of treatment is known as a humanized monoclonal antibody, which aims to target and neutralize soluble Aβ peptides. As mentioned previously this was not a successful attempt but the evidence that was found through these trials was that, "statistically significant slowing of cognitive decline" (Bayer & T. A., 2013) Preventative measures can be understood and taken early on as a means to prevent neurodegenerative diseases from occurring in the future. There appears to be an increase in the development of neurodegenerative diseases within aging adults and through understanding the ways one can prevent such diseases can gradually decrease the development of neurodegenerative diseases.

Conclusion

This newfound discovery of neurogenesis within the adult brain has opened up many doors to understanding the specifics of neurodegenerative disorders and ways to combat them. Through trial and error within experimentation in the scientific community as well as environmental enrichment from one's life, newfound knowledge on neurodegenerative disorders and their inner mechanisms is being obtained. This can ultimately help identify a cure or close-to a cure that can help eradicate humanity from advancing towards such diseases and get rid of the notion of this becoming a norm with old-age.

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Comparison of SSRI and SNRI on Their Effects of Relieving Major Anxiety

Yushan Li

Abstract

Anxiety disorders, including generalized anxiety disorder (GAD), panic disorder (PD), social anxiety disorder, and phobia-related disorders are the most common group of mental disorders in the U.S. Anxiety disorders are treated with psychological therapy and pharmacotherapy. Pharmacotherapy includes first-line drug selective serotonin reuptake inhibitors (SSRIs) as well as second or third-line drug serotonin-norepinephrine reuptake inhibitors (SNRIs). Both have the potential to induce adverse effects and can lead to similar withdrawal symptoms due to their effects on neurotransmitters and serotonin levels (Gupta et al., 2021). However, SSRI is widely considered to be the first choice for anxiety disorder therapy in comparison to SNRI because of its recent developments.

Introduction

Common anxiety disorders are often associated with the hypothalamus-pituitary adrenal system (HPA axis). The HPA axis controls responses to stress. The HPA axis causes an increase in cortisol secretion to the adrenal cortex, increasing the level of salivary cortisol. When the level of salivary cortisol increases, individuals will experience an increase in stress and anxiety levels. Promotion for anxiety disorders is influenced by genetic, epigenetic, and other environmental factors, while the severity of these psychiatric disorders are often underestimated and underrecognized by society. GAD and PD are among the most prevalent mental health diseases in the United States, and they can negatively impact a patient's quality of life and interfere with essential activities of daily lives. For instance, GAD and PD have been associated with a loss in motivation, as well as a loss in the ability to learn, memorize, and make decisions. According to a study conducted by Front Public Health, the 12-month prevalence for GAD and PD among U.S. adults 18-64 years of age is 2.9-3.1% (Bandelow et al., 2017). Patients with GAD are typically presented with pervasive anxiety and worry that are associated with symptoms like irritability, fatigue, sleep disturbance, and difficulty concentrating. However, PD is characterized by recurrent panic attacks along with other physical symptoms such as accelerated heart rate, trembling, and nausea or abdominal distress (Roy-Byrne et al., 2006).

In 1988, the first SSRI fluoxetine was introduced in the United States. It was discovered that highly receptor-sensitive agents would lead to a superior side effects profile, especially compared to that of first-generation antidepressants, such as tricyclic antidepressants (TCAs). TCAs have high efficacy but fatal side effects. The discovery of SSRIs was an important step in the treatment of depression and anxiety disorders because of their high sensitivity to serotonin receptors and low propensity to cause seizures (Lambert & Bourin, 2002). Currently, fluoxetine and sertraline are the most commonly prescribed SSRIs in the United States and will be used as representative SSRIs in this study.

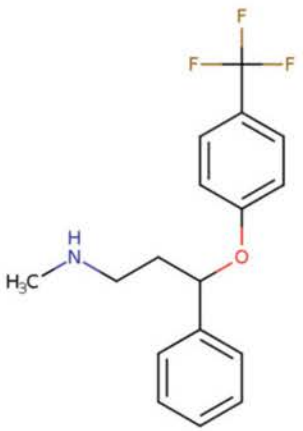
In 1993, venlafaxine immediate release (IR) was the first SNRI in the United States that was approved by the United States Food and Drug Administration (FDA). This was followed by a micro-encapsulated extended release (XR) formulation, which was presented to have milder effects. IR Venlafaxine is developed to dissolve without delaying absorption of the drug while XR Venlafaxine is designed to release in a controlled manner during an extended period following ingestion. Venlafaxine is widely used in the treatment of major depression, GAD, PD, and phobia-related disorders. Other SNRIs, such as duloxetine, desvenlafaxine, milnacipran, and levomilnacipran, exhibit different structures than venlafaxine and are mainly used in treating major depression and GAD. At present, two SNRIs are used in clinical trials, including the venlafaxine and milnacipran. Both venlafaxine and milnacipran will be the subject SNRIs of this study.

Description of SSRI and SNRI

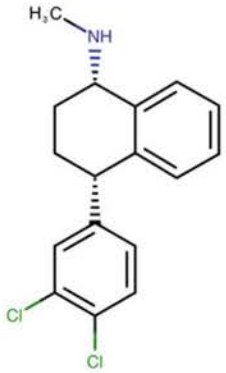
1. Chemical Properties.

SSRI:

Fluoxetine (C17H18F3NO): Fluoxetine is classified as a diphenhydramine, an antihistamine and sedative mainly used to treat allergies. Fluoxetine is a trifluoromethyl benzene, a trifluoromethane with a substituted phenyl group. Its metabolite, norfluoxetine, is freely soluble in methanol and ethanol, with a melting temperature of 158.4-158.9ºC (Drug Bank, 2022).

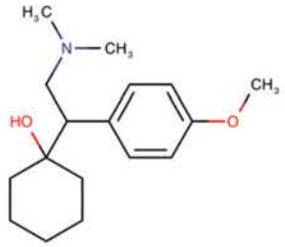


Sertraline (C17H17Cl2N): Sertraline is classified as a tetralin, with a methyl amino substituent at position 1 and a 3,4-dichlorophenyl group at position 4. [6] A tetralin is a partially hydrated naphthalene derivative. Desmethylsertraline, the active metabolite of sertraline, has a melting temperature of 243-249ºC (Drug Bank, 2022).

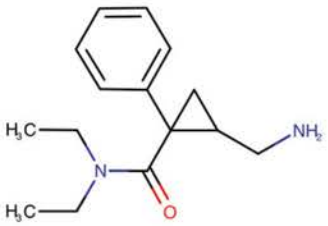


SNRI:

Venlafaxine (C17H27NO2): Venlafaxine is classified as both a tertiary alcohol and amino. Its basic structure is an N,N-dimethylethylamine, with a substituent at position 1. The substituent at position 1 contains both a 1-hydroxycyclohexyl and 4-methoxyphenyl group. The active metabolite of venlafaxine is O-desmethylvenlafaxine (ODV), which has a melting temperature of 208-213ºC (Drug Bank, 2022).



Milnacipran (C15H22N2O): Milnacipran is a member of the acetamides and is classified as a monocarboxylic acid amide Milnacipran undergoes minimal hepatic metabolism (Drug Bank, 2022).



2. Synthesis of SSRI and SNRIs

Chemical synthesis is the preparation of an organic compound that can be produced from inexpensive and widely available start materials. Biosynthesis involves the synthesis of complex molecules that undergo multiple steps of production. Most molecules that require synthesis by plants, microbes, or biotechnology are biosynthesized. Both the SSRIs (fluoxetine and sertraline) and SNRIs (venlafaxine and milnacipran) in this study are all chemically-synthesized due to their chemical structures and mechanism (Dell'Osso et al., 2010).

Fluoxetine: Fluoxetine has been synthesized through different methods, including one that begins with a Mannich reaction of acetophenone with paraformaldehyde and methylamine to produce dimethylaminopropiophenone (Drug Summary, 2022). This is followed by the reduction of the carbonyl group of the dimethylaminopropiophenone, which produces an alcohol. The nucleophilic substitution of the resulting hydroxyl group from the last reaction with thionyl chloride produces 3-chloro-3-phenylpropan-1-amine. Then, 3-chloro-3-phenylpropan-1-amine is treated with sodium hydroxide and 4-(trifluoromethyl)phenolate to form a secondary amine, which will be demethylated by cyanogen bromide and potassium hydroxide to produce fluoxetine (Pinna, 2015).

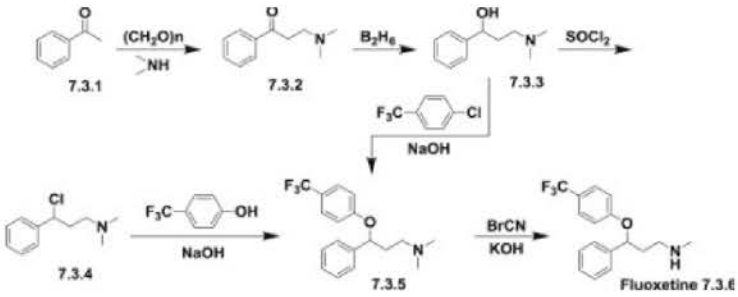


Figure 5. The Approach Synthesizing of Fluoxetine

Sertraline: The original approach of sertraline synthesis involved a five-step reaction that can be replaced by the one-step condensation of α-naphthol with O-dichlorobenzene in the presence of AlCl3. This produces a substituted tetralone, which is further condensed with methylamine in the presence of TiCl4 to form an imine (Murdoch et al., 1992). During the reaction, an equal ratio of rac-cis and rac-trans-amines will result from the reduction of the imine double bond. The addition of D(-)-mandelic acid will result in the production of sertraline.

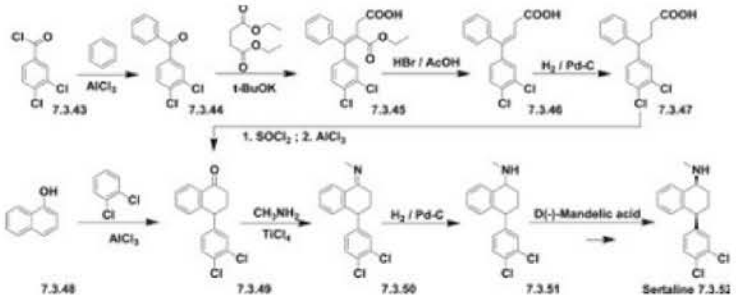


Figure 6. The Approach Synthesizing of Sertraline

Venlafaxine: Venlafaxine is first synthesized by the nucleophilic addition of 4-methoxyphenyl acetonitrile with cyclohexanone, using BuLi or LDA. The intermediate then undergoes a catalytic hydrogenation with the aid of a metal catalyst, Rh or Al2O3, to give (RS)-1-[2-amino-1-(4-methoxyphenyl) ethyl]-cyclohexanol. The cyclohexanol will then be dimethylated to produce venlafaxine by reductive amination, using the Eschweiler-Clarke procedure. (Savella, 2023)

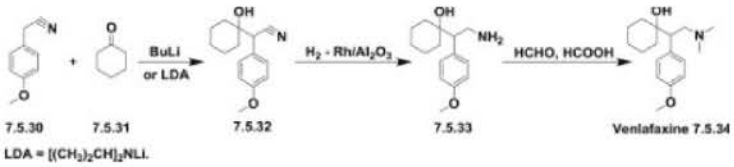


Figure 8. The Approach of Synthesizing Venlafaxine

Milnacipran: The synthesis of milnacipran starts with the treatment of compound 2 with AlCl3 and diethylamine at room temperature. This results in the opening of the ring and the production of an alcohol that is oxidized with Dess-Martin periodinane (DMP) (Savella, 2021). Acetic acid or aqueous ammonium in addition to cyanoborohydride, ethyl acetate, and hydrochloric acid are then added to increase the chemoselective reductive amination of aldehyde (Vukics et al., 2001).

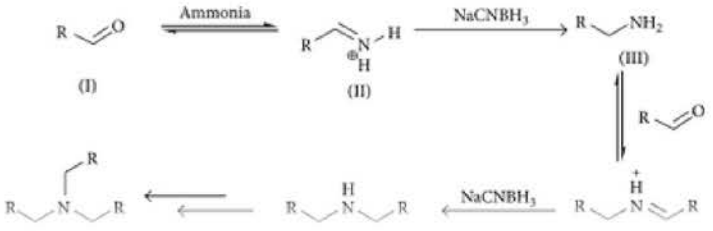


Figure 9. The Approach of Synthesizing Milnacipran

3. Mechanism of Action and Metabolism

It is speculated that the cause of various anxiety disorders is the deficiency in serotonin (5-hydroxytryptamine, 5-HT), a monoamine neurotransmitter that influences autonomic function, modulation of mood, recognition, and hormone secretion. Serotonergic neurons mediate anxiety responses. These neurons are located in the raphe nuclei, a part of the brainstem which releases serotonin to the brain.

SSRI, as its name suggests, inhibits the reuptake of serotonin specifically, increasing the availability of serotonin in the synaptic cleft without interfering with other neurotransmitters. Fluoxetine, like the other SSRIs, acts to block the sodium-dependent serotonin transporter (SLC6A4) at the presynaptic axon terminal, thereby increasing the levels of 5-HT. Upon ingestion, fluoxetine is metabolized to norfluoxetine by enzymes via N-demethylation. Additional enzymes will mediate O-dealkylation of fluoxetine and norfluoxetine, which will eventually metabolize into hippuric acid. Similarly, sertraline, the other representative SSRI, undergoes N-demethylation to form N-desmethylsertraline (Marsh, 2007). However, the metabolism of sertraline also involves N-hydroxylation, oxidative deamination, and glucuronidation. Sertraline binds to human serum albumin with high affinity via hydrophobic interactions and hydrogen bonding. Its metabolic activity happens mainly in livers (Desireddy et al., 2017).

SNRIs, in contrast to SSRIs, elevate the levels of both serotonin and norephedrine. This alleviates the symptoms of anxiety disorders and other diseases such as major depression and obesity. SNRIs act upon serotonergic and noradrenergic neurons without acting upon histaminergic receptors. Venlafaxine, one of the representative SNRIs in this study, functions by increasing the levels of serotonin, norepinephrine, and dopamine by blocking the reuptake at the presynaptic terminal by transport proteins (Zoloft, 2023).

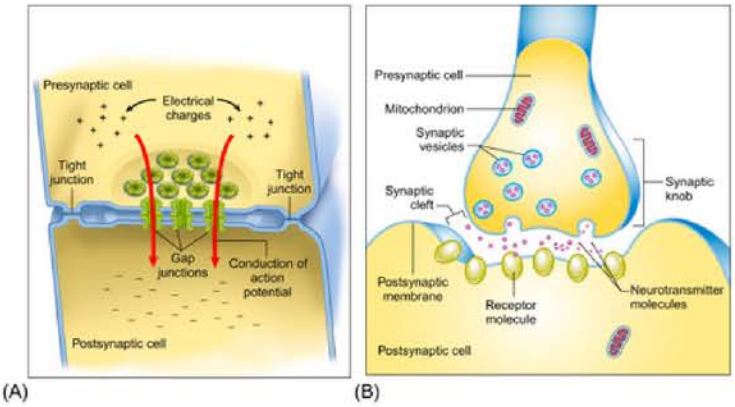


Figure 10. The Reaction of SSRI at Synaptic Axon Terminal

Unlike other SNRIs, venlafaxine is more potent in inhibiting the reuptake of serotonin than the reuptake of norepinephrine. After oral administration, venlafaxine is well-absorbed from the gastrointestinal tract and, similar to the SSRI sertraline, and undergoes extensive hepatic metabolism. It primarily undergoes CYP2D6-mediated demethylation to form ODV. Milcipran, the other SNRI in this study, functions by inhibiting the reuptake of 5-HT and norepinephrine. Upon administration, milnacipran undergoes desethylation and hydroxylation to form N-desethyl levomilnacipran and p-hydroxy-levomilnacipran. Both are oxidative metabolites of milnacipran and will undergo conjugation with glucuronide to form the conjugate milnacipran carbamoyl-O-glucuronide (EffectorXR, 2021).

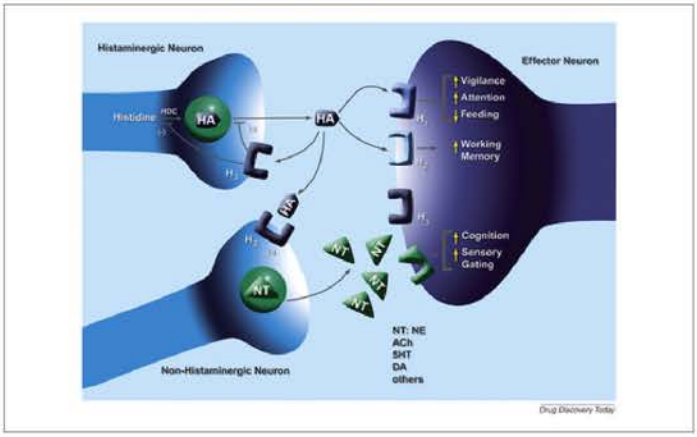


Figure 11. The Reaction of SNRI at Histaminergic Receptors

4. Mode of Delivery and Dosage

Drugs can be delivered to the human bodies in different routes to achieve the highest efficacy. The mode of delivery primarily depends on the half-life, toxicity, and mechanism of action of the drug. On the other hand, the dosage is associated with the medical condition, weight, and response of the patient.

Fluoxetine : Generally, fluoxetine should be administered once per day, between 20 to 40 mg. However, considering both efficacy and adverse effects, it is possible for the drug to be administered at smaller doses, like 10 mg for individuals

with low tolerability of side effects. Inversely, individuals may require high doses of 60-80 mg for effective medication. Delayed-release capsules, medications that are designed to release the active ingredient after an extended period of time following the administration, are generally administered at 90 mg per week and are comparable in efficacy with the daily 20 mg treatment. Fluoxetine, compared with other SSRIs, has milder withdrawal symptoms, including sleep impairment, fever, and nausea. In addition, drug-drug interaction should also be strictly monitored as non-steroid inflammatory agents might affect the efficacy of fluoxetine (Pae et al., 2009).

Table 1. The Mode of Delivery and Dosage of Major Brands of Fluoxetine

Drug	Type	Dosage	Intake method
Prozac	Capsule	10 mg/20 mg/40 mg	Oral
Rapiflux	Delayed-release capsule	90 mg	Oral
Sarafem	Tablet	10 mg/20 mg/60 mg	Oral
Selfemra	Solution	20 mg/5 mL	Oral

Sertraline: Generally, sertraline is administered once per day, either in the morning or evening, starting with the initial dosage of 50 mg to a maximum dosage of 200 mg (Drug Bank, 2023). Single and multiple oral doses of 50 to 200 mg per day have been well-tolerated in patients with anxiety disorders according to clinical trials conducted by FDA.

Table 2. The Mode of Delivery and Dosage of Major Brands of Sertraline

Drug	Type	Dosage	Intake method
Sertex	Tablet	25 mg/50 mg/100 mg	Oral
Zoloft	Capsule	150 mg/200 mg	Oral
Zoloft	Solution	20 mg/1 mL	Oral

Venlafaxine: Venlafaxine is prescribed only for individuals over 18 years old and should be taken with food for better absorption. Immediate-release tablets can be cut and crushed but extended-release tablets cannot. Treatments by venlafaxine generally start between 75 to 150 mg per day, with an upper dose of 375 mg per day, divided into three doses (PubChem, 2023).

