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Resiliency Against Stressful Life Events and the Progression of Depression from Adolescence

Neil V. Sen

Philadelphia College of Osteopathic Medicine

Philadelphia College of Osteopathic Medicine

Graduate Program in Biomedical Sciences

Department of Biomedical Sciences

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Abstract

Major depression is the second most impactful condition on overall health in the United States, affecting approximately 9 million commercially insured Americans. Depression rates are increasing as time goes on, especially in adolescents (12-17). From 2013, the rates of teen depression has risen 63 percent (47 percent for boys and 65 percent for girls).[1] Depression is a complicated condition and there are many factors that play into the progression of the disease, including environmental, genetic, and psychological factors. Adolescence is a developmentally critical period of brain development for plasticity and maturation of stress systems. Studies have suggested that there may be developmental periods in which exposure to stressful life events (SLEs) cause cell sensitivity to epigenetic changes that affect gene and protein expression long term.[2] Thus, these changes may render adolescents more vulnerable to psychiatric disorders. Variations of certain genes may allow certain people to be more susceptible to psychiatric disorders when exposed to an early SLE than others. However, there are studies that have demonstrated that children who have had SLE have no particular mental health problems in adulthood.[3] These individuals would be considered "resilient". Resilience is the brain's capacity to cope with environmental stress and achieve stable psychological functioning in response to prolonged stress.[4] Understanding the numerous neuronal mechanism in different parts of the brain is pertinent, in that it may lead to the susceptibility of depression. A literature review was done on stress's effects in different regions of the brain and how it may contribute to the progression of depression later into adulthood.

Introduction

Major depression is a mental disorder that affects the quality of life of many people. Individuals with depression are affected in varying degrees. Those who have this disorder describe it as having a sense of emotional numbness and a lack of motivation. These emotions can be great enough to feel as though life is not worth living and may go as far to take their own life. Affected individuals who live day to day with this mentally and physically debilitating disease find it difficult to maintain their familial, social, and working relationships and see themselves neglecting their daily chores and routines. Unfortunately, rates of depression seem to be steadily increasing in all age groups in the United States, especially between those under the age of 35. Specifically, between 2013 and 2016 diagnoses increased 63 percent among adolescents; a 65 percent increase for girls compared to a 47 percent increase in boys.[1] This increased rate of diagnosis among adolescents can have serious implications on a growing society upon entering adulthood.

Adolescence is a critical time period for physiological, behavioral, and neurobiological development preparing the child for adulthood.[5] This is the time point where they are most susceptible to many stress related diseases such as anxiety and depression. The areas of the brain involved in stress regulation have distinct maturational processes between early life and adolescence in the human population.[6] Children who fall susceptible to these stressors may experience an impact on their mental state and eventually lead to depression later in life. It has been reported that individuals who had previous episodes of depression in adolescence are more likely to suffer depressive episodes in adulthood.[5] Currently, studies for developmental stress at various stages of adolescence are being explored and still remain limited in scope in terms of human studies. Studies that have been conducted in rats and monkeys to address chronic stress in

adolescence and follow the impacts into adulthood may give us perspective into how this may affect humans.

From birth, to the continuing developmental periods, to adolescence, biological processes, environmental conditions, and experiences have influence on the brain's development. One neuroconstructivist viewpoint is that the brain has plasticity and the connections that are made occur from experiences we have as we grow up.[7] These experiences that we have affect gene expression of neuronal cells within the brain. Studies have shown that genetic effects play a substantial role in development of depressive symptoms and may explain the variance of the many symptoms that manifest. For example, one of the most studied genetic markers for susceptibility of depression is the polymorphisms in the promoter region of the serotonin transporter gene, SLC6A4. The serotonin transporter is responsible for the uptake of serotonin from the synaptic cleft, as it is considered to play a role in emotional response and regulation in the brain. Associating single polymorphisms to the explanation of genetic risk for depression have been criticized since it has produced unreliable findings. Studies that focus on single polymorphisms fail to consider the complexity of genetic pathways and their interactions with other genes.[8] Also, carrying a genetic risk does not imply that individuals will develop depression. Looking at genetic effects only provides a small window of what is occurring at a neurobiological level. There are many other factors at play, such as the environment one is raised in and the experiences he or she has had. It is crucial to understand the effects of a detrimental environment or a stressful life experience on the brain during adolescence.

Emotional regulation is clearly not developed in a growing child. Experiencing stressful situations may interfere with this process of regulation over time. Examples of negative emotional regulation consist of rumination and heightened emotional reactivity. It has been documented that

adults who have experienced child maltreatment do not cope with daily stress well compared to adults who did not have this childhood adversity.[9] The associations between stressful life events and the internalizing psychopathology have bidirectional influences and cascading effects over time, making this a complex issue. The stress sensitization hypothesis states that exposure to early adversity in development heightens vulnerability for developing depression and anxiety following subsequent stressors.[2] However, not everyone who experiences early life stress in their childhood develop mental disorders in their adulthood. This can be explained as resilience.