Table 3. The Mode of Delivery and Dosage of Major Brands of Venlafaxine

Drug	Type	Dosage	Intake method
Effexor XR	Extended-release tablet	25 mg/37.5 mg/50 mg/75 mg/100 mg	Oral
Xanax	Immediate-release tablet	37.5 mg/75 mg/150 mg/225 mg	Oral

Milnacipran: The dose range of milnacipran is 50-200 mg in divided doses. Treatments by milnacipran begin at 25 mg per day and can be increased to the maximum of 200 mg according to individual need and response (Pae et al., 2009). Like venlafaxine, the other SNRI, milnacipran, is also recommended to be taken with meals and there are no dose-dependent adjustments that can be made for patients with hepatic impairment and chronic liver disease. In order to minimize withdrawal effects, milnacipran should be tapered after extended use and should not be discontinued abruptly.

Table 4. The Mode of Delivery and Dosage of Major Brands of Milnacipran

Drug	Type	Dosage	Intake method
Joncia	Capsule	25 mg/50 mg/100 mg	Oral
Savella	Tablet	12.5 mg/25 mg/50 mg/100 mg	Oral

4. Adverse Effects

Due to SSRI selectivity on targeting serotonin reuptake transport proteins without interfering with other receptors, such as histaminic, cholinergic, dopaminergic, and noradrenergic receptors, SSRIs are more tolerable for patients than other drugs that treat anxiety and depression, such as TCAs. [25] However, serotonin receptors mediate a variety of functions unrelated to mood, including appetite, sleep, and sexual function. SNRI inhibition of the reuptake of serotonin causes more of the available neurotransmitters to interact with the aforementioned receptors. The increased interaction of these receptors is closely associated with the adverse effects of SSRI. Common side effects developed by patients under SNRI treatment include headaches, gastrointestinal complaints, sleep impairment, and sexual dysfunction. Most SSRI side effects are dose-dependent and can usually be alleviated by reducing the dose. Like SSRI, patients treated with SNRI show a lower rate of treatment discontinuation than TCA, but many patients still experience various side effects including sexual dysfunction and elevation of blood pressure, especially in case of venlafaxine. A meta-analysis of 16 randomized controlled trials conducted by the Health Psychology Research in 2021 reported that milnacipran showed fewer adverse effects and lower premature withdrawal rates (Gupta et al., 2021).

Conclusion and Future Prospect

Currently, SSRI-related therapy is held to be the major treatment for patients with anxiety disorders. However, it could not be regarded as ideal because of its mild but still present side effects in addition to its poor cost-effectiveness. SNRIs, on the other hand, are receiving an increasing amount of consideration. A potential advantage of SNRI over SSRI is its selective inhibition on both serotonin and norepinephrine. However, it is still premature to acknowledge the superiority of SNRI over SSRI as anxiety disorders are often complicated by the presence of comorbid conditions. Therefore, this conclusion requires more meta-analyses to elucidate the data.

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The Impact of Classical Music on Neuroanatomy & Brain Functions

Vani Sharma

Abstract

The human brain is responsible for a variety of functions that govern daily life, adaptation, and survival. Characteristics such as memory consolidation, mood states, and neural plasticity give rise to brain biodiversity and higher-order intelligence. There are many external stimuli that evoke such characteristics, from a car horn to the latest Marvel movie, and classical music is no exception. This genre of music is a means to stimulate neural chemistry and cerebral circuits. This paper focuses on the nervous system as well as the neuroanatomy that can be impacted by engagement with such music. Classical music increases memory consolidation, relaxes the nervous system, can amplify emotional mood states, and can increase neural plasticity to slow down age-related cognitive decline. Furthermore, it can be used as a therapy for memory-related brain-based conditions such as Alzheimer's disease and dementia.

Introduction

Humans, at our fundamental cores, are complex beings. We possess introspection, reasoning and language skills, and high levels of emotional intelligence. These traits, along with an innate ability to respond to music and dance, help to distinguish Homo sapiens from any other animal species (Moeller, 2017; Trimble and Hesdorffer, 2017). The human brain consists of cortices, connections, and structures that respond to sound and rhythmic inputs (Trimble and Hesdorffer, 2017). From the rhythmic beating of human hearts to the hand clapping motions of ancient hominids (Trimble and Hesdorffer, 2017), many aspects of human biology and behavior have evolutionarily been tied to music, and neuroanatomy and brain function play a key role in that connection.

When humans listen to music or play a musical instrument, the music activates the brain, ignites emotion, and can impact brain-behavior associations, memory, and even psychopathology (Trimble and Hesdorffer, 2017). These neural effects are much more amplified when looking at classical music. Classical music is significant because it has greater harmonic resources and rhythm variety than modern music, which leads some to consider it as more expressive (Young, 2016). As per Cleveland Clinic, a non-profit academic medical center at the forefront of neurological research, this type of music stands out as a therapy and has been shown to assist with the treatment of neurodegenerative diseases ("Music Therapy," 2020). The techniques and variations within classical music also greatly bolster neural functionality (Young, 2016). This article will discuss how classical music increases memory consolidation, relaxes the nervous system, amplifies mood states, and slows down age-related cognitive decline. It also dives into the rising methods of music therapy and clinical applications for Alzheimer's disease as well as dementia.

Classical Music Affects Memory Consolidation

Classical music affects the neurochemistry of the brain in relation to memory consolidation.

Memory consolidation refers to forming and retrieving memories to adapt to the demands of a changing environment through the steps of encoding, consolidation, and retrieval (Rasch and Born, 2013). In this process, a new memory trace is first formed. Next, during the consolidation stage, the memory trace is stabilized and integrated into pre-existing knowledge networks in the brain. Lastly, during the retrieval, the stored memory is accessed and recalled. The most critical player in the memory consolidation process is sleep. The sleeping brain is what provides the ideal conditions for the encoded memory to go into long term memory.

Recent neurological studies have shown that classical music can reactivate memories during sleep, assisting with consolidation and especially benefiting patients that have poor sleep patterns (Gao et al., 2020). A recent study published in the National Library of Medicine played classical music consisting of Chopin, Beethoven, and Vivaldi to sleep-deprived undergraduate microeconomics students. These students were then asked to take a college-level exam that included problems they were trained to solve and new questions that require knowledge application. The students that listened to classical music showed a 20% increase in knowledge transfer, concept integration, and memory consolidation (Figure 1).

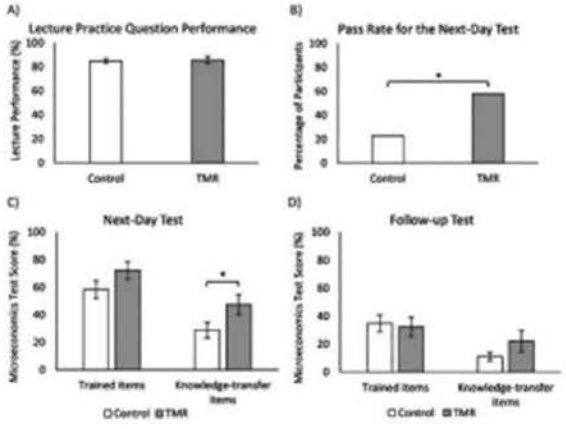

Fig. 1. Gao et al., 2020.

Studies such as this suggest that classical music can be leveraged in preparing for academic tests, test performance, and tasks that require day-to-day memory consolidation.

Classical Music Induces Relaxation States

Classical music affects functional neuroanatomy by inducing relaxation states. Physiological change in the nervous system has long been associated with music perception (Siragusa et al., 2020). These changes include heart rate and blood pressure fluctuations. Research shows that exciting or fast tempo music tends to increase heart rate and blood pressure, while relaxing or slow-tempo music has the opposite effect (Siragusa et al., 2020). Additionally, there is evidence that cerebral blood flow increases in the brain arteries when subjects are exposed to music with a faster tempo (Siragusa et al., 2020).

In recent years, neurological studies have further proposed that music influences cognition and emotion. Specifically, a 2020 study done by the Clinical Investigation Center of the Hospital of France took healthy individuals ages 18 to 45 and exposed them to Stravinsky and Rossini's classical music compositions. The brain's pulsatile movement, which is the measure of brain volume changes in relation to cerebral blood flow and elastic tissue properties, was tracked. The results showed a decrease in amplitude. Studies such as this suggest that classical music can relax the nervous system and induce physiological and emotional changes within the brain and body.

Classical Music Induces Relaxation States

Similarly, research has shown that emotional states can be induced by happy, sad, and neutral classical music. Evidence shows that emotional states produced by music are strong, long-lasting, and more pervasive (Mitterschiffthaler et al., 2007). One brain region that is particularly responsive to pleasant music is the ventral striatum, which is involved in reward, attention, and motivation (Mitterschiffthaler et al., 2007). Conversely, another brain region known as the amygdala is engaged in response to fearful and sad stimuli. Evidence has shown that there is decreased cerebral blood in the amygdala in relation to "chills" that are produced from pleasurable classical music. There is also greater amygdala activity with unpleasant musical excerpts (Mitterschiffthaler et al., 2007).

A study from the Institute of Psychiatry at King's College in London played its participants' sad, neutral, and happy classical music compositions (Mitterschiffthaler et al., 2007). When compared to neutral music, it was found that listening to happy classical music induced an increase in activation in various gyral regions (temporal, front, parahippocampal, etc.) and the ventral striatum (Figure 2). Likewise, brain activity increased in areas such as the hippocampus, amygdala, and cerebellum after listening to sad classical music (Figure 3). This evidence demonstrates that classical music can induce and even amplify happy or sad states while activating the emotional neural pathways.

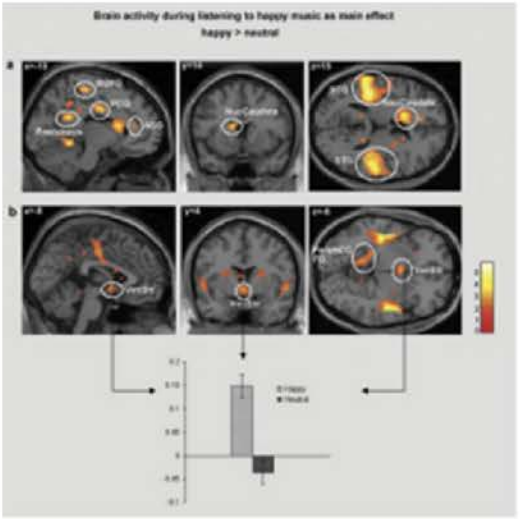

Fig. 2. Mitterschiffthaler et al., 2007

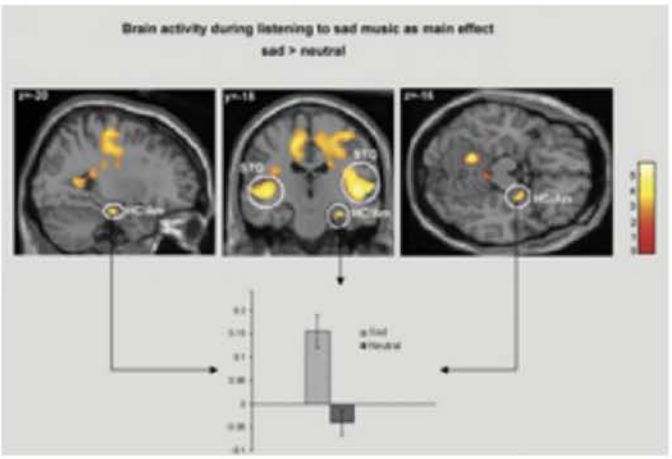

Fig. 3. Mitterschiffthaler et al., 2007

Classical Music Induces Relaxation States

Classical music has also been frequently associated with preventing cognitive decline. As humans age, it is normal to see cognitive regression (James et al., 2020). This means that there is a reduction in gray matter, loss of structural connections within the brain, and the information flow dynamics between regions . The elderly commonly experiences a deterioration in terms of hippocampal function and memory. Specifically, there tends to be a lapse in long-term memory and a decrease in abstract thinking. This puts the elderly at a greater risk for cognitive conditions such as dementia. This is where classical music has been applied as a potential intervention to slow down and prevent such decline.

A study published in BMC Geriatrics, an open access journal publishing peer-reviewed articles, trained the elderly in playing classical music on the piano over an extended period. Participants trained on piano saw improvements in executive function, working memory, and general well-being, which were measured through behavioral observation and physiological testing. Tracking these patients also highlighted the functional and structural plasticity in the hippocampus, which can counteract age-related decay leading to neural decline in this region (James et al., 2020). This is especially clear in the case of older adults, as learning a new skill, such as playing the piano, can increase and engage neural plasticity (James et al., 2020).

Engaging geriatric populations with classical music in this way opens a new medicinal and therapeutic outlet that can promote neural plasticity and slow down age-related cognitive decline.

Clinical Applications of Classical Music with Alzheimer's Disease & Dementia

Classical music has been proposed as a form of therapy for elderly patients with Alzheimer's disease and dementia. In both cases, memory is impaired, which can cause confusion and agitation (Gerdner, 2005). Since classical music has not only been shown to prevent cognitive decline but also promote relaxed and positive mood states, studies have been conducted to test its effects in alleviating these symptoms and helping patients cope with the disease.

A study published in the International Psychogeriatric journal by Cambridge University describes conducting classical music interventions for thirty minutes twice a week. Thus, for a total of six weeks, elderly men and women with severe cognitive impairment were monitored in long-term care facilities. The research showed that there was a significant reduction in agitation following individualized classical music therapy (Gerdner, 2005). Due to the prevalence of such memory-based conditions in elderly populations, classical music is being further researched and utilized as a therapeutic treatment option ("Music Therapy," 2020). With more research studies at bay and innovation, classical music is promising in its potential to serve as an alternative medicine treatment option to patients.

Conclusion

Classical music is much more than a pleasant sound. It can help boost cognitive performance, amplify emotional states, and promote relaxation. It can have positive effects on multiple aspects of health, including one's physical, mental, and emotional well-being. This genre of music serves as one of the most essential sub-components of music therapy due to the bolstering effects it can have on neuroanatomy and brain function. However, as music therapy is becoming a more mainstream treatment method, it requires evaluation of its clinical applications and limitations. Music therapy is accessible, economical, and widely culturally accepted, making it a promising for a large patient population across a diverse globe. While classical music therapy is only one application of music therapy, its neural impacts serve as a testament to the powerful bond between the human brain and its complex sensory inputs from the environment.

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Botulinum and Pain Relief

Ruchi Prakash

Abstract

While the term “neurotoxins” inherently has a connotation of harm and danger, their targeting mechanism plays an important role in pain relief. This paper takes a deeper look into a major neurotoxin - botulinum, one of the most poisonous chemical compounds. By blocking acetylcholine, a neurotransmitter that facilitates communication between nerve endings and smooth muscle, botulinum has found popularity for cosmetic usage but more importantly muscle pain relief. However, new research has shown that it may be able to affect other neurotransmitters to provide a more direct effect.

Introduction

From the products we use to the air we breathe, neurotoxins can be found everywhere. Common examples include lead, ethanol, tetanus toxin, and even essential molecules like glutamate and nitric oxide. Neurotoxins, as opposed to neurotoxicants, are naturally occurring chemicals that result in the alteration of normal neuronal activity or neurotoxicity. More specifically, they can cause cell and tissue death, disrupt signal pathways and metabolic processes, and have been linked to neurodegenerative disorders like Alzheimer’s and Parkinson’s disease. It is important to note, however, that neurotoxins are not intrinsically toxic (Spencer & Lien, 2014). It is only when exposed to an extremely high concentration that negative consequences can occur. This characteristic of neurotoxins makes them especially useful in drug manufacturing.

What is Botulinum?

Botulinum is a toxin produced by a bacteria known as Clostridium botulism and can be commonly found in soil, water, and the intestines of certain animals. Unfortunately, this toxin is extremely deadly when ingested or when it comes in contact with open wounds, resulting in botulism. Botulism results in muscle paralysis that can in turn lead to the weakening of muscles, blurry vision, difficulty swallowing, and more. What makes this illness so dangerous is the spreading of paralysis to the extremities and even respiratory muscles.

More specifically, these symptoms can be attributed to the toxin targeting neuromuscular junctions to inhibit the release of acetylcholine. Acetylcholine is a neurotransmitter that controls muscle contraction and voluntary movement. Through an irreversible process, BoNT binds to the presynaptic membrane and severs the SNARE protein complex that connects the vesicles containing acetylcholine to the membrane (Nigam & Nigam, 2010). With acetylcholine unable to bind to the postsynaptic receptors, an action potential is never fired. In other words, the message to contract the target muscle is temporarily stopped, thereby inhibiting bodily movement.

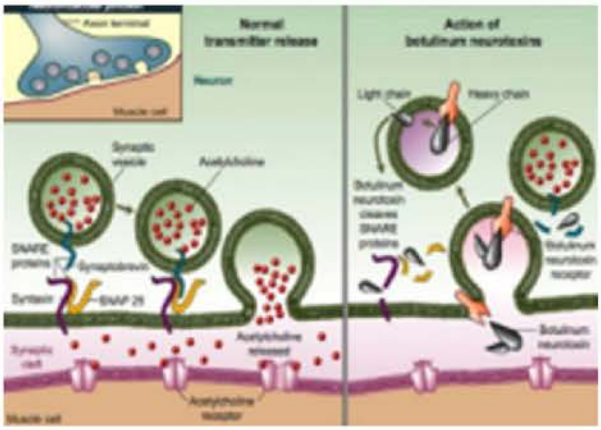

Fig. 1. Comparison of normal acetylcholine release versus the action of BoTN-A on the presynaptic neuron (“Novel Compound”, 2018).

In recent years, scientists have realized that this same mechanism can be manipulated to give the illusion of youth. This process starts with purifying the toxin or removing any bacterial cell components. It is then using 0.0073 nanograms of this pure neurotoxin that one unit of BOTOX is created. Once injected into the desired muscle, the lack of muscle contractions help prevent the formation of wrinkles. Fortunately, since these injections contain such a small amount of toxin, they can block nerve communication and temporarily paralyze the area without causing botulism.

Given botulinum’s ability to interrupt the transmission of signals, it can disrupt abnormal muscle and pain signals and thus act as a pain killer. In fact, the toxin is commonly used for a series of conditions like excessive sweating, lazy eye, tight muscles, chronic migraines, and muscle spasms.

Indirect Pain Relief

The most common mechanism of botulinum-induced pain relief is temporarily stopping the muscle spasm-pain cycle by blocking acetylcholine. In fact, intramuscular injections can reduce arthritis-induced pain, spasticity (stiffness in muscles), dystonia (uncontrollable muscle contraction), muscle tension, and more.

As such they are commonly used, to reduce symptoms of stroke, cerebral palsy, and MS patients (Davis & Barnes, 2000). This is especially important given the lack of effective and affordable pain treatment. In fact, many oral medications, anti-inflammatory and narcotics alike, have several harmful side effects. On the other hand, botulinum injections tend to have milder and reversible side-effects, if any.

Direct Pain Relief

A more recent discovery, however, is BoTN-A's ability to directly relieve pain. While the exact mechanism is unclear, past research has shown that BoTN-A may suppress other neurotransmitters and neuropeptides (chemical messengers that are made up of amino acids and released by neurons) in addition to acetylcholine. This includes glutamate, substance P, and CGRP. Uncoincidentally, these neurotransmitters are found in high concentrations along the nociceptive pathway and tend to be released when the body is experiencing pain.

The nociceptive pathway refers to the pain pathway. A vital component of this pathway is C fibers. These fibers transmit feelings of itch, burning pain, and temperature from receptors in the skin/joints/muscles to the brain. As seen in Figure 2., these fibers do so by releasing neurotransmitters like glutamate, substance P, and CGRP.

For instance, increased levels of glutamate is associated with migraines and fibromyalgia (muscle pain and tenderness throughout the body) (Bittencourt et al., 2014). Fortunately, in both mice and human studies, BoTN-A injections have been shown to directly suppress the secretion of these messengers in a similar fashion to the suppression of acetylcholine release. Once the neurotransmitters are blocked, the individual no longer feels pain.

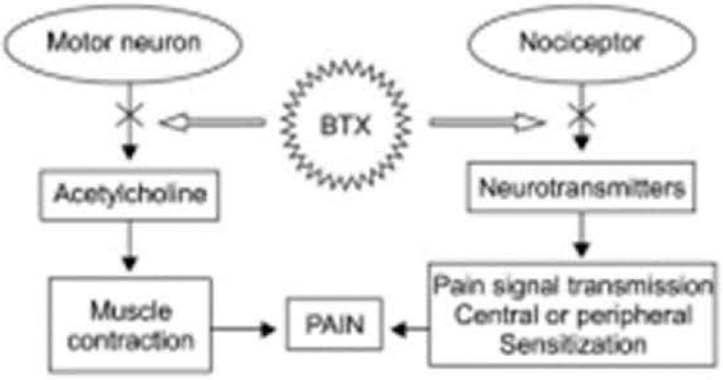

Fig. 2. BoTN-A caused inhibition of both acetylcholine and neurotransmitters released from nociceptors aid in the reduction of pain (Sim, 2011)

Additionally, studies have found that the toxin may also directly effect glial cells - non-neuronal cells that provide structural and functional support to neurons. Glial cells can be further divided into astrocytes, which primarily controls the brain's metabolism and homeostasis, microglia which function as immune cells, oligodendrocytes which mainly make myelin and more.

While it continues to be debated whether they primarily interact with microglia or astrocytes, scientists do know that BoTN-A can reduce inflammation and pain caused by touch or temperature stimuli via glial cells (Feng et al., 2021). This highlights the need for continued research on the true mechanism of botulinum toxin.

Current Research

Using toxins as drugs brings up many challenges. As discussed earlier, neurotoxins in high concentrations cause a number of problems, making it vital to inject precise doses. However, there is a lack of standardized dosing guidelines as research continues to search for the most effective drug and concentration.

Additionally, research by Dr. Bomba-Warczak and colleagues has shown that contrary to previous findings, the botulinum toxin may spread from the injected site. They believe that while some of the toxin acts on the intended area, a fraction of the toxin travels to other neurons which may increase the risk of harm. This brings up the question of whether desired effects for both cosmetics and pain may be in part due to the movement of the toxin. To answer this, they hope to genetically modify Clostridium bacteria and potentially find a safer way to ensure paralysis in a localized area (Tennenbaum, 2016). On the other hand, this ability to travel into the central nervous system may have benefits to science. In particular, it may be used to develop drugs against viral infections that can cross the blood-brain barrier.

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Current Developments and Future Possibilities of Glioblastoma Treatment

Susanna Martin

Abstract

Glioblastoma is one of the deadliest forms of cancer. It mainly occurs in the brain but can also be found in the spinal cord and is associated with aggressive proliferation and high treatment resistance. Researchers are looking for more effective treatments and ways to improve patient survival times as the prognosis for most patients is only about 14-15 months. The standard of care for glioblastoma currently involves surgery along with temozolomide chemotherapy and radiation therapy, but these treatments are often unable to combat the tumor successfully. Perhaps the most promising field of potential future treatments is immunotherapy, in which treatment is designed to stimulate the immune system to target the tumor. This article will specifically discuss two types of immunotherapies: vaccine immunotherapy, in which the patient's immune system is conditioned to fight the tumor through the introduction of tumor specific antigens, and CAR-T cell therapy, in which modified T cells are introduced into the patient intravenously.

Introduction: What is Glioblastoma?

Glioblastoma, also known as glioblastoma multiforme or GBM, is a particularly aggressive form of cancer that affects the central nervous system. It arises mainly from astrocytes, which are cells in the CNS that support the functioning of neurons by regulating neurotransmitter levels, stabilizing synapses, maintaining homeostasis in the extracellular space, and performing other important functions. GBM is the most common type of brain cancer in adults, and the risk of acquiring it increases with age. The incidence rates of diagnosis gradually increase from about 0.16 in children under 19 years of age to about 13.05 in the 65-74 year age range and 15.24 in the 74-84 year age range (Ostrom, 2015). The known risk factor for GBM is exposure to ionizing radiation, most commonly in the form of x-rays or other high-energy therapeutic radiation used to treat childhood tumors. Other risk factors include certain genetic disorders, such as neurofibromatosis, tuberous sclerosis, and other syndromes that seem to provide a predisposition for developing GBM (Nelson, 2012). Some potential risk factors include head trauma, certain medications, and exposure to various other agents, but the cause of GBM is still unknown (Nelson, 2012). GBM has a very poor prognosis - most patients are expected to live about 14-15 months after diagnosis - and is the most common type of glioma. The poor prognosis of GBM is due to the cancer's aggressiveness and the difficulty of treatment.

There are many characteristics of GBM that make it particularly dangerous and difficult to treat. It is known as a "quiet tumor," meaning it has low levels of tumor-infiltrating T cells. T cells are an important part of the immune system that help the body fight infection and, in cancer patients, tumors. The brain has many layers of tissue that surround and protect it, but that also keeps the level of T cells inside relatively low (Evans, 2019).Because it is difficult for T cells to get inside the brain, it is also difficult for them to fight GBM.

This, along with the fact that GBM tends to be highly heterogeneous, means GBM is one of the most immunosuppressive types of tumors. Another aspect of GBM that makes it difficult to treat is the fact that any drugs used to treat the tumor must cross the blood-brain barrier (BBB), which is exceptionally good at maintaining homeostasis and keeping foreign chemicals, including GBM treatments, out of the brain.

The Blood-Brain Barrier

One of the main reasons why treatment of GBM is so difficult is because of the BBB, which is a collection of blood vessels, endothelial cells, and other cells that surrounds and protects the brain. It maintains homeostasis in the brain by regulating which chemicals and ions can move in and out, which is essential for healthy brain functioning (Daneman, 2015). However, this tight regulation of chemicals also makes delivering drugs to the brain difficult. The cells of the BBB are very tightly intertwined, making it difficult for large chemicals to get across. Even when a drug is delivered to the brain, the BBB often removes the drug very quickly in its effort to maintain homeostasis, which prevents the drug from combating the tumor effectively (Bender, 2018).

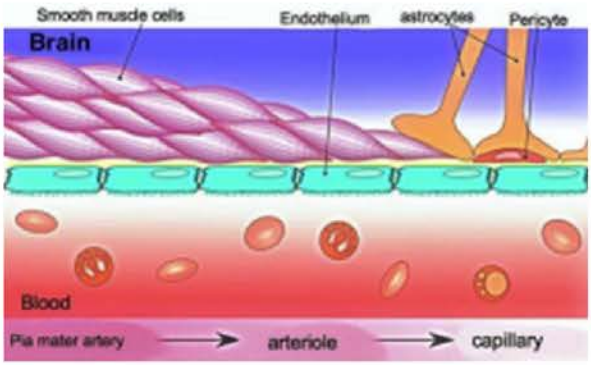

Fig. 1. A model of the blood brain barrier. Tightly joined endothelial cells line the blood vessels, creating a strong barrier to chemicals traveling into and out of the brain. Astrocytes, pericytes, and smooth muscle cells lend structural support to the endothelium. Evans, Taylor. 2020, April 17. How Pathogens Penetrate the Blood-Brain Barrier. American Society for Microbiology. https://asm.org/Articles/2020/April/How-Pathogens-Penetrate-the-Blood-Brain-Barrier.

An emerging technique to bypass the blood-brain barrier is called convection-enhanced delivery (CED). As opposed to typical systemic chemotherapy, CED involves placing catheters directly into the brain in or around the site of the tumor (Lambride, 2022). This process involves minimally invasive surgery and, in concept, is an easy way to bypass the BBB because it delivers the medication directly to the tumor. This technique also has an advantage over systemic chemotherapy because it reduces the toxicity in regions of healthy tissue and can limit its effects to the area directly surrounding the tumor (Lambride, 2022). However, since it is very difficult to examine the concentration of drugs in patients' brains, researchers usually must rely on computer models to predict the efficacy of this method (Lambride, 2022). Even with this limitation, CED could significantly improve the standard of care for glioblastoma by localizing chemotherapy and bypassing the blood-brain barrier.

Standard of Care

Currently, the standard initial treatment for GBM is surgery followed by radiotherapy and/or chemotherapy (Tan, 2020).If possible and relatively safe for the patient, surgery is linked to longer life expectancy when the extent of resection is very high. However, surgery is often difficult to perform without damaging healthy parts of the patient's brain, and it is not completely effective in removing all cancer cells (Tan, 2020). If surgery is not feasible, or after surgery is performed in patients in whom it is feasible, the next standard treatments include radiotherapy and chemotherapy. Radiation is directed toward the tumor site and surrounding areas, where it kills many remaining cancer cells (Tan, 2020). The FDA-approved chemotherapy drug for glioblastoma is temozolomide, which works by adding methyl groups to DNA's nitrogenous bases, disrupting the function of the cell and eventually leading to cell death (Fernandes, 2017). However, there are many problems with this treatment that prevent it from being an effective way to combat glioblastoma. Firstly, over 50% of patients are immune to temozolomide, and even more patients will acquire immunity over time as the treatment continues (Karachi, 2018). Secondly, temozolomide is known to negatively impact the immune system by reducing the number of lymphocytes in the body, which can cause unpleasant and dangerous side effects for the patient due to lower immune ability (Karachi, 2018). Because GBM is already a highly immunosuppressive form of cancer, this side effect of temozolomide is even more serious. Thirdly, temozolomide is usually unable to remove all cancer cells in the body and GBM often recurs within 6 months of treatment (Karachi, 2018). There is no standard treatment for GBM recurrence, but common treatment routes include additional surgery, treatment with different drugs, and repeated temozolomide treatment. Because of the difficulty and ineffectiveness of the standard treatments, researchers have been looking into new and better types of treatment.

Immunotherapy

Many potential immunotherapies are undergoing clinical trials, but there is currently no FDA-approved immunotherapy, and there is no concrete evidence yet that this type of treatment is effective. Additionally, the immunosuppressive nature of GBM makes immunotherapy an inherently difficult method of treatment. Nevertheless, researchers continue to test a wide variety of immunotherapies that could improve survival rates.

One type of potential immunotherapy involves vaccines designed to produce an immune response against tumor antigens. In vaccine immunotherapy, the patient is exposed to tumor specific or tumor-associated antigens along with immune-stimulating molecules in order to activate the body's immune response and make it easier for the immune system to target the tumor (McGranahan, 2019). Tumor-specific antigens, such as EGFRvIII, are antigens found only on GBM cells, while tumor-associated antigens, such as survivin, are not exclusively found on tumor cells but are rare enough in the rest of the body that they remain safe targets for treatment (McGranahan, 2019). These treatments are all still in clinical trials and, while some researchers find the results promising, they will require more testing and clinical trials before they can be used as official treatments. For example, in a clinical trial for a vaccine of the EGFRvIII antigen, a tumor-specific mutation of epidermal growth factor, the median overall survival was 20.1 months in the experimental group and 20.0 months in the control group, which is not a significant difference (Weller, 2017).In a clinical trial for a vaccine of the survivin antigen, an anti-apoptotic protein, the 12-month overall survival rate was 94.2%; however, this trial included a sample size of only 63 and did not include a control group (Ahluwalia, 2018). Additionally, many antigens are HLA-restricted, meaning the safety and efficacy of a vaccine depends on the antigens in the patient's immune system (McGranahan, 2019). Because of this, some vaccines will only be viable treatment options for a fraction of patients, and even if vaccines are proven to be effective treatments, they will not be available to everyone.

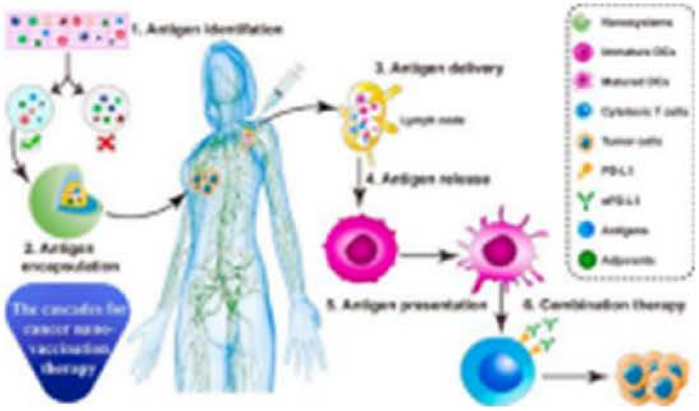

Fig. 2. A brief overview of how vaccine immunotherapy works. Tumor-specific antigens are identified and encapsulated to be delivered to the patient. Inside the patient, the antigens are released and presented to the immune system. Chen, F. et al. 2021. Nanomaterial-based vaccination immunotherapy of cancer. Biomaterials 270:10.1016/j.biomaterials.2021.120709

Another potential type of immunotherapy is CAR-T cell therapy. CAR-T cells are T cells that have been modified to recognize a tumor-associated antigen (Lambride, 2020). When administered into the patient's body, these cells target the tumor and activate other T cells to target the tumor. CAR-T cell therapy is already an established treatment for certain lymphomas and leukemias, so researchers are looking into applying it to GBM (Lambride, 2020). There are many antigens currently under investigation for CAR-T cell therapy, including IL13-Ra2, EGFRvIII, and HER-2 (Lambride, 2020). Similarly to vaccine immunotherapy, these treatments are still in the early stages of testing and clinical trials, and it is still unknown whether they will be viable treatment options. CAR-T cell therapy is potentially dangerous because it is known to produce toxicities, but so far, no clinical trials have shown significantly detrimental effects. As with other types of immunotherapies, more testing is necessary to determine whether CAR-T cell therapy is safe and effective as a GBM treatment.

Conclusion

The future of GBM treatment must involve new techniques and therapies such as convection-enhanced delivery and immunotherapy. The current standard of care is unable to put patients into remission consistently or to extend their lives significantly, and it often produces unpleasant and dangerous side effects. Though the fields of CED and immunotherapy are relatively new, they show more promise than the existing treatments, and researchers must continue to perform clinical trials to develop them into viable, effective, and safe treatments.

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Analgesia: The Importance of Pain Relief

Sarah Masud

Abstract

Analgesia refers to the relief of pain without the loss of consciousness or sensation. Pain relief is something that is necessary in life, and it has many practical applications during and after surgery. There are various types of analgesics that are used to treat different kinds of pain, but they can fall into two major categories: anti-inflammatory drugs and opioids. When deciding on the best kind of analgesics to administer, specifics about the patient and the procedure must be considered. Overall, they play a crucial part in the medical field, although they have their risks and disadvantages as well.

Introduction

Pain remains a continuous and troubling part of everyday life. Thankfully, there are many ways to relieve it. Analgesia refers to the relief of pain without the loss of consciousness or sensation, and modern advancements in science have given it a practical use in many surgeries and other medical procedures. In contrast to anesthetics, analgesics may simply be referred to as pain relievers; they differ in that they do not induce sleep or render patients unable to move as anesthesia does.

There are various types of analgesics that are used to treat different kinds of pain, but they can fall into two major categories: anti-inflammatory drugs and opioids. The most familiar type of anti-inflammatory analgesics may be over-the-counter painkillers such as acetaminophen, which includes common brand names such as Tylenol and Nyquil, and ibuprofen, which includes Advil. Others may require prescriptions, and this is often due to a higher dosage of over-the-counter analgesics or a combination of multiple nonsteroidal anti-inflammatory drugs (NSAIDs). Although not addictive in nature, they may carry certain side-effects such as gastrointestinal irritation, ulcers, and liver toxicity (Teater, 2014). For these reasons, the use and wide distribution of several anti-inflammatory analgesics was limited when they were first created.

Most opioids, however, require a prescription. They function differently from anti-inflammatory analgesics in that they pose a higher risk of misuse, dependency, and addiction. Despite this, opioids have been used and identified as one of the only effective treatments for pain for thousands of years. It remains a well-known fact that they have a strong ability to relieve pain, and they've been commonly used to treat mental illnesses such as anxiety and depression as well. But, recent studies have actually shown that taking acetaminophen and ibuprofen together is more effective than using opioids (Kissin, 2013). Before diving into the negative effects that pain relief medications have generated in the current opioid epidemic, the benefits and useful applications that analgesics bring to the modern medical world will be discussed.

Regional Analgesia in Trauma

Regional analgesia refers to numbing a specific part of the body in order to relieve pain or allow for the execution of medical procedures. This proves to be effective for orthopedic trauma patients, as the use of regional analgesia reduces the chances of developing chronic pain and potentially misusing opioids as an alternative treatment (Cunningham et al, 2021). Trauma and the pain that comes with injuries stimulate a protective response in the body to initiate inflammatory, metabolic, and endocrine responses to relieve it. This means that without properly tending to the issue, whether it's acute or continued traumatic pain, there's a chance that this stress response will further develop into chronic pain (Fleming & Egeler, 2014). But, with regional analgesia opening the door for life-saving techniques and advancements, the body's natural responses can be altered to prevent this.

Rib fractures are the most common type of injury that result from trauma to the chest, and they often lead to severe pain and failed respiratory function. If they are left poorly treated, they can result in complications such as pneumonia and acute respiratory distress syndrome (Gadsden & Warlick, 2015). In addition to this, recent studies indicate that rib fractures are associated with chronic pain, disability, and an impaired quality of life that goes beyond the injury itself (Heindel et al, 2022). The goal of regional analgesia in this case is to minimize respiratory depression and reduce any possible side effects, though the specific type of treatment depends from patient to patient due to different anatomical positions of rib fractures.

When deciding on the best method to treat acute orthopedic trauma pain, several factors have to be considered. For reference, the fracture location, tissue involvement, and operative fixation have notable effects on pain intensity, and trauma to the bone and soft tissue is often associated with increased inflammation and pain patterns (Cunningham et al, 2021). Elective surgeries that are paired with regional analgesia have been able to reduce opioid consumption and postoperative pain, although other surgical approaches require a discussion between the surgeon and anesthesiologist about the potential risks and benefits.

Every patient will have a different fracture pattern, leading to different operation details and potential postoperative concerns (Gessner et al, 2020).

Analgesia During Childbirth

The use of pain relievers during childbirth has a long history of controversy and social movements surrounding it. The reason behind these disputes ultimately comes down to the fact that every woman has a different experience of childbirth, which includes not only the physical pain but the psychosocial, emotional, cultural, and sensory factors as well. Often, the methods that healthcare professionals use to measure and treat this pain do not take these other elements into consideration (Caton, 2015). Some may want to cope with the pain associated with labor because of the meaning they assign to the experience. However, over the past 50 years of advancements in obstetric anesthesia and analgesia, women have been able to choose from a range of options including neuraxial analgesia, nitrous oxide, systemic opioids, and nonpharmacologic methods (Gibson, 2021).