Resilience is the brain's capacity to cope with environmental stress and achieve stable psychological functioning in response to prolonged stress. The neurophysiological coping mechanisms of stress resilience are still unknown. Thus, this creates opportunity for creating treatment options that address the dysregulating factors causing depression since diminished resilience may serve as a mechanism underlying the effects of childhood trauma and genetic risk factors common to different psychiatric disorders. Researchers who have attempted to understand the mechanisms of stress resilience have looked into the ventral tegmental area (VTA) dopamine (DA) neurons in mice, the natural reward circuitry of the brain. This paper will be a literature review on the effects of stress in different regions of the brain during adolescence and how it may contribute to the progression of depression later into adulthood. It will focus on effective coping mechanisms that may help alleviate the symptoms of depression.

Literature Review & Discussion

Major depressive disorder (MDD) is a dysregulation within the neural circuits of emotional processing and regulation. Emotion processing and regulation function to influence the duration, intensity, and type of emotion experienced through personally relevant significant events.

Generally, emotions can be processed in two ways: implicit (automatic) or explicit (effortful). Emotions processed automatically involve initial reactivity of lower limbic structures, whereas explicit processing initially requires a higher level of cortical structures.[10] This bidirectional relationship engages a common neural network within the brain involving structures such as the prefrontal cortex (PFC) and limbic structures: the nucleus accumbens (NAc), the ventral tegmental area (VTA), hippocampus, and amygdala. The relationships with all these structures demonstrate the complexity and heterogeneity of this mental disorder upon structural dysregulation

Stress Plasticity of the VTA

While the exact cause of depression is unknown, the four main contributors to depression are biological, genetic, environmental, and psychological. However, through much evidence, researchers have elucidated the link between stress and depression. There are different forms of stressors that a person can experience, such as a stressful live event or an ongoing chronic stressor in their life. Experiencing stress has profound effects within the brain and can contribute to the structural plasticity changes in the brain. There are different forms of stress in terms of duration, physical or psychological, and intensity of stress. These different manifestations of stress impact the brain in different ways.

Dopamine (DA) is one of the neurotransmitters that is greatly affected by stress. The VTA is mainly populated with DA neurons which are responsible for linking internal states with appraisal of environmental stimuli forming emotional-motivational valuations. It is neuronally connected to limbic structures and medial prefrontal cortex (mPFC).[11] Appraisal processes direct the decision to cope with stress and requires the mPFC to exert a top-down executive control over the lower structures of the brain located in the brainstem. There are two types of behavioral coping strategies: active and passive. An active coping strategy occurs when the person acts upon the

stressor to eliminate it and to defend the 'self'. But, when they cannot terminate the stressor by themselves, it becomes a passive coping strategy which relies on others to solve the emotional aspects of the stressful experience.[11] However, if both strategies cannot resolve the stressor, the stress becomes toxic and increases the vulnerability to mood disorders, such as depression.

The VTA-DA circuitry is important in the limbic system, as it plays a significant role in learning and memory processing. This ultimately leads to its role of differentiation between good and bad.[11] Experience-dependent plasticity of VTA-DA circuits is beneficial in that it helps the person adapt to certain environmental situations through learned associations. These learned associations determine the value of different options in order to promote the wellbeing of the person when they experience specific stressful situations. Also, the VTA-DA circuitry evolves over time in terms of reinforcement learning. So, when faced with adversity, formerly neuronal stimuli can reinforce or strengthen behaviors through certain cues. This occurs so that the person can predict future events and thus enables him or her to choose an action that is appropriate to the situation.[11] Unfortunately, a mismatch between predicting stimuli and reality can create a prediction error.[11] An accumulation of these errors can result in improper adaptations to such events of stress, which eventually leads to behavioral rigidity in coping with stress.

Adaptive behavior in stressful situations require VTA-DA circuitry. The VTA-DA circuitry is quite complex, and the adaptive behavior is strongly dependent on the type of activity the neurons perform. VTA-DA neurons are either active or inactive. Active neurons have two types of firing patterns, asynchronous low frequency tonic firing or high frequency phasic activity. The tonic firing pattern fires at a frequency of 1-4 Hz and has a single action potential discharge.[12] The high frequency or burst firing has a frequency range of 15-30 Hz.[12] There is a balance of excitatory and inhibitory pre-synaptic inputs that determine the neuronal state of the VTA-DA neuron.

Excitatory control is mainly regulated by glutamatergic neurons which can induce a burst firing pattern. On the other hand, inhibitory control is mainly regulated by GABA-ergic neurons which can place VTA-DA neurons in a hyperpolarized inactive state.

It is important to note that phasic firing VTA-DA neurons require tonic activity to be present, otherwise phasic firing will not occur.[12] This can be thought of as tonic firing acting as a spontaneous low background pacemaker for DA activity. The activity of these neurons is labeled as population activity. DA neurons must be spontaneously active to respond to behaviorally relevant phasic inputs, therefore a change in population activity better reflects the responsivity of the system.[11] Through optogenetic manipulations, it was found that active coping strategies are linked to a greater population activity and passive coping strategies are linked to a decrease in population activity in VTA-DA neurons. This suggests that the amount of neuronal tonic activity has a role in behavioral decision making.