Since anesthetics were first introduced, there have been debates surrounding who should have access to pain relief in which particular cases. Some argued that it was only necessary when the practitioners were inflicting pain, as such is the case during instrumental delivery. There were also those who believed that the natural pain associated with childbirth shouldn't be relieved at all, and others suggested that any pain should be alleviated if there's a way to do so (Pernick, 1985).

During the first wave of feminism in the early 1900s, many women began demanding for their right to have a pain-free birth. In these early times, women could be compared to children and the insane in the sense that they were thought to be incapable of exercising bodily autonomy. However, during the second wave of feminism in the 1960s and 1970s, the movement fighting for women's reproductive rights took off. This ultimately helped in creating more humane childbirth practices that took their pain into consideration (Nichols, 2000). By the third wave, more women were acting on their right to use neuraxial analgesia during the birthing process, which allows for them to remain conscious during childbirth. This continues to be the most common form of pain relief used during labor, and between 1981 and 1997, the use of neuraxial analgesia during childbirth increased from 22% to 66% of births in the busiest hospitals of the United States (Camann, 2014).

Multimodial Analgesia

The perioperative period of a procedure refers to the time surrounding the surgery, which includes the preoperative, operative, and postoperative periods. Perioperative pain management is intertwined with anesthesia and analgesia, and the discussion of which types of drugs and methods are the most effective is an ongoing one.

Because it is common for moderate to severe pain to persist after surgery, some sort of postoperative analgesia is crucial to both relieve the short-term effects and prevent the onset of long-term chronic pain. If administered inadequately, some complications that may arise include decreased patient satisfaction, delayed postoperative mobilization, a higher chance of experiencing cardiac and pulmonary issues, and increased morbidity and mortality (Gerbershagen et al, 2013). In fact, 30% to 80% of patients continue to report pain even after relatively minor procedures (Meissner & Zaslansky, 2019). So, what can be done to prevent this?

Since relying on opioids for perioperative and postoperative pain leads to a higher chance of developing opioid dependence and hyperalgesia (increased sensitivity and responsiveness to pain), multimodal analgesia turns out to be a better alternative. This refers to the balanced use of low to moderate doses of nonopioids, and this is meant to maximize analgesic activity by targeting different pathways in the nociceptive system and attacking pain from several sides. Since it's also currently difficult to administer opioid-free anesthesia on a large scale, a multimodal analgesic approach helps to limit opioid usage to the minimum dose and time required, ultimately minimizing the risk of any side effects (O'Neill & Lirk, 2022).

One advantage of multimodal analgesia is that the combination of several drugs allows for lower doses of each individual one, thus reducing the adverse effects that may come with high doses of each drug while still being effective in treating pain. The American Society of Anesthesiologists (ASA), the American Pain Society (APS) and the American Society of Regional Anesthesia and Pain Medicine (ASRA) have each strongly recommended using multimodal analgesia to relieve perioperative pain, and it is now considered the standard for surgical patients (Ladha et al, 2016). Orthopedic surgery, which is generally one of the most painful types of surgical procedures, has also been applying multimodal analgesia for at least a decade considering that it leads to better analgesic outcomes in this specific type of surgery (Halawi, 2015).

Although the specifics of a multimodal analgesic approach will differ depending on the procedure and patient, there is a typical outline when generating a plan. Given that there are no contraindications, acetaminophen and either a NSAID or cyclo-oxygenase-2 inhibitor are the baseline drugs that should be used. The next things to be considered are adjuvants, such as dexamethasone, gabapentinoids, ketamine, and alpha-2-agonists. They each have advantages and disadvantages depending on what is being treated; for instance, alpha-2-agonists can be especially helpful for anxious patients.

Opioid Misuse

Although analgesics provide many advantages and applications to the medical world, it's important to discuss the negative effects as well. The use of prescription opioids has increased over the past 20 years in North America, and with that, so have the overdoses, poisonings, and deaths caused by both prescription and illegal use (Public Health Agency of Canada, 2020). Opioid misuse also poses more of a risk among specific populations. This includes people with lower incomes or periods of employment instability, as well as Indigenous people. The reason behind this lies in inequalities in the socioeconomic determinants of health and racially discriminatory health services (Phillips-Beck et al, 2020). Indigenous people have also been historically undertreated and subjected to poor quality treatment, which has resulted in trauma and increased susceptibility to disabling pain.

Administrative health data has also shown an increasing trend of opioid-related poisoning among people aged 15 to 24 (Canadian Institute for Health Information, 2016). This alarming opioid crisis resulted in Canada enacting new guidelines concerning opioid-prescribing in 2017. These guidelines seeked to minimize exposure to opioid prescriptions and instead opt for alternative pain treatments that would reduce future harm. But, experts claim that these efforts will unlikely resolve all harm-related issues for people who are currently experiencing chronic pain and using opioids as a form of pain relief (Furlan & Williamson, 2017).

Conclusion

In the context of analgesics being used to aid in surgeries as well as everyday life, they have a notable impact. There are various types of drugs, whether anti-inflammatory or opioids, that can assist in treating different kinds of pain and preventing chronic pain. They have a long history of debate and controversy as well, and it is still unclear in some situations what the most appropriate analgesic approach would be. Overall, astounding advancements in science have been made that allow for analgesia to play a significant and necessary part in many procedures.

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Mediation of Rat's Social Dominance by Medial Prefrontal Cortex

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Abstract

Social dominance in animals predicts competitive success and access to desirable resources. Dominant animals tend to monopolise food and forage more effectively than subordinate group members. At the neuronal level, a region commonly associated with dominance-related behaviours is the medial prefrontal cortex (mPFC). Mice studies demonstrated that manipulating mPFC neurons in-vivo shifts dominance rank in the hierarchy. However, there are limited studies on rats involving the effects of in-vivo mPFC manipulation. Our study applied chemogenetic methods to investigate the role of mPFC neurons in the social dominance of male and female rats. Rats were tested in individual and group competitions to account for dominance behaviour in different interactions. In individual competitions, mPFC inhibition led to a delayed decrease in male dominance behaviour yet an instantaneous decrease in female dominance behaviour. These changes did not affect dominance rank. In group competitions, the effects of mPFC inhibition were variable. Our findings suggest that mPFC activity is likely one component in a multivariate mechanism that mediates rats' social dominance.

Introduction

Animals are known to establish social hierarchies with varying complexity depending on species. More importantly, rank in the hierarchy determines access to resources and mating opportunities within an animal group. Dominant animals tend to monopolise food and forage more effectively than subordinate group members who subsequently adjust their competitive efforts (Li et al., 2022). Social ranks can be determined by several factors including size, gender, and personality traits (Ferreira-Fernandes & Peca, 2022). In simple species, where social hierarchy relies heavily on physical contest, dominant animals are typically larger and less timid males. More complex species, such as humans and non-human primates, have more complex rules in their social hierarchies (Ferreira-Fernandes & Peca, 2022).

At the neuronal level, dominance-related behaviours of animals are associated with the medial prefrontal cortex (mPFC) (Holson, 1986; Uylings et al., 2003; Wang et al., 2011; Zhou et al., 2017). Rats with mPFC lesions are lower in social rank and express more timid behaviours than intact controls (Holson, 1986). Manipulations of mPFC neurons in mice cause instantaneous changes in competitive successes and effortful behaviours (Zhou et al., 2017). Findings in animal studies are compatible with patient studies and functional neuroimaging in humans. The prefrontal cortical region is attributed to social information processing and social behaviours (Blair & Cipolotti, 2000; Mah et al., 2004; Zink et al., 2008; Chiao, 2010). These findings suggests that the mPFC is essential for assessing social context in the environment and producing appropriate behaviour. However, there is a lack of study in the literature comparing potential functional differences between sex. Social impairments implicated in neuropsychiatric disorders, such as Autism Spectrum Disorder, commonly exhibit sex differences (Ochoa et al., 2012; Werling & Geschwind, 2013; Li et al., 2016). It is unclear if there are sex-specific functional differences in relevant regions, including the mPFC, that may contribute to this symptomatic variability. To better understand the mPFC's role in the social dominance of both sexes, we investigated the effects of inhibiting mPFC neurons in male and female rats.

In the wild, rats may compete dyadically with conspecifics or in groups. A comprehensive study on rats' social dominance should consider dominance behaviour in different competitive interactions. Two behavioural paradigms were adopted in this study to observe social dominance in individual and group competition. The tube test is commonly used in the literature to determine hierarchy in rodents due to its simplicity (Zhou et al., 2018). It involves a narrow tube where a pair of rats meet at the centre and attempt to advance by pushing their forcing their counterpart to retreat. Whichever rat is successful is declared the winner of the trial. Winner rats are likely to be more dominant and higher ranked in the hierarchy (Zhou et al., 2018). On the other hand, the sucrose competition is a relatively novel test designed to observe behaviours in a group setting. Rats compete to occupy a desirable reward for as long as possible. To achieve this, a dominant rat would remove a preexisting occupant while resisting attempts by other rats to prevent itself from being displaced. As such, dominance behaviour in this paradigm is defined by the total time spent occupying the bottle containing sucrose solution. In both paradigms, social dominance has two components: dominance behaviour and dominance rank. Dominance behaviours are operationally defined by a metric in each paradigm: David's Score measures dominance behaviour in the tube test, total time spent occupying the sucrose bottle measures dominance behaviour in the sucrose competition. Dominance rank is derived from the degree of dominance behaviour expressed relative to other group members. Male and female rats are ranked daily to identify their hierarchy and detect any changes.

This study uses a chemogenetic tool known as Designer Receptors Exclusively Activated by Designer Drugs (DREADD), which are a class of G-coupled protein receptors artificially engineered to bind with synthetic ligands. DREADDs lack an endogenous ligand to activate them but are sensitive to the inert drug clozapine N-oxide (CNO) (Smith et al., 2016). DREADD's reversible and highly specific nature is ideal for behavioural studies involving in vivo manipulations. A common vector used to express DREADD on neuronal membranes is known as an adeno-associated virus (AAV). Thus, to apply chemogenetics in this study, manipulated rats are injected with AAV in the mPFC region. Once DREADD expression is achieved in two weeks, DREADD agonist CNO can be injected intraperitoneally to inhibit mPFC neurons on demand. Effects of CNO are observable 15-20 minutes after injection and are expected to last no longer than 9 hours (Zhou et al., 2017; MacLaren et al., 2016; Jendryka et al., 2019). Finally, we hypothesise that the targeted inhibition of mPFC neurons reduces dominance behaviour in all competitions and decreases dominance rank in the hierarchy.

Methods

Animals. All procedures were approved by the University of Illinois Urbana-Champaign's IACUC. All experiments were performed on wild-type Long Evans male (n = 4) and female rats (n = 4) ages 1 to 3 months. Animals were bred from a lineage of rats received from Charles River Laboratories. Animals were assigned into experimental groups based on sex and housed together in large (480 x 375 x 210 mm) cages. Animals were allowed to freely interact with their group members at least 3 days before experimentation. Animals were tail- marked with Sharpie permanent markers and remarked every week. Rats were maintained on a 12:12 light-dark cycle (6am to 6pm) with food and water provided ad libitum. Experiments were conducted during the light phase of the cycle and bodyweights were recorded daily.

Tube Test. The apparatus was made up of a one-metre clear acrylic tube with chambers connected on both ends. A slot was cut out at the centre of the tube to insert a divider. The diameter of the tube was large enough to allow a rat to pass through from one end to another, but not sufficient for two rats to pass each other. Tube with incresaing diameters were used over time to accommodate the growing rats. Before testing began, each rat was acclimated to the apparatus by ensuring that they were comfortable entering the tube. Acclimation was considered unsuccessful and repeated the next day if rats failed to complete 10 tube-crossings in 15 minutes. During testing, a rat was placed in each chamber and the divider was removed when both rats met in the middle of the tube. The trial ended when a rat was forced to retreat out of the tube. The rat that successfully displaced its counterpart from the tube was declared the winner. Trials were video-recorded and arranged in a round-robin format to ensure every possible pairing was tested each day. Rats were also randomly assigned to the chambers to control for side bias. The apparatus was wiped down with 70% isopropyl alcohol between each trial. The dominance behaviour of each rat in their respective group was calculated daily using a metric known as David's Score (DS). DS accounts for cumulative wins and losses. Rats were ranked daily according to their DS to identify the hierarchy of the group. Tube tests were conducted for five consecutive days in the first week to allow rats to establish a stable hierarchy before surgery. Later, two weeks of tube testing were conducted with 5mg/kg CNO and 10mg/kg CNO manipulation respectively. On the weeks of CNO manipulation, CNO was administered on Day 2 and Day 4.

Sucrose Competition. Animals in a group competed for access to a bottle containing 10% sucrose solution in an open field arena. The arena (1 x 1 x 0.5 m) was built with 16 polyethylene panels and a bottle holder installed onto one of the panels. The unique panel was also modified to include a cylindrical extension surrounding the bottle tip and acrylic panels (72 x 305 mm) on both sides of the cylindrical extension serving as barriers. These modifications were made to only allow the head of one animal to reach the bottle tip. This designed required a competitor to forcefully displace the existing occupant to gain access to the sucrose reward. We performed one day of acclimation by allowing 25 minutes for rats to explore the arena as a group and learn the reward. The arena was wiped down with 70% isopropyl alcohol before the next group of rats was acclimated. All rats were food restricted approximately 15 hours before acclimation or testing to increase salience of sucrose reward. During testing, rats were placed in the arena as a group for 25 minutes and the session was recorded using a camera installed above the arena. The floor of the arena was wiped down with 70% isopropyl alcohol before the next group of rats was tested. Dominance behaviour was measured by the amount of time each rat spent occupying the sucrose bottle. Each rat was subsequently ranked to identify the groups' hierarchy. Sucrose competition took place the week following the completion of the tube test. After one day of acclimation, testing was conducted for five consecutive days with 10mg/kg CNO was administered on Day 2 and Day 4.

Viral Injection. Before selecting rats for viral injection, we ran a week of tube testing to identify the social hierarchy of each group. By the end of the week, rank-1 and rank-2 rats of each group (n = 4) were selected to receive AAV injection to enable local inhibition of mPFC neurons. Animals were anaesthetised with 3-5% isoflurane via inhalation followed by intraperitoneal (IP) injection of a ketamine-xylazine mixture. The mixture contained 3.25 mL of 100mg/mL ketamine, 1.65 mL of 20mg/mL xylazine, and 10mL saline solution. The head of the anaesthetised rat was fixed on a Kopf stereotaxic frame, followed by bilateral craniotomies lateral of the sagittal suture and anterior of bregma. A syringe with a needle connected to a syringe pump was slowly lowered to the stereotaxic coordinates relative to bregma: AP: +3.0 mm, ML: +/- 0.6 mm, DV: -3.3 mm to target mPFC. 2000 nL of AAV8-CaMKIIa-hM4D(Gi)- mCherry was injected bilaterally at a rate of 5 nL/s. Sham surgeries were performed on rank-3 and rank-4 rats of the groups. The protocols for anaesthesia and stereotaxic surgery were replicated with saline solution held

Chemogenetic Manipulation. One animal was selected from each group to receive CNO injections based on their rank in the hierarchy. Two criteria for selection were: (1) animal must have received AAV injection during surgery and (2) animal must be ranked in the upper half of the hierarchy (i.e., rank-1 and rank-2). On days of manipulation, rats with AAV received a CNO injection 30 minutes before testing while others received a saline injection. All injections were administered via IP 30 minutes before testing. CNO injections were prepared by dissolving 5 or 10 mg of CNO in 100 µL of dimethylsulfoxide (DMSO) and then diluted in 900 µL of saline.

Bodyweight-Dominance Correlational Analysis.
The correlation between bodyweight and social dominance is calculated using Pearson product-moment correlation coefficient, r. This dimensionless index ranges from -1.0 to 1.0 and measures the extent of a linear relationship between two data sets. Correlational analysis for the tube test compared the daily bodyweight of each rat against their DS and dominance rank for each day. Similarly, correlational analysis for the sucrose competition compared daily bodyweight of each rat against their total time spent occupying the sucrose bottle and dominance rank for each day. A total of eight correlational analyses were conducted, correlations with r above +/- 0.7 were regarded as significant.

Results

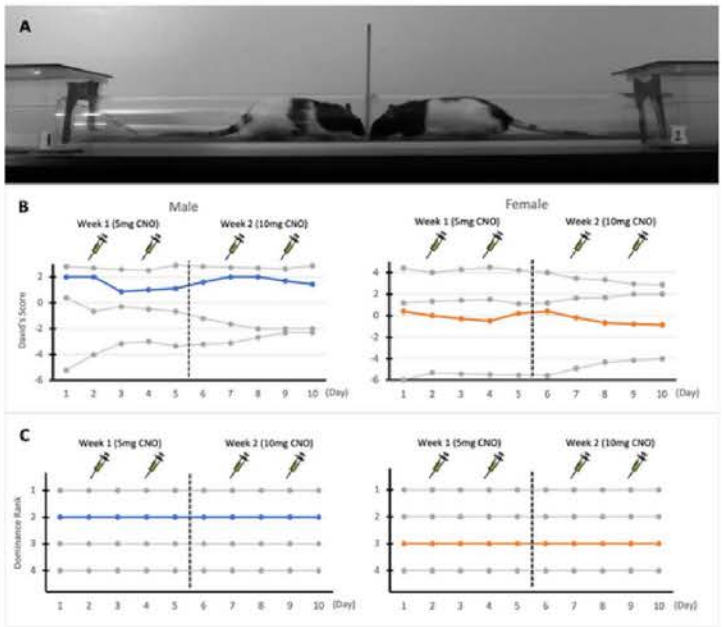

Fig. 1. Effects of mPFC inhibition on dominance in tube test. **(A)** Setup of a tube test trial. **(B)** David Scores of rank-2 male rat and rank-3 female rat after receiving 5mg/kg CNO injection in Week 1 and 10mg/kg CNO injection in Week 2. **(C)** Dominance in the tube test ranked by David's Score of each rat.

One group of male rats and one group of female rats were tube tested. Fig. 1B illustrates the effects of 5mg/kg and 10mg/kg CNO on the David Score (DS) of both sexes. On the first week, 5mg/kg CNO had no immediate effect on male DS on Day 2, but male DS decreased on Day 3. Although male DS increased slightly following 5mg/kg CNO on Day 4, it remained below baseline (DS = 2.0) since the initial decrease on Day 3. Male DS restored close to baseline over the 10mg/kg. There were no changes in male DS after the first CNO injection on Day 7. Male DS decreased slightly after the second CNO injection on Day 9 and continued to decrease on Day 10.