Connections to the VTA

Parts of the VTA's complex afferent and efferent neuronal network has been elucidated mainly through animal models. Generally, these models involve mice or rats that perform different tests to induce phenotypes that match as closely as possible to depressive-like symptoms. For example, researchers use tail suspension tests (TST) and forced swim tests (FST) on mice to quantify their motivation. They observe and record the amount of time spent in trying to escape. The less time they spend struggling has been interpreted in the community as a sign of behavioral despair or passivity. Another form of testing for anhedonia is a sucrose preference test (SPT). This test quantifies how many licks on a spout containing sucrose would occur compared to a spout containing just water. This presents the level of pleasure the mouse experiences when it licks a certain spout. Thus, this reflects the animal's hedonistic potential or 'liking'. Since stress has been

a major link to depression, different stress paradigms are used on mice. Particularly, a chronic social defeat stress model (CSDS), chronic mild stress model (CMS), and chronic restraint stress (CRS) were performed. In the CSDS model, mice are exposed to a similar more aggressive and larger mouse. They were then separated by a clear physical barrier that allowed them to see and smell each other. Depressive phenotypes this model produced are anhedonia, anxiety, and social avoidance behaviors.[11] The CMS model is applied in an unpredictable or semi-random fashion with a variation of physical and psychological micro-stressors that are applied for several days or weeks. This phenotype is portrayed as a decrease in reward responsiveness. The CRS model restrains the animal for at least three weeks for 1-6 hours a day. The downside of this model is that the animals become habituated to the stress as they can predict when they will be restrained. More advanced techniques are also performed, such as optogenetics. This method combines pharmacological, fiber optics, and genetic techniques to activate or inhibit certain neurons within the brain, giving researchers a precise tool to study specific neural circuits.

One major connection that is well known to be involved with the VTA is the nucleus accumbens (NAc). The NAc is a critical mediator of depression-related outcomes as it integrates information from afferent inputs leading to motivation and reward functions. Surprisingly, clinical studies have shown how deep brain stimulation of the NAc can provide antidepressant effects for individuals who are treatment-resistant, showcasing the importance of NAc for mediating emotional behaviors in depression.[13] The connection between these two structures can have very different results depending on what type of stress is implicated on the subjects.

For example, one study explored the activity of VTA DA neurons when mice were subjected to a subthreshold social defeat stress model. During the stress exposure, the researchers optogenetically stimulated VTA DA neurons (either phasic or tonic) and measured social

interaction and sucrose preference. The mice that received phasic stimulation showed a decrease in social interaction and sucrose preference, demonstrating depressive-like behaviors.[14] This model bypasses the CSDS model by using optogenetics instead of required repeated bouts of social defeat stress, and shows that stimulation of VTA DA neurons induces depressive-like behaviors. Next, using a ten-day social defeat paradigm, the researchers wanted to see if mice who are deemed 'resilient' to stress would revert to depressive-like behaviors when induced with phasic stimulation of the VTA. When optogenetic phasic stimulation was performed, they saw that the resilient mice spent less time interacting with each other and had a decrease in sucrose preference. Seeing that by affecting the VTA resulted in behavioral changes, they performed the subthreshold social defeat stress model once again on mice and phasic stimulated the VTA-NAc pathway which induced depressive-like behaviors.[14] When they inhibited the VTA-NAc pathway, they saw resilient behaviors suggesting a strong link between these two structures in stressful situations.

However, another group of researchers performed slightly different experiments and saw conflicting results. First, they inhibited VTA DA neurons and found a significant reduction of struggling in mice during TST and FST, as well as a reduction in preference for sucrose. This shows that selection inhibition of VTA DA neurons can induce depression-like behavior.[15] This finding conflicts with what was previously stated in terms of a stimulated VTA showing depressive- like behaviors. Next, they used the CMS model to induce stress in mice delivered twice daily for 8-12 weeks. Then, the researchers stimulated the VTA DA neurons through phasic photoactivation and saw the mice struggled more during TST, indicating that activation of these neurons rescued stress induced (chronic mild stress) depressive-like behaviors. To see if the NAc was involved, they blocked dopamine receptors in the NAc and subjected the mice to CMS. They saw that phasic photoactivation had no effect on mice, as they still exhibited depressive-like

behavior.[15] They suggested that the NAc neurons encode phasic activation of the DA neurons in the VTA and the effects of NAc on depression-related behaviors are affected by the activity of the VTA, indicating that VTA-DA neurons modulate the NAc for escape behaviors.[16]

There are two main projection neuron subtypes called the medium spiny neurons (MSNs) of the NAc. The MSNs have two different types of dopamine receptors, D1 and D2, and are classified as D1-MSN and D2-MSN. NAc D1-MSN project to the VTA, the ventral pallidum, and globus pallidum internal, while NAc D2-MSN