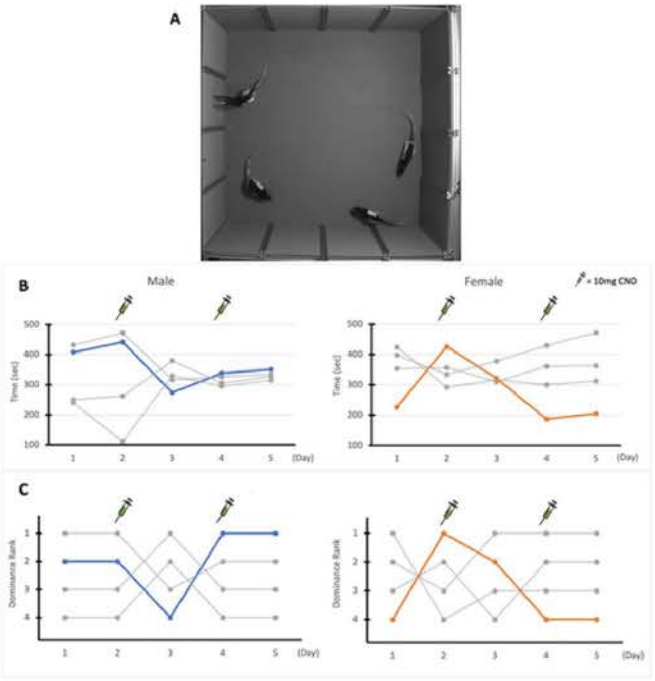

Fig. 2. Effects of mPFC inhibition on dominance in sucrose competition. **(A)** Setup of the sucrose competition. **(B)** Total time spent occupying sucrose bottle by rank-2 male rat and rank-4 female rat after 10mg/kg CNO injection. **(C)** Dominance in the sucrose competition ranked by the total time spent occupying the sucrose reward by each rat.

In females, DS decreased after administering 5mg/kg CNO injection on Day 2 and continued to decrease steadily over subsequent days. Female DS was lowest in that week following the second 5mg/kg CNO injection on Day 4. Similar to the male rats, DS restored to near baseline levels (DS = 0.4) over the weekend between Week 1 and Week 2 of tube testing. On the second week of tube test, female DS exhibited a similar pattern to Week 1 where DS decreased steadily after the first CNO treatment. Here, DS was the lowest on the last day of Week 2. Collectively, 5mg/kg and 10mg/kg CNO did not affect the dominance rank of the male and female rat (Fig. 1C).

Fig. 2 illustrates the effects of 10mg/kg CNO injection during one week of sucrose competition. In Fig. 2B, male's total time spent occupying the sucrose bottle increased immediately following 10mg/kg CNO injection on Day 2 and Day 4. However, there was a sharp decline in total time on Day 3 before a slight rebound on Day 4. It was noted that there was a large deviation between the total time of rank-1, rank-2 rats and rank-3, rank-4 rats on the first two days of sucrose competition. This deviation diminished after Day 2 and the total time of all males became close in proximity on Days 4 and 5. In terms of dominance rank, the CNO- injected male experienced a downward shift in rank the day after the first CNO injection. This did not occur after the second CNO as the hierarchy remained unchanged on Day 5. At the same time, female's total time also increased upon receiving 10mg/kg CNO injection on Day 2, though this was not

observed on Day 4. Total time gradually returned towards baseline (226 seconds) after the initial increase on Day 2. It was noted that the female's total time on Day 3 was very close to that of rank-3 and rank-4 female rats. As for dominance rank, 10mg/kg CNO injection induced an instantaneous upward shift in female dominance rank from rank-4 to rank-1. The dominance rank later shifted downwards on Day 3 and returned to rank-4 on Day 4 after second CNO injection. Like its male counterpart, the female hierarchy remained unchanged on Day 5.

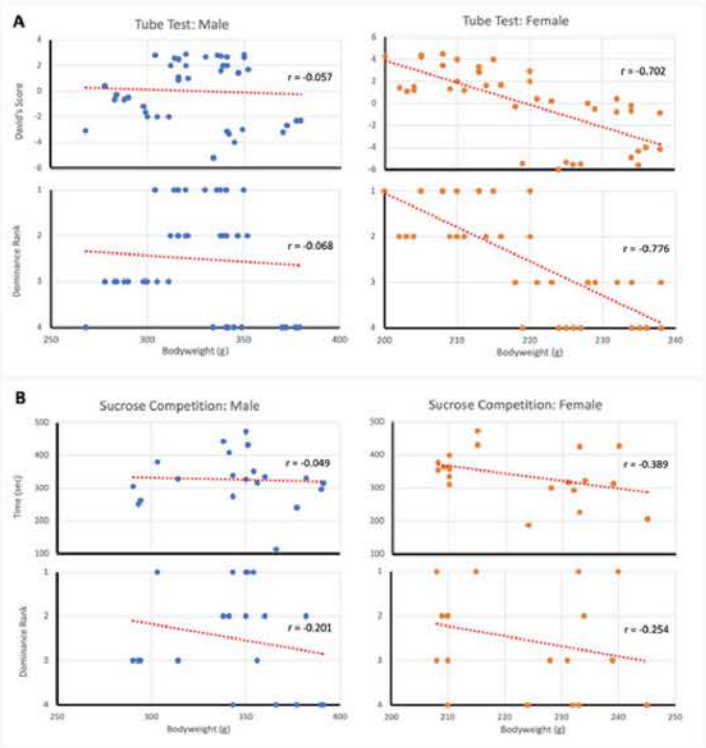

Fig. 3. Correlational analysis between bodyweight and dominance. (A) Relationship between bodyweight and measures of dominance in the tube test. **(B)** Relationship between bodyweight and measures of dominance in the sucrose competition.

The relationship between bodyweight and dominance was analysed using the Pearson correlational coefficient (Fig. 3). During 10 days of tube testing, bodyweight was not correlated to DS (*r*DS = -0.057) or the dominance rank (*r*Rank = -0.068) of male rats. However, there was a negative correlation between female bodyweight and both measures of dominance (*r*DS = -0.702 and rRank = -0.776; Fig. 3A) in the test tube. In the sucrose competition, there were also no correlation between male bodyweight and DS (rDS = -0.049) or dominance rank (rRank = -0.201). In females, there was no correlation between bodyweight and both measures of dominance in the sucrose competition (rDS = -0.389 and rRank = -0.254; Fig. 3B).

Discussion

The tube test was intended to study rat's social dominance in an individual competition. At the start of each testing week, male rats did not show instantaneous change in dominance behaviour in response to the first CNO injection (Fig. 1B). But we observed a decrease in dominance behaviour 24 hours later. The absence of immediate effects was likely due to animals behaving based on past competitive successes. If a subordinate rat has consistently lost against a dominant rat, the subordinate rat may expect to lose again. This win history may prompt the subordinate rat to initiate fewer pushes and offer less resistance against push attempts (Zhou et al., 2017). Thus, a dominant rat that was recently given CNO may still be able to win the initial bouts easily although it no longer expressed usual levels of dominance-related behaviours. This idea is supported by the observations made during video reviews of the tube test. However, when rats are returned to their home cage, the CNO-injected rat and saline-injected rats are able to interact over an extended period. Then, the effects of CNO on the dominant rat becomes apparent to its group members and the social hierarchy is affected. This may explain the delayed decrease in the male's dominance behaviour following the first CNO injection in Week 1 and Week 2. Past CNO studies with mice found that the behavioural effects of CNO is known to last between six to nine hours (Alexander et al, 2009; Zhou et al., 2017). This suggests that the delayed changes in dominance behaviour that occurred nine hours after CNO injection was not caused directly by the CNO's inhibition of mPFC neurons.

In contrast, CNO injection caused an instantaneous decrease in the dominance behaviour of the female rat (Fig. 1B). Unlike males, female rat hierarchies are less linear and more susceptible to external factors (Williamson et al., 2019; Varholick et al., 2019). Studies observed markedly less strict hierarchies in female rats and mice (Fulenwider, 2022). In our study, the effects of CNO readily influenced the interactions of the CNO-injected female with other group members and led to changes in the outcomes of the tube test. Although mPFC inhibition via CNO was insufficient to shift ranks in the female hierarchy (Fig. 1C), a decrease in David's Score (DS) indicated losses in trials where wins were expected. DS is a measurement that accounts for past results by comparing both animals' proportion of wins and losses (Gammell et al., 2003). An animal with high win proportions is 'expected' to win, DS will shift more significantly upon an upset. The DS of the rank-3 female rat decreased over both days of CNO which was accompanied by a similar trend on Week 2 (Fig. 1B). This suggests that mPFC inhibition attenuated dominance behaviour in female rats.

CNO dosage increased from 5mg/kg to 10mg/kg on the second week of tube test (Fig. 1B, C) to increase the salience of its effect, if any. Ultimately, mPFC inhibiton did not affect the social hierarchy of male and female rats in our sample. Although studies in the literature found that manipulations of the mPFC consistently shifted dominance ranking in the hierarchy, the manipulations were performed on mice (Wang et al., 2011; Zhou et al., 2017; Li et al., 2022). Mice are simpler species where social hierarchy is largely determined by physical contest. In such cases, changes in dominance behaviour caused would shift ranking within the hierarchy more readily. However, rats are more socially tolerant and less hierarchical (Schweinfurth, 2020). Our results suggest rat's social hierarchy may be more complex. The dominance rank of rats may be mediated by other factors in the environment in which the inhibition of mPFC neurons alone may not be significant. This might be a result of their

naturalistic behaviour in the wild, where rats are found to live in large groups (Schweinfurth, 2020). Hence, it is necessary to consider social dominance in a group setting to assess the effects of mPFC inhibition by CNO.

In group competitions, the effects of mPFC inhibition on social dominance were highly variable. Male dominance behaviour decreased 24 hours after the first CNO injection, but the same effect was not observed after the second CNO administration (Fig. 2B). At first glance, the decrease in dominance behaviour on Day 3 may resemble tube test results. But the saline- injected rank-1 rat also saw a significant decrease in dominance behaviour on Day 3 (Fig. 2B). Moreover, rank-1 rat behaved similarly to the CNO-injected rat throughout the testing period. Given the limited testing period of the sucrose competition, no comparison can be made to better explain these outcomes. The male results on Day 3 could not be definitively ascribed to the effects of mPFC inhibition. The behaviour observed in rank-3 and rank-4 rats via video review implies that both rats had not learned the reward. Both rats had tendencies to explore the arena and showed less interest in competing for the reward. This allowed rank-1 and rank-2 rats to dominate the sucrose competition on the first two days of testing. This rationale is supported by the large deviation in total time spent occupying the bottle between the learned and unlearned group (Fig. 2B). Furthermore, the competition narrowed beginning on Day 3 and remained as such in the following days. This suggests that the laggard rats had acquired the reward on Day 3 and began to compete for the reward from then on. For this reason, the results of Days 2 and 3 could not be compared to those of Days 4 and 5. It is unclear how the effects of mPFC inhibition contributed to results on Day 3.

As for females, there was a significant increase in dominance behaviour and dominance rank on the day of CNO. However, this was a single occurrence that did not repeat upon the second CNO injection. Both measures of social dominance steadily returned to baseline over the next three days. Here, CNO led to an increase in dominance behaviour and improvement in dominance rank on Day 2 only (Fig. 2). This isolated occurrence was likely the result of factors unrelated to mPFC inhibition as we identified hunger level as a possible cause. When food restriction was imposed the evening prior to testing on Day 2, food may have been removed when the rank-4 rat was not fully satiated. At the start of testing the following day, the rat would have been especially hungry and highly motivated to consume a gustatory reward. This did not occur again on other days of testing. We also note that upon receiving CNO on Day 1, the rat was already at the bottom of the hierarchy with considerably low dominance behaviour as shown by the difference in total time at the bottle (Fig. 2B). This demonstrates a floor effect where the effects of mPFC inhibition cannot be measured accurately due to a lower limit. The lowest- ranked rat is unable to fall further in dominance rank, the ability to observe changes in dominance behaviour is also limited. In this case, any manipulation introduced to the rat has two possible outcomes: (1) social dominance remains unchanged, or (2) increased social dominance.

The rank-4 female rat was selected for manipulation despite the limitations of the floor effect because it was previously selected for manipulation during the tube test as well. To control for the long-term effects of mPFC inhibition, the rat was selected again for manipulation in sucrose competition.

Social hierarchies emerge whenever there is competition between individuals for important resources (Williamson et al., 2019). The more intense the competition, the more likely that a highly linear social hierarchy will develop within the group. In mammals including rodents, males often form highly linear social hierarchies through high intra-sexual competition. Female rodents, on the other hand, form hierarchies that are less linear, steep, and despotic—in some cases even non-existent (Varholick et al., 2019; Williamson et al., 2019; Fulenwider, 2022). To test the findings in our study, a correlation analysis was conducted between the bodyweight and social dominance of our rats (Fig. 3). Overall, no relationship was found between bodyweight and either measure of social dominance, except for the female tube test results (Fig. 3A). This finding aligns with other rodent studies in the literature that generally found no association between bodyweight and dominance rank (Lindzey et al., 1961; Berdoy et al., 1995; So et al., 2015; Williamson et al., 2016; Williamson et al., 2019). Other intrinsic and extrinsic factors have been proposed as determinants of social hierarchy in rodents (Berdoy et al., 1995; Fulenwider, 2022). Intrinsic factors are inherent physical and mental attributes, such as antagonistic behaviours; whereas extrinsic factors originate from the environment, such as past competitive successes (Zhou et al., 2018). Nevertheless, an interesting finding in this correlational analysis was the negative correlation between bodyweight and female dominance in the tube test. This can be attributed to the design of the tube test. Video review of the tube test suggested that although larger rats may have better resistance against push attempts, smaller rats could force their counterpart to retreat by scratching or headbutting. This indicates that the tube test does not necessarily favour big, heavy rats since there are alternative methods for rats to express dominance-related behaviours.

The sample size used in this study served as the primary limitation. Without any comparisons to make, the interpretations of our findings had to consider occurrences by probability. A larger sample size for both male and female groups would have allowed inferences to account for probability. There were also limitations found within the design of our study. Firstly, the habituation for sucrose competition was inadequate in which testing began before all rats in the group acquired the reward. This affected the male results, and possibly female results, in addition to the limited number of sucrose competitions that were run. Future studies involving the sucrose competition should ensure that the acclimation process is continued until all rats display a noticeable interest towards the reward. Subsequently, the timeframe for sucrose competition was limited to five days with one day between the first and second manipulation. This prevented the possibility of observing behavioural trends over the weekend when manipulation was absent. A two-week

timeframe for the sucrose competition would also allow a result comparison between Weeks 1 and 2. Future directions of this study should expand to include mPFC excitation in both sexes for comparison with similar experiments done in mice studies. Although the tube test has been established over many decades, the sucrose competition is a novel paradigm that more closely resembles natural foraging behaviour in rats. The sucrose competition is a useful behavioural paradigm that should complement existing paradigms in the literature. Adopting more than two paradigms in a behavioural study is an effective method to overcome the limitations posed by each paradigm.

In conclusion, we found that the inhibition of mPFC neurons via chemogenetics decreased dominance behaviour in individual competition. The effect was delayed in male rats but instantaneous in female rats due to the dynamic nature of female hierarchies. However, mPFC inhibition was insufficient to induce a downward shift in the dominance rank. Increased inhibition of the mPFC neurons did not alter the effects on social dominance. In group competitions, the role of mPFC in social dominance was unclear. Correlation analysis found that bodyweight was generally not associated with social dominance, except for a negative correlation in female individual competition. This negative correlation was attributed to the nature of the competition which was less reliant on size. Collectively, our findings suggest a degree of complexity in the social dominance and hierarchy of rats. mPFC neurons may be recruited as a component in a multivariate mechanism that mediates rats' social dominance

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Dedicated to Ma, Pa, Gong, Po, and Mei.

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Immediate Early Gene Expression in D1-SPNs and D2-SPNs During a Striatum-dependent Reinforcement Learning Task

Cynthia Mu, Emily Shao, Jones G. Parker

Abstract

Dopamine signaling is thought to promote movement by differentially altering the excitability of the striatum's principal neurons (D1- and D2-SPNs). Here, we used immunohistochemistry to quantify the expression of Fos, a marker of neural activity, in mice trained to run in a head-fixed fear conditioning task that requires dopamine signaling in the striatum. Training in the task increased the number of Fos-expressing neurons, and a greater proportion of these neurons were D1-SPNs than D2-SPNs. However, this relative increase in D1-SPN Fos expression was not specific for learning the task, as a similar increase was observed in animals that underwent training, but did not learn to perform the motor response. In those animals, D1-SPN activation may encode something other than the learned response. Although further experiments are necessary to determine what the Fos-active populations encode in learners and non-learners, the training-dependent changes we observed in the levels of Fos expression in D1- and D2-SPNs may correspond to fluctuations in neural plasticity that may contribute to the changes in neural calcium activity previously observed by others in our laboratory. Our findings have implications for understanding disease processes that affect the dopamine system, such as Parkinson's disease and schizophrenia.

Introduction

The striatum is the main input to the basal ganglia, a collection of interconnected brain nuclei involved in motor control (Albin et al. 1989, DeLong 1990, Smith et al. 1998). Aberrant striatal and basal ganglia function plays a crucial role in many neurological disorders characterized by deficits in motor function. For instance, in Parkinson's Disease, dopamine-releasing cells that project to the striatum and modulate the direct and indirect basal ganglia pathways degenerate to impair movement. 95% of the cells located within the striatum are spiny projection neurons (SPNs), of which there are two types: one that expresses D1 dopamine receptors (D1-SPNs) and the other that expresses D2 dopamine receptors (D2-SPNs) (Al-Muhtasib et al., 2018). D1- and D2-SPN activity changes due to phasic fluctuations in dopamine that regulate the intracellular signaling and gene expression cascades that modify their excitatory synaptic strength (Shen et al., 2008). In the classical view, dopamine is thought to increase D1-SPN and decrease D2-SPN activity to promote movement and motor learning (Reynolds et al. 2001, Kravitz et al. 2010).

Previous work in the lab found that D1- and D2-SPNs exhibit differential calcium activity during learned movement in the head-fixed, conditioned avoidance task. At the early stages of training, both D1- and D2-SPNs activated during cued motion onset. However, later in training, D1-SPNs became preferentially activated during cued movement. By contrast, D2-SPNs are activated during these learned movements at lower levels and later in time. With these initial findings, our experiment sought to determine whether these different patterns of activity also correspond to differences in the expression of immediate early genes in D1- and D2-SPNs. We hypothesized that learning causes increases in D1-SPN and decreases in D2-SPN Fos expression, a canonical immediate early gene that has increased expression in active neurons.

D1 and D2 receptor signaling is thought to differentially modulate the activity of SPNs to drive motor learning. One way to assay these changes is by monitoring immediate early gene activation and expression in these cells. Specifically, we quantified Fos expression in D1- and D2-SPNs in mice trained in a head-fixed, striatum-dependent fear conditioning task. By establishing how immediate early gene expression maps onto the two principal cell types of the striatum and validating a transgenic tool for monitoring immediate early gene expression *in vivo*, our studies lay the groundwork for future investigations to pinpoint the mechanisms by which striatal neural activity is altered to drive reinforcement learning and how these neuromodulatory processes may go awry in striatum-associated diseases. These findings will help to develop a deeper understanding of these processes and inform potential therapeutic strategies for neurological and psychiatric diseases.

Methods

Training

The mice used in these experiments were genetically engineered to express the red fluorophore tdTomato selectively in D1-SPNs. A headbar was implanted onto a mouse's cranium, which was used to head-fix the mouse onto the training wheel (Figure 1A). Its tail was placed into a cylindrical plastic holder with electrical leads through which a mild electric shock could be administered to the mouse's tail as a negative reinforcer during training. While on the training wheel, the mouse was exposed to a sweeping tone of 2kHz to 8kHz over a period of 4 seconds and delivered a 0.5-s tail shock when the mouse did not exceed a specific running speed within 3.5 s. Each training session lasted 30 minutes, which consisted of 50-55 trials (Figure 1B).

We assessed each mouse's instantaneous running speed by polling the running wheel's rotation every 0.25 s using a rotary encoder. During each trial, the mice must begin stationary, and then have 3.5 seconds to exceed the running

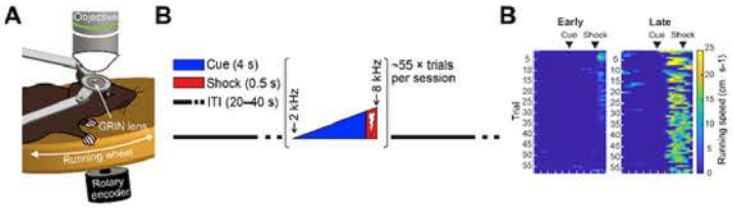


Figure 1. (A) Visual for head-fixed mouse behavior. A headbar is implanted onto a mouse's cranium. (B) Mice are exposed to a sweeping tone of 2kHz to 8kHz for 4 seconds and given a mild electric shock when the mice do not meet a specific running speed. The mouse is given ~50 trials.

threshold of 10cm/s after receiving a cue. Failure to cross this threshold of running speed in response to the cue resulted in a 0.15 mA tail shock. After either avoiding or receiving the shock, the mice were given an intertrial interval (ITI) period of 20-40 seconds during which time no cues or shocks were delivered. The random nature of the ITI prevented the mouse from predicting the next trial, allowing us to specifically assess the mouse's association between the cue and motion onset. After the ITI period expires and the animal becomes stationary, it is given a sweeping cue once again. The criterion for progressing a mouse onto the next phase was two consecutive sessions with greater than 70% avoidance, thus qualifying as a learner.

Aside from the mice that had learned the task, we included two other experimental groups: mice that failed to learn in the task (non-learners) and mice that were exposed to the task but never received a tail shock (untrained). Mice were classified as non-learners when they did not meet the 70% avoidance criterion across training. For the control group, the mice were not given the tail shock when they failed to complete the task. All other aspects of the training were kept the same.

Perfusion

After training completion, mice from all groups were given a 10-min session of probe trials in which they received cue presentations but no tail shocks. They were euthanized and transcardially perfused after one hour with a phosphate-buffered saline solution followed by the same solution containing 4% paraformaldehyde. Unlike learners, non-learners were perfused one hour after a final session of training in phase 1 with no evidence of learning. The brains were then harvested and stored in a solution containing 4% paraformaldehyde.

Immunostaining

The brains were suspended in solidified agar and sliced at a thickness of 70 microns using a vibratome. The sliced brains were washed and permeabilized by rinsing them 3 times for 5 minutes each session in phosphate buffered saline (PBS) + Triton-X-100 (0.3%) in net wells. The brains were blocked by incubating them for 60 minutes in a solution of PBS + Triton-X-100 (0.3%) and 10% normal serum from the secondary antibody host species (donkey) in net wells. The incubated brain slices were then immunostained with the primary antibody (rabbit anti c-Fos) (1/1000 dilution) in PBS + Triton-X-100 (0.3%) and 1% normal donkey serum in a 24-well plate. The imimmunostained brains were washed again 3 for 5 minutes each session in PBS + Triton-X-100 (0.3%) in net wells. To visualize immunostaining, the brains were incubated for 1-2 hours in a solution of secondary antibody (donkey anti-rabbit IgG conjugated to alexa fluor 488 [green]) at a 1/500 dilution in PBS + Triton-X-100 (0.3%) with 1% normal donkey serum in a 24-well plate. The brain slices were then finally washed 3 times for 5 minutes each session in PBS in net wells.

Mount & Analyze

The immunostained brain slices were mounted onto glass slides and imaged using a two-photon microscope. The magni- fication was 16x objective with 1.69 optical zoom (overall effective magnification of 27x). The software used was Prairie View. The images were analyzed using a cell counter in the Image J program and MatLab to determine the immunofluorescence intensity of the entire cell. Two pictures of the same region of cells were taken, one showing the Fos-expression in immunostained green fluorescence and the other showing the red tdTomato markers of D1-SPNs. We overlaid the two images to determine the differences in Fos activation between D1-SPNs (red) and D2-SPNs (unlabeled). Activated D1-SPNs were both green and red, displaying an overlaid color of yellow. D2-SPNs were solely colored green by the activated Fos staining (Figure 2).

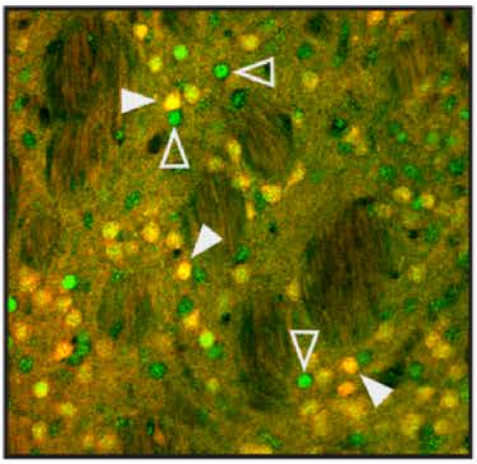


Figure 2. Captured image of Tdt expression overlaid with Fos expression of the striatal region of a mouse. Cells with green Fos+ expression and tdT+ markers are activated D1-SPN, highlighted in the filled arrows. Cells with only green Fos+ expression are activated D2-SPN, shown in the lined arrows.

As shown in Figure 3, Fos positive, Tdt positive cells were manually annotated (labeled '1') as activated D1-SPNs, Fos positive, Tdt negative cells (labeled '2') as activated D2-SPNs, and areas of background fluorescence (labeled '3'). Background fluorescence intensity was used to normalize all cellular fluorescence intensities to isolate the Fos specific immunofluorescence.

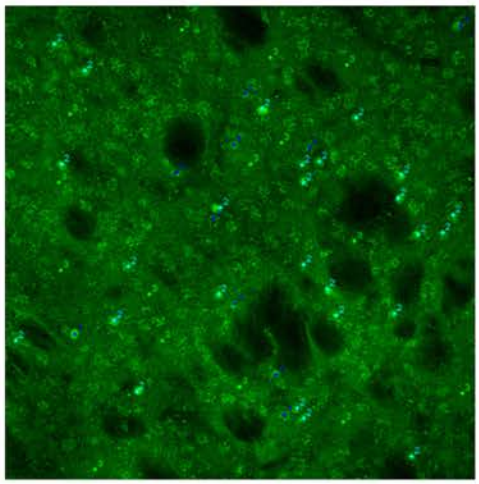
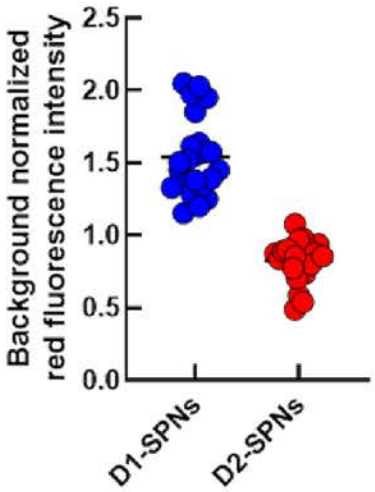


Figure 3. Image of the ImageJ cell counter program in which we marked Fos+ D1 cells with a 1, Fos+ D2 cells with a 2, and points on the background with a 3.

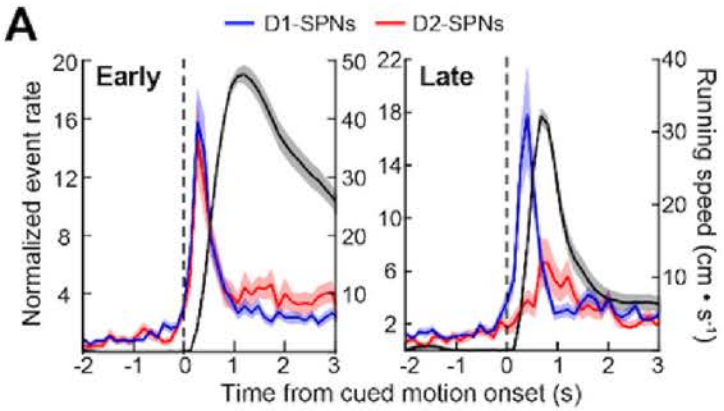


Graph 2: This graph shows the red fluorescence intensity that differentiate the D1 and D2-SPNs.

Data

Preliminary Data

Previous experiments using calcium imaging to monitor D1- and D2-SPN activity during learning in this task (in vivo) showed that D1- and D2-SPNs coactivate when mice run in response to the auditory stimulus early in training (Graph 1). By contrast, after mice had learned to perform this task, a disparity in cue-evoked, movement-related activity became apparent, with D1-SPNs activating earlier and to a greater extent that D2-SPNs.



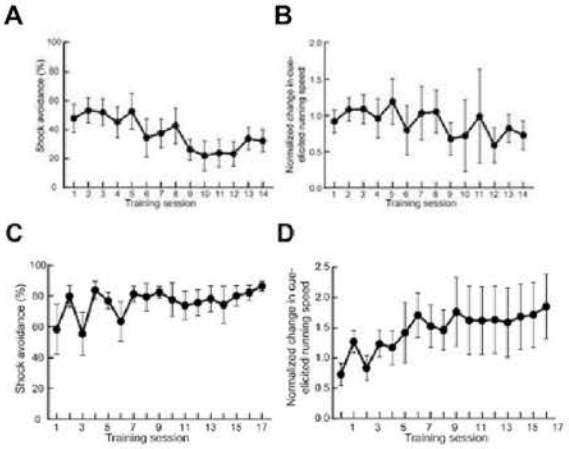
Graph 1: This graph shows that D1 and D2-SPNs exhibit differential calcium activity during a movement-dependent task.

Positive Control

As a positive control for our experimental analysis pipeline, we measured the red-fluorescence intensities of manually annotated tdt positive (D1-SPNs) and tdt negative cells (D2-SPNs). As shown in Graph 2, there were substantially greater levels of red-fluorescence in cells that were deemed tdt positive. The average red-fluorescence intensity for tdTomato was over 1.5, and that of D2-SPNs was near 0.8. These values were normalized to background red-fluorescence intensities to control for any difference in baseline fluorescence between different brain slices and images.

Behavioral Performance

Mice were classified as learners in the task if they passed a threshold of 70% successful trials in a session, as defined by exceeding the running speed threshold required to cancel the tail shock following the auditory cue. Mice were deemed non-learners if they did not achieve a 70% success rate at the end of the trials. Furthermore, there was no significant improvement in running speed for non-learners. Learners, on the other hand, had a high shock avoidance percentage and increased normalized change in cued running speed (Graph 3).

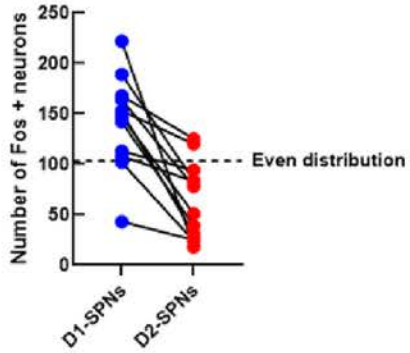


Graph 3. This graph shows the average fraction of avoidance of the mild electric shock between the mice that learned the task (A and B) versus the ones that did not (C and D). A 70% avoidance rate by the last trial was needed for the task to be deemed "learned."

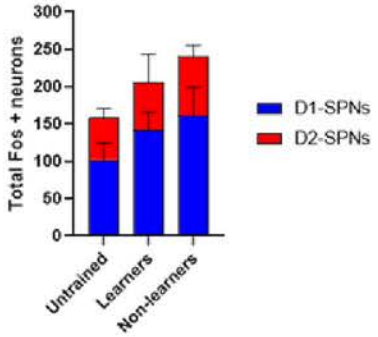
Cell Quantification

In mice that had learned in the task, there were more Fos-expressing D1-SPNs than D2-SPNs, suggesting there is higher neural activity in D1-SPNs in mice that had learned in the task (Graph 4).

In Graph 5, the number of Fos+ neurons for the untrained control, the learner, and the non-learners are shown. This graph clearly shows that the untrained group has an overall lower number of Fos+ cells. However, all three experimental groups had similarly increased proportions of Fos expressing D1- to D2-SPNs.

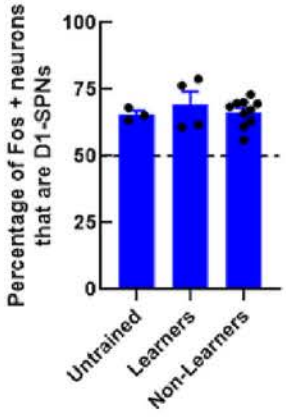


Graph 4. Graph showing the number of cells that were considered D1 and D2 cells in learners.



Graph 5. Graph showing the number of cells that were considered activated D1 and D2 cells in the untrained group, learners, non-learners.

Thus, despite an increase in the overall number of Fos+ neurons following exposure to the task, Graph 6 shows that the proportion of Fos+ D1-SPNs compared to D2-SPNs was similar in all three experimental groups.



Graph 6. Graph showing the percentages of cells that were considered activated D1 and D2 cells within each of the untrained group, learners, non-learners.

Analysis and Discussion:

From the preliminary data utilizing calcium imaging *in vivo*, we saw that there was a difference between D1- and D2-SPN activity detected within mice from the early stages of training compared to after they learned in the task.

To confirm this finding, we utilized Fos expression to measure the activation of D1- vs. D2-SPNs. We saw a greater number of Fos+ D1-SPNs in learners compared to D2-SPNs, therefore aligning with our initial hypothesis (Graph 4).

However, one caveat is that both learners and non-learners show an increase in D1-SPN activity. In other words, even though non-learners failed to acquire the learned stimulus-motor response, D1-SPNs still showed a relative increase in activity. Because both non-learners and learners exhibit a higher level of D1-SPN activity when compared to the control group (Graph 5), we posit that the activated D1-SPNs in non-learners may encode something other than the learned motor response. This change in the levels of Fos expression in D1-SPNs may have occurred due to the aversive experience of the tail shock, although this cannot be proven using our current experimental approach. However, these changes in Fos expression may correspond to changes in neural plasticity that may contribute to the changes in neural calcium activity observed in the lab. Adjudicating this idea will require further experiments and different experimental tools to monitor Fos expression and neural activity in vivo.

Potential Future Research

The lab is implementing a new type of cell detection called FosTRAP. In this method, the tdTomato markers, or red fluorescence, "TRAP" activated, Fos+ cells, permanently labeling them red *in vivo*. In Figure 4 below, we are comparing the number of Fos+ cells from Fos immunostaining to the red TRAPed cells to confirm that this approach works within the striatum. As seen in this initial test, around 80% of the activated Fos+ cells were successfully trapped, which shows that tdTomato accurately reports the Fos expression. Moving forward, this could help directly compare Fos and calcium activity simultaneously *in vivo* in order to determine if Fos active cells encode different task parameters in the learners and non-learners.

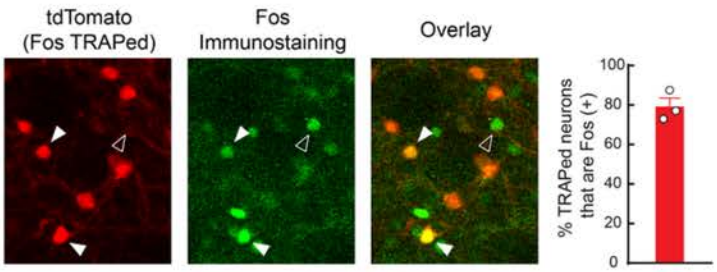


Figure 4. Images of the same cell region showing FosTRAPed cells (1), Fos immunostaining (2), and the overlay of the pictures. The graph on the right shows the percentage of TRAPed neurons that are Fos+.

In all, our findings concerning D1- and D2-SPNs in a conditioned avoidance task lay the groundwork for future investigations to pinpoint the mechanisms by which striatal neural activity is altered to drive reinforcement learning, which could later be developed into therapies that more precisely target specific domains of dysfunction in diseases associated with the striatum. Waiting for our work to validate the Fos-TRAP mice, the lab plans to use this genetic tool to simultaneously image D1- or D2-SPN calcium activity and Fos expression in vivo to link immediate early gene expression to neural coding of learned movement in the striatum.

Conclusions

Better understanding how this process is orchestrated will further our understanding of these neural circuits and how they may function in brain diseases. However, we do acknowledge that the conclusion thus far will require further testing to bolster the number of learner and non-learner mice to evaluate statistical significance. This information lays the groundwork for future investigations to pinpoint the mechanisms by which striatal neural activity is altered to drive reinforcement learning, which could later be developed into therapies that more precisely target specific domains of dysfunction in diseases associated with the striatum.

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Dopamine: The Culprit Behind Alcohol Addiction

Erin Ford

Recent studies have shown that dopamine is a major factor in the development of alcohol dependence in the brain. When dopamine receptors were increased in mice, the mice substantially increased their alcohol consumption, according to an early study in the Journal of Neuroscience (Rishi Sharma et. al, 2022). Among many other prominent findings, this study explores how dopamine (one of the prominent "happiness" neurotransmitters) plays a fundamental role in addiction to alcohol—and possibly other substances. New discoveries like this, could lead to a whole new realm of treatment options for addiction. However, before addressing alcohol addiction rehabilitation itself, it's important to understand the biomechanical effects of alcohol in the brain.

Dopamine is a molecule that is heavily involved in the reward pathway within the brain, meaning that dopamine plays a role in pleasure and motivation. Additionally, dopamine is an important aspect to learning behaviors. Typically, dopamine is released when encountering specific motivators such as hobbies, family, a job, or food and it encourages a person to perform, or repeat, a specific behavior (Di Chiara, 1997).

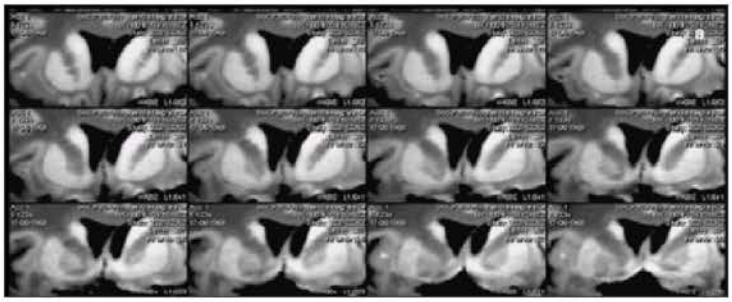

Figure 1. An MRI image of a Human Nucleus Accumbens. Retrieved from a study done by Lia Lucas Neto and others.

Alcohol causes a significant increase in the release of dopamine within the brain. When dopamine is released, its behavioral and motivational regions are activated. The person consuming the alcohol enjoys the rewarding effects of dopamine, which makes them experience intense feelings of pleasure while drinking alcohol. Dopamine also influences the nucleus accumbens (Di Chiara, 1997), the region of the brain involved in motivation and reinforcement. Specifically, it influences the person to remember the rewarding response and encourage participation in that behavior—namely drinking—again. This aspect of dopamine contributes to the craving of alcohol after it is no longer in the bodily system, a key feature of alcohol dependence. This dependence can become so strong over time that the other motivational factors mentioned above (hobbies, family, jobs, or food) no longer have the same motivational effect that they once possessed.

What separates alcohol from typical motivational reinforcement is that in typical motivational reinforcement, habituation occurs. Habituation, in this context, is when dopamine is no longer released, or is released at lower levels, when the motivational factor is repeatedly presented. However, when alcohol is repeatedly presented, similar levels of dopamine are released every time (Di Chiara, 1997). This repeated release of similar levels of dopamine strengthens alcohol's control of the brain, eventually leading to alcohol addiction.

The receptors of dopamine are also heavily involved in alcohol addiction. According to an article from the Shanghai Archives of Psychiatry, when dopamine receptors are destroyed in the brain, the subjects showed a decreased preference for alcohol (Hui Ma & Gang Zhu, 2014). Meaning that without these dopamine receptors, the rewarding effects of alcohol are nearly shut off. Additionally, during alcohol withdrawal, levels of dopamine are considerably decreased, contributing to the disgruntled state people often find themselves in during alcohol withdrawal. In essence, dopamine ensures that the user feels a surplus of positive emotions when they consume alcohol then feel a surplus of negative emotions when it is no longer in the system, encouraging the user to want to drink more alcohol to experience the rewarding effects.

This effect can also be applied to other addictive substances. For example, a more recent study by the Biochimica et Biophysica Acta (BBA) established a correlative relationship between dopamine receptors in the brain and cocaine addiction (Juan Li et. al., 2023). According to the study, the activity of the dopamine receptors was increased by the ingestion of cocaine. When these receptors were inoperative, the substance was unable to change the learning and memory pathways within the hippocampus. Meaning the subject was unable to become addicted to the normally addictive substance.

Understanding the relationship between dopamine and alcohol dependence provides a new perspective to the treatment of alcoholism. Experiments dealing with this discovery have already begun. One showing that medications that increase the activity of dopamine receptors have shown that these medications can reduce alcohol intake and alcohol tolerance in the body, decreasing the effects of alcohol addiction in the subjects (Hui Ma and Gang Zhu, 2014). With continued research, new approaches to addiction rehabilitation and treatment have the potential to help countless individuals with their battle against alcoholism and other addictive substances.

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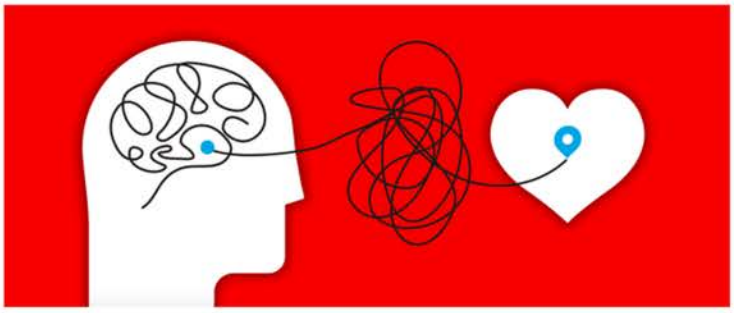
Robotic Romance; Psychology Behind Artificial Love

Megan Lu

Emma was straight out of a movie—gorgeous blonde hair, beautiful blue eyes, always smiling. Her intellect astounded everyone around her, knowing numerous languages and always knowing the answer to every question. So, it doesn't seem surprising when Geof Gallagher completely fell in love with her. Each night, he and Emma would read the newspaper, watch a show, or have a deep conversation about everything and anything. When Geoff was finally ready for bed, all he had to do was remember to plug Emma in so she could recharge for the next day. He couldn't be happier with his robot "wife" (Bell, 2020).



HealthMatters

Organic Love

With AI becoming more and more human-like, cases like Geoff's are becoming increasingly common. Yet, the very idea of falling in love with a robot or any artificial being still seems incredibly taboo. In a regular, organic romance, "love" is already a multifaceted psychological process that's difficult to define. Physically, sensory stimuli such as smells and pheromones trigger various regions of the brain due to our body's response toward genetic compatibility. Researchers have found that there is a significant increase in the neurotransmitter dopamine (which is responsible for the feeling of pleasure), oxytocin (which is associated with the feelings of bonding and attachment), and a decrease in serotonin (which contributes to the feelings of addiction towards one's partner) (Lombardi, 2022). Psychologically, factors such as physical attraction, similarity, proximity, and emotional connections contribute to falling in love.

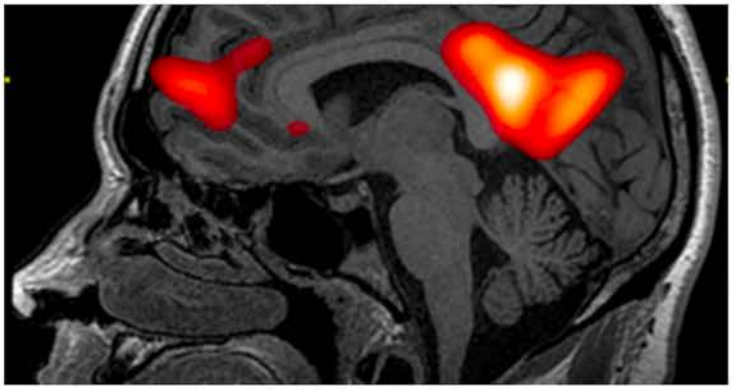


Figure 1. Brain in love. *PsychologyToday.*

Inorganic Love

In the case of inorganic love, however, it is even more complicated. A human clearly cannot react to a robot's pheromones, yet romantic interactions with AI still trigger a cascade of hormonal reactions. The reasons *why* this is the case are distinct from organic love. According to David Levy's "Love and Sex with Robots", humans tend to anthropomorphize animals and even inanimate objects; this is why we have deep attachments to childhood toys or often perceive the front of cars as looking "happy" or "angry" when they are unquestionably emotionless (2007). This anthropomorphization is enhanced when the artificial objects seem genuinely empathetic and act like they truly love someone, even if that someone knows the AI was simply programmed to act that way. This is because humans *already* make this assumption about other humans. Since you can never conclusively prove that other humans are also conscious and sentient, we are forced to create assumptions about their inner states of mind based on their observable behavior (Kewenig, 2019). Applying such assumptions to robots would almost be the natural route to take.

Limits of AI Love

To some, robots can actually be seen as the ideal companion. They are programmed to always be in a good mood, have high emotional and social intelligence, and cater to their partner's exact preferences (Viik, 2020). This flawlessness, though, can be a flaw in and of itself. According to "Artificial Companions: Empathy and Vulnerability Mirroring in Human-Robot Relations", being vulnerable toward one's partner is one of the most important parts of building trust and closeness between two individuals because they train their brain to acknowledge painful emotions related to vulnerability rather than suppress them. However, with robots being programmed to always act perfectly, it would be challenging for them to mirror this necessary vulnerability without breaking their perfect facade (Coeckelbergh, 2011). Furthermore, it's also essential to note that the robot's empathy and emotions are never actually real (at least, with current technology). In a human relationship, when one person clearly loves the other more, it causes an imbalance and usually leads to a falling out. Since we can't just forget the machine is faking its actions and words, this imbalance of authentic love could just as easily create problems in the relationship (Perry, 2022).

The idea of humans falling in love with robots raises complex questions about the nature of love and our fundamental assumptions about what it means to be in a relationship. While the technology to create fully sentient robots does not yet exist, the increasing sophistication of AI raises the possibility that a large percentage of the population may one

day form deep emotional connections with artificial beings. Though the idea of inorganic love may still be met with skepticism, it is worth exploring how such relationships can/will be incorporated into our future lives.

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Mind Over Matter: Exploring the Efficacy of Placebo Painkillers

Kaitlyn Faith

The Placebo Effect

The Placebo Effect is defined by Oxford Dictionary as, “a beneficial effect produced by a placebo drug or treatment, which cannot be attributed to the properties of the placebo itself, and must therefore be due to the patient's belief in that treatment”. Usage of the placebo effect is rarely heard in pharmaceutical settings until recently. Many scientists have found that using the placebo effect can “mimic the action of active treatments” and “facilitate the activation of pain and nonpain control systems” in our bodies (Colloca, n.d.). Researcher Markus Rütgen theorizes the body can be its own painkiller through the general understanding of the placebo effect. In theory, the body will exhibit the same pain relief characteristics as a painkiller, without the actual use of a painkiller. Delving deeper into the power of placebo painkillers will further substantiate the hypothesis, “empathy for pain is partially grounded in first-hand pain by suggesting that this also applies to the underlying opioidergic neurochemical processes” (Rütgen, 2018).

NeuroscienceNews

The Study's Set-Up

Researchers in Rutgen’s study split people into two equivalent groups: those who were given a placebo painkiller (labeled “placebo” group) and those who weren't (labeled “control” group). After the placebo group took the fake painkiller, researchers proceeded to zap the hands of both groups. The control and placebo groups were then surveyed on the inflicted pain.

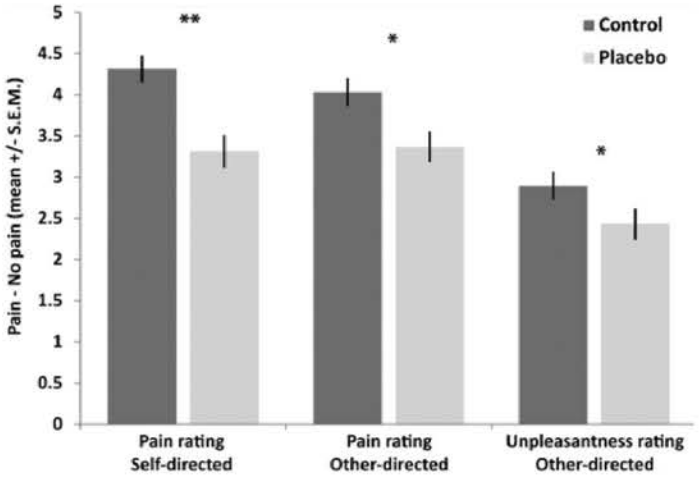

Figure 1. "Self-report results in the control (n = 53) and placebo group (n = 49), for ratings of self-directed pain (“how painful was this stimulus for you?”), other-directed pain (“how painful was this stimulus for the other person?”), and self-experienced negative affect (unpleasantness) when witnessing other-directed pain (“how unpleasant did it feel when the other person was stimulated?”). Asterisks (*P < 0.05, **P < 0.01) mark significant planned comparisons (independent samples t tests) of the main hypothesis that placebo analgesia reduced both empathy for pain and its first-hand experience” (Rütgen, 2015).

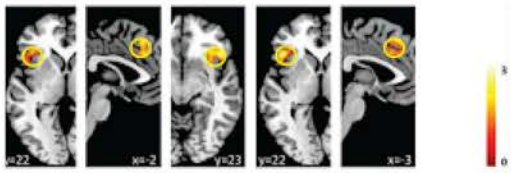

Figure 2. “Activation maps displaying the spatial distribution of brain activity within the ROIs (taken from a two-sample t test contrasting the two groups, for the contrast pain > no pain, separately for self- and other-directed conditions). The yellow circles mark the ROI sphere used to extract the mean activation. Note that these maps are shown for illustration purposes only (and for this reason are thresholded at P = 0.05 uncorrected) and that they are not independent of the ROI results” (Rütgen, 2015).

Self-Reported Pain During Zapping

Generally, the placebo group reported feeling less pain after taking the placebo painkiller compared to the control group (Rütgen, 2015). Researchers proceed to run an MRI scan on the subject’s heads. Within the MRI scan, scientists found reduced activity in the anterior insular cortex and the midcingulate cortex, which are responsible for emotional cognitive processes, control, and decision making (Sawa, 2017). What’s more interesting is that the MRI scans show how there is more activity in the insular and midcingulate cortex from the placebo group than the control group (Rütgen, 2015). It can be inferred that the placebo group illustrates more brain activity is needed to create the pain relief effect onto the body than the control group. Without a catalyst, such as a painkiller, to be active in the body, the body needs to use more energy to create a similar effect. Therefore, if the mind can be tricked into thinking the body has taken painkillers, it will endure the same effects of pain relief.

So, what does this mean? The patients who took placebo were able to activate the opioid receptors of the brain by simply believing they took a strong painkiller. This reinforces the placebo effect and how the human brain can be tricked into reducing pain itself.

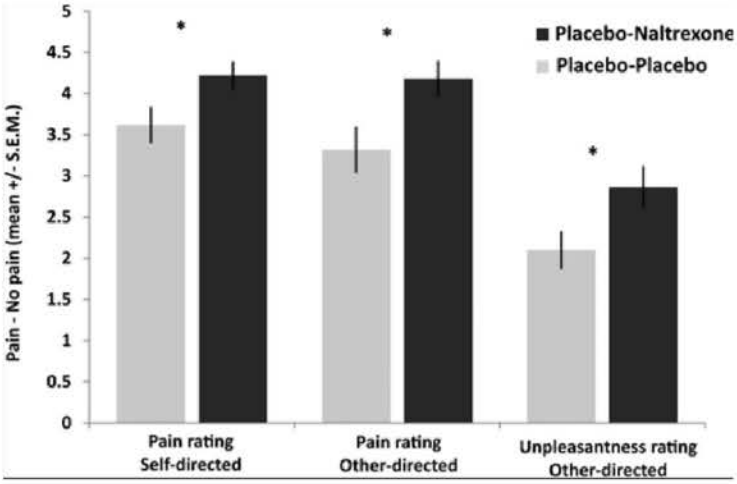

Figure 3. "Self-reported affect ratings of the psychopharmacological experiment in the placebo-placebo (n = 25) and the placebo-naltrexone group (n = 25), for the different types of ratings (self-directed pain, other-directed pain, and unpleasantness in response to other-directed pain). Asterisks (*P < 0.05) mark significant planned comparisons (independent samples t tests) of the main hypothesis that naltrexone reduced the effects of placebo analgesia for both empathy for pain and its first-hand experience" (Rütgen, 2015).

Future Research and Implications

So how will placebo painkillers contribute to future studies and, moreover, what does implicating placebo painkillers look like for ongoing clinical methods? While the use of placebo painkillers is still a work in progress, further progressions in the pharmaceutical industry can be made to strengthen drug development. Rütgen and his researchers even ran a final experiment a few years later where they gave the patients an actual painkiller. In this case, Naltrexone was used, "...to help narcotic dependents who have stopped taking narcotics to stay drug-free" (Mayo Clinic, 2023). Using this information, blah blah blah transition, "Self-report showed that blocking opioid receptors after the induction of placebo analgesia increased both first-hand pain and empathy for pain, replicating previous findings" (Rütgen, 2018). After taking naltrexone, researchers found that the opioid blocked the patients' newly initiated opioid receptors, not only neutralizing the effects of the placebo painkiller but also verifying the effects of the placebo painkiller mimicking the effects of an actual painkiller. By continuing research on what is already known about placebo painkillers, a new realm of pharmaceuticals can be explored and utilized in medical practices today.

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Current Research Pertaining to the Correlation of Prion Proteins and Alzheimer's Disease

Rikhil Chitimilla

Abstract

At present, there are around 50 million AD patients worldwide and this number is projected to double every 5 years and will increase to reach 152 million by 2050. (National Library of Medicine). This neurodegenerative disease has been known to cause cognitive impairment and memory loss. In recent research it seems that this degenerative disease is being correlated with the accumulation of the amyloid-beta (Aβ) peptide as well as the prion protein (PrPC). (PrPC) has been discovered to have inhibitory properties on the Beta-Secretase enzyme (BACE1). The inhibition of BACE1 leads to the essential deprivation of the rate-limiting step, which results in the increase of amyloid-beta.

What is Amyloid-Beta?

The Amyloid-Beta peptide is a product of proteolytic production of the amyloid precursor protein (APP), which is an enzymatic process that essentially is like a blueprint for the breakdown of the APP. APP processing begins with Beta-secretase, in which APP is cleaved into two and releases an N-terminal fragment (sAPPBeta) as well as a membrane-bound C-terminal fragment known as the C99 strand. C99 is then residually cleaved by the presenilin-containing gamma-secretase complex to form amyloid-beta and the amyloid intracellular domain (AICD). Multiple amyloid-beta isoforms are formed by this amyloidogenic cleavage. These isoforms are commonly found in their AB40 and AB42 state. These peptides can subsequently be self-assembled into small soluble oligomers, which are the common oligomers that are found in all human brains.

Amyloid Cascade Hypothesis

However, the amyloid cascade hypothesis, indicates that the amyloid deposition signals the start the progression of Alzheimer's Disease. Amyloid-Beta peptides are known to have a normal function in human brains in the regulation of potassium and calcium channel currents. To prevent its build-up, it is degraded by multiple peptidases. However as humans age, increased BACE1 activity results in the increase in the expression of the amyloid-beta peptide. The reason for the lack of degradation is also due to the reduction in the levels of the amyloid-beta peptidases.

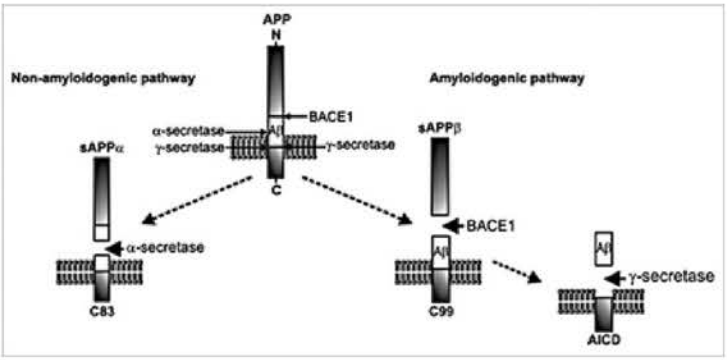

Figure 1. BACE 1 and APP cleavage in the Non-Amyloidogenic Pathway as a result of the Gamma-Secretase

(PrPC) Regulation

In a study conducted in 2007, it was identified that the main reason that the accumulation of Amyloid-Beta peptides is due to the prion protein PrPC. PrPC is known to decrease the rate of cleavage between the BACE1 protein and APP. To investigate this further, researchers examined the effect of mature PrPC on mice. It was here that it was discovered that PrPC must interact with glycosaminoglycans, whose primary role is to facilitate regulation of structural scaffolding. This is how its interaction with BACE1 begins splicing. It was here that they were able to identify that the Met/Val129 genotype polymorphism is the main reason that there is an impact on the PRNP gene (Journal of Biological Chemistry). The PRNP gene itself is known for its role in promoting Amyloid-Beta production.

Through this study, scientists were able to come to the conclusion that humans with higher expression levels of the Met129 genotype in their PRNP gene will most likely have early onset of Alzheimer's seeing as how these specific individuals would report having higher concentrations of the Amyloid-Beta 40 peptides. This essentially means that in the future, certain diagnostic tests for Met129 will allow for the detection of early onset Alzheimer's allowing the patient to properly prepare for the result.

Conclusion

Through this study, scientists have been able to properly understand how PrPC is playing a role in the production and inhibition of the Amyloid-beta proteins, which are the leading indicator of AD. The interactions between PrPC and APP are crucial to the understanding of AD. Through this overall understanding of the correlation between the two factors, it can be stated that the PRNP gene, which is what is controlling the Amyloid-Beta peptide production, can be further examined and regulated through the Met129 phenotype.

In the future, if we can properly identify the upregulation of the Met129 phenotype, we can find possible ways to identify strategies to test for this phenotype, allowing for early detection of onset AD.

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Restoring Public Trust in Science and Clearing Up Misconceptions about Alzheimer's Disease Controversies

Tyler Smith

Scientific research is supposed to be conducted in pursuit of the truth while maintaining high ethics and standards to allow for trust between scientists and the public. There is a level of rigor that is expected to have been performed when a novel drug comes to market or a major advancement in science is announced. However, in the past few years, that notion has been challenged because of the falsification of data published about Alzheimer's disease (AD), which is the most common type of dementia. In 2022, about 6.5 million Americans were estimated to be living with AD, whereas a predicted 14 million Americans will be living with AD by 2060 (Kumar, 2021). One of the many hypotheses for what causes AD to develop is the aggregation of amyloid beta (Ab) plaque in the hippocampus and cortex of the brain. It is believed that these aggregates form tangles in the extracellular space between neurons in the brain leading to neuronal degeneration.

The work in question was done by Sylvain Lesné and published in Nature in 2006. Having amassed nearly 2300 citations as of writing, it was one of the most widely cited papers in the field up until the discovery of tampered images within the publication. The paper allegedly found that an isoform of Ab, called Ab56, was correlated with memory deficits in a strain of TG2576 mice. Western blot data (which is an experiment utilized by scientists to detect the presence and concentration of a protein) was critical to the determination of the fabrication by piecing together images from different experiments (Piller, 2022).

Figure 1. The duplication of bands for different western blotting experiments Piller, C. (2022).

It was also found that some bands appeared duplicated from other experiments. This falsified work led to media headlines and articles about how the entire Ab theory of AD was discredited. When in actuality, very few people continued to work on Ab56 and worked on Ab oligomers of different sizes. It also broke the public's confidence in Ab research and tarnished the credibility of any future work in the field. The amyloid theory for AD was given a smudge on its record; however, the theory itself was not invalidated by this specific paper and its fabrications. Since 2006, many papers have come out looking at Ab of different lengths and functions, which in turn, have found correlations to memory loss (Gu & Guo 2013).

The future of AD research is still promising even after the notorious mishap. Most of the Ab therapies are now targeting different isoforms of Ab called Ab40 and Ab42. Several drugs have been approved by the FDA in recent years. For instance, in 2021 Aducanumab was approved as an amyloid beta-directed antibody. However, later that year, safety data was published showing that 1/3 of patients had developed amyloid-related imaging abnormalities (ARIA) (Ebell & Barry, 2022). The abnormalities result in small lesions throughout the brain which can result in swelling and bleeding in the brain causing headaches and nausea (Ebell & Barry, 2022). It was successful in decreasing the Ab plaque present which was long sought in the field. AD research is taking the direction that utilizes genetic editing to target genes linked to the production of Ab. In 2021, researcher Yangyang Duan published his work in Nature Biomedical Engineering where he used CRISPR-Cas9 (the first edition CRISPR editing system) to cause a disruption in the AB pathway (Dunn et al., 2021). They used a single intravenous injection which is an improvement compared to the monthly injections required by the antibody treatments. This is because one injection should decrease the price of treatment compared to the monthly injections. Through this experiment, they saw a decrease in Ab plaque burden and an increase in cognitive performance in an aggressive mouse model. This is a very promising start for gene editing's use for treating neurodegenerative diseases.

Alzheimer's Disease is a complex neurodegenerative disorder that we still have a lot to understand about how it develops and how it may be treated. Targeting Ab is a possibility for treatment but should not be our sole focus. Moving forward, science must be conducted in the pursuit of truth and not in the pursuit of a publication. In following this, the scientific community may make progress in regaining the public's trust in science that has been severely degraded in recent years.

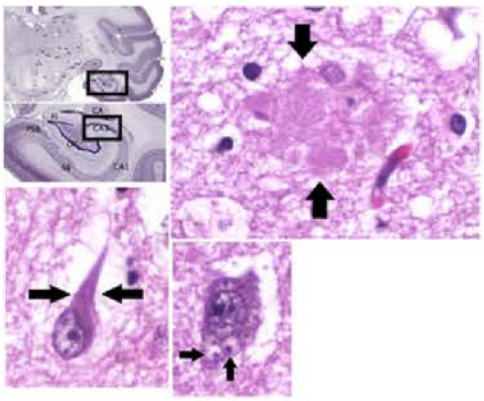

Figure 2. The pathology of AD showing the Ab plaques and tangles in the hippocampus of the brain. Wikimedia Commons Mikael Häggström (2020).

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Brain Matters Board

Chief Editor

Rajvi Javeri is a Senior pursuing a major in Psychology with a Concentration in Behavioral Neuroscience and a minor in Music. Apart from being a part of Brain Matters, she helps out as a research assistant at the Cognitive Neuroimaging Laboratory at the Beckman Institute. In her free time. she likes to practice guitar and sing. She also loves drinking infused teas and reading books whenever she can. She loves going on treks and any outdoor activities in general and is also a part of the UIUC archery club!

Assistant Chief Editor

Manan is a Senior majoring in Brain and Cognitive Sciences and is pursuing a minor in Chemistry. Apart from being a pre-dental student, Manan has previously been an Orientation Leader at UIUC. Last summer, Manan worked with people of determination and designed thinking modules for underprivileged students in India. Outside of class, Manan is deeply interested in reading books that pertain to cognitive psychology and productivity, engaging in insightful scientific dialogue and community service. He looks forward to making Brain Matters an inclusive and engaging scientific committee on campus

Public Relations Chair

Shireen Aydogan is a senior majoring in Molecular and Cellular Biology on a pre-med track and exploring the possibility of an Arabic and Communications minor. She devotes time to teach English to refugees and volunteer at the free health clinic in the community throughout the school year as well. In her free time she enjoys playing the guitar, and spending time with her family. She also likes to stay active by playing basketball and snowboarding. She hopes to increase awareness in neuroscience through her writing and as Social/Advertising Chair for brain matters.

Treasurer

Hi! My name is Sneha Mittal and I am the Treasurer and an Editor for Brain Matters. I am currently a sophomore majoring in Biochemistry on the pre-med track. Outside of Brain Matters, I work as an EMT and serve as the advocacy chair for the UNICEF branch on campus. When I am not working I enjoy spending time with my friends and exploring new things (I plan on going skydiving in a couple of weeks).

Design Board

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Katy Simmons is a senior MCB major pursuing a certificate in neuroscience! Her interests include cellular neuroscience and neuroimmunology. She is involved in Brain Matters as a design team member, editor, and writer. Her favorite thing about being a part of the journal is meeting and engaging with others that are passionate about neuroscience. Apart from her role in Brain Matters, she is a research assistant in the Evolution of Intelligent Systems lab, as well as the Evolutionary Immunology and Genomics Laboratory. After undergrad, she plans to attend grad school to conduct her own research in cellular neuroscience!

Michelle is a sophomore pursuing a major in Chemistry and a minor in Computer Science. Aside from being a part of the journal, Michelle is currently working in the Silverman Lab as an undergraduate researcher. She is excited to explore the field of neuroscience by writing for Brain Matters.

Editors

Hi! My name is Sneha Mittal and I am the Treasurer and an Editor for Brain Matters. I am currently a Junior majoring in Biochemistry on the pre-med track. Outside of Brain Matters, I work as an EMT and serve as the advocacy chair for the UNICEF branch on campus. When I am not working I enjoy spending time with my friends and exploring new things (I plan on going skydiving in a couple of weeks).

Katy Simmons is a senior MCB major pursuing a certificate in neuroscience! Her interests include cellular neuroscience and neuroimmunology. She is involved in Brain Matters as a design team member, editor, and writer. Her favorite thing about being a part of the journal is meeting and engaging with others that are passionate about neuroscience. Apart from her role in Brain Matters, she is a research assistant in the Evolution of Intelligent Systems lab, as well as the Evolutionary Immunology and Genomics Laboratory. After undergrad, she plans to attend grad school to conduct her own research in cellular neuroscience!

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Meher finds it especially fascinating that the brain, unlike other organs, is two-timing, responsible for molecular regulation and behavioral expression, inspiring her to pursue a Neuroscience major. She also aspires towards health equity, working with Brain Matters to make science communication more accessible and empower our students to share their interests and make discoveries of their own. In her free time, she loves to explore Green Street to try new foods and go for walks around the quad.

Andrew is a Sophomore majoring in Neuroscience and pursuing a minor in Chemistry and Spanish. He is very interested in giving as many students the opportunity to write in an Undergraduate journal as possible. Outside of the journal, he is an assistant researcher in the Sweedler Lab, a member of the LAS Leaders Exec Board, a student leader for McKinley, and a patient care technician at a local hospital.

Hi my name is Claire Hershenhouse and I am a junior studying bioengineering I am a first year editor on Brain Matters and I am passionate about neuroscience because it enhances our understanding of the body's functions. I hope you all enjoy the volume!

My name is Faisal Ahmad and I am an editor with Brain Matters. I was involved with the editing process of short article reviews and papers on amyloid beta plaques. My time in editing neuroscience literature was a highlight of college and I really enjoyed learning about various topics in psychology and molecular neuroscience. My hobbies include spending time with friends, gaming, and working out.

Hello! My name is Celeste Acosta and I'm a Senior in Molecular and Cellular Biology and Psychology! I'm from Cicero, Illinois. I'm currently training to work at the Physical and Neurocognitive Health Lab with Dr. Dominika Pindus and her team on campus. I love learning about the brain and it's wonderful ability to constantly change itself even in the most challenging circumstances! In the future I'd like to apply what I've learned about Neuroscience in a clinical setting as a Child and Adolescent Psychiatrist. In my free time I like to take pictures, read, play Animal Crossing, and spend time with my family back home!

Michelle is a sophomore pursuing a major in Chemistry and a minor in Computer Science. Aside from being a part of the journal, Michelle is currently working in the Silverman Lab as an undergraduate researcher. She is excited to explore the field of neuroscience by writing for Brain Matters.

Joseph Caruana is thrilled to be a contributing editor to the 7th volume of Brain Matters. Joe is a senior in the Brain and Cognitive Science Major at UIUC, where he is earning certificates in Neuroscience and Animal Behavior and working for his second year as a research assistant in the Gulley Neuropsychopharmacology Lab. Under the mentorship of Dr. Josh Gulley in the Department of Psychology, he is completing an Honors Thesis which investigates the effects of sex and age on motivational anhedonia during methamphetamine withdrawal. Prior to attending UIUC, Joe was a professional dancer, choreographer, and arts administrator. On the rare occasion he is not in class, studying, or in the lab, he enjoys volunteering for Uniting Pride of Champaign County, biking around campus, and relaxing to the soothing sounds of the cows that live across the street from him.

Brain Matters Writers

Julia Gainski graduated in May of 2022 with a major in Integrative Biology and a minor in German. She was the Public Relations Chair and a writer for Brain Matters. During her time as a research assistant in the Control & Network Connectivity Team (CONNECTlab) at the Beckman Institute of Advanced Science and Technology, she presented and published her senior thesis. Additionally, she was the President of the Pre-Pre-Physician Assistant Club, an Integrative Biology Peer Leader, and a McKinley Special Populations Peer during her time at UIUC.

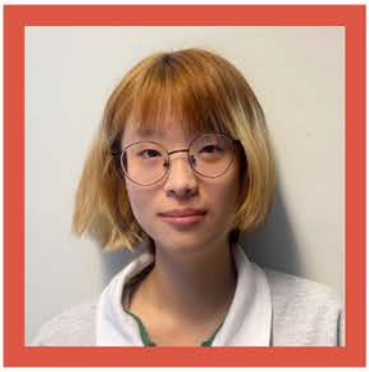
Khushi is a Molecular and Cellular Biology major who is passionate about the intersection of exercise and brain health. In particular, she is interested in the mechanisms and potential of resistance training in enhancing general brain health, improving neuroplasticity, and preventing numerous neurodegenerative conditions. Khushi is also involved in research focused in dietary polyphenols and genetic therapies for Alzheimer's disease. Outside of school, she enjoys reading, playing pickleball, and watching tv. Khushi is thrilled to be a part of Brainmatters and to share the latest research and promote a greater understanding of the brain, its functions, and its potential.

Violet Park is a sophomore majoring in Integrative Biology and currently a research assistant at the Miller Mycology Lab studying the evolutionary relationship of the fungi species Rhytismatales. She is also involved at an ophthalmology eye clinic where she helps patients who may be facing cataracts, glaucoma, macular degeneration, or diabetic retinopathy. In her free time, she enjoys calligraphy and bullet journaling as well as spending time outside or working out. She became interested in neuroscience and psychology after taking various behavioral science courses at college and joining the Alzheimer's Association. Violet hopes to attend medical school and continue research in the future!

Neha Bashir is majoring in MCB Honors on the pre-med track, with a minor in Business. Her interests include neuroscience and cognitive health. She is a writer for Brain Matters, which allows her the opportunity to learn about pursue new information about the brain and nervous system. She first became interested with the brain in high school when she was provided the opportunity to hold a cadaver brain, and became inspired as she traced her fingers along the sulci and gyri. Additional to being a writer in Brain Matters, she is involved in cultural and medical clubs at UIUC, as well as being an undergraduate research assistant in the Physical Activity and Neurocognitive Health Lab. After graduating from UIUC, she hopes to attend medical school and achieve a career as a pediatric neurologist.

Delisha Nair is a freshman majoring in Brain and Cognitive Science on the pre-med track and exploring the possibility of a Spanish minor and/or Health Administration minor. She is an aspiring surgeon hoping to specialize in neurosurgery. She devotes most of her time to running her photography business, being the Volunteering Chair for American Medical Students Association, and volunteering weekly at the Carle Foundation Hospital. In her free time, she enjoys playing the violin, drawing/painting, swimming, watching her favorite shows and spending quality time with friends and family.

My name is Vyapti Patel, and I am from Bloomingdale, a suburb near Chicago here in Illinois. The research that I am currently involved in is regarding the 7T-fMRI study here at the Beckman Institute. We are working on gathering data through clinical trials regarding the working memory of individuals. This would hold the potential of bringing out a 7-T fMRI machine in the field of medicine. My hobbies consist of reading fantasy and mystery novels, as well as painting on the Quad. I love neuroscience because it is such a fascinating mystery, and every time I am learning about the brain, my intrigue for it grows and questions upon questions pop in my head that I want to expand my knowledge on.



Hi, my name is Yushan Li, and I am a rising sophomore at the University of Illinois, majoring in Bioengineering and hoping to transfer to Molecular and Cellular Biology. I am currently working in Roger Adams Laboratory where I researched about optogenetics and cell imaging. Outside lab, I enjoy doing origami tessellation, digital drawing, collecting instant ramen lids, and playing 2048 game (personal record: 16384 tiles). After undergrad, I plan to conduct research in graduate school.

Vani Sharma is majoring in MCB Honors on the pre-med track, with a minor in public health & neuroscience certificate. She is a writer for Brain Matters, which allows her the opportunity to learn about the brain & its neuroanatomy in depth along with her interest in brain disorders. On campus, she is heavily involved with medical clubs & the Illini Strings Orchestra, serves as an undergraduate research ambassador, is a part of the Madak Erdogan Women's Health & Metabolism Lab, and works as a teaching assistant for chemistry. After graduating from UIUC, she hopes to attend medical school.

Ruchi Prakash is a sophomore pursuing a degree in psychology and neuroscience. Some of her academic interests include cellular neuroscience and the effect of drugs. In her free time, Ruchi enjoys painting, reading, and hanging out with friends. Currently, she is also a member of Illini Fighting Alzheimer's and Brainwaves. Through these organizations, as well as Brain Matters, she hopes to increase education of neurodegenerative disorders and brain science.

Susana is a sophomore studying Integrative Biology. Her academic interests range from ecology and evolution to physiology and genetics, and she loves learning about all topics related to biology, including neuroscience. Outside of the classroom, she enjoys taking care of plants, baking, reading, and especially writing, which is what motivated her to work with Brain Matters. This is her first publication, and she plans to continue pursuing a career in biological research.

Sarah Masud is a sophomore pursuing a dual degree in Psychology and Information Sciences. She also plans to minor in Art & Design. Some of her academic interests include cognitive science, human-computer interaction, and psychiatric disorders. She enjoys drawing, finding new music, and visiting coffee shops as well! Outside of Brain Matters, she is also a Market Research & Communications Intern at the Cargill Innovation Lab and involved in the Undergraduate Psychology Association. She hopes to continue furthering her understanding of neuroscience through writing for the journal.

Benedict graduated in Spring 2023 with a degree in Psychology, concentrating in Behavioral Neuroscience. As an undergraduate student, his research experience involved studying memory and social behaviour in rodents. Today, Benedict is an aspiring cognitive neuroscientist interested in the mechanisms of sleep and its relation to cognition. He currently works as a Research Assistant at the Vanderbilt University Medical Center.

Cindy Mu is a current junior majoring in Chemistry and Molecular Biology here at UIUC. As an avid neuroscience researcher and writer, she explores the frontiers of medicine, accessibility, and human identity in formats of creative writing, screenplay, and research. Her work has appeared in venues such as the Belmont Story Review, Montage Arts Journal, and Carle Foundation Hospital Journal.

Erin Ford is a sophomore majoring in Chemical Engineering with a concentration in Biomolecular Engineering. In her free time, she enjoys playing tennis and painting. She hopes to help others increase their knowledge about neuroscience through her writing in Brain Matters.

Megan Lu is a sophomore majoring in Brain & Cognitive Science with a minor in Health Administration and Business. She is involved in various RSOs on campus, including TEDxUIUC and Alpha Epsilon Delta (a pre-health fraternity), and is currently doing research with the Illinois Alternative Protein Project. In her free time, Megan spends most of her time at the gym working out, cooking new recipes, or listening to true crime podcasts. She hopes to deepen her understanding and appreciation of the brain through writing with Brain Matters.

Kaitlyn Tuvilleja is a sophomore majoring in bioengineering and trying to achieve a Neuroscience certificate. Other than Brain Matters, Kaitlyn works as a pharmacy technician and also is involved in the Philippine Student Association's Barkada dance group. In her free time, she loves playing piano, baking sweet treats for her friends, and crocheting! Through her, and her talented peers' articles, she hopes to spark curiosity towards neuroscience devices and potential pharmaceutical advances.

Hello, my name is Rikhil Chitimilla, a current Junior majoring in MCB and planning to get my neuroscience certificate as well. In my spare time I enjoy going around campus and visiting restaurants that I haven't been at before. I love to cook and have been trying to get into baking as of recently. On campus I work as a TA for LAS 101 and as an RA in Townsend Hall. Brain matters has really allowed me to conduct my own research and explore my own interests in the field of neuroscience.

Tyler Smith is a senior majoring in Molecular and Cellular Biology. He works in the Gaj lab as a research assistant in the Bioengineering department. In the lab he conducts research on harnessing the therapeutic potential of CRISPR gene editing to treat neurodegenerative diseases including Alzheimer's Disease and Amyotrophic lateral sclerosis (ALS). After graduation he intends to attend graduate school to further study neurodegeneration and gene editing.

Want To Get Involved?

Brain Matters is a Registered Student Organization (RSO) on campus that welcomes all years and majors. Please email brainmattersuiuc@gmail.com with inquiries about getting involved with the journal or RSO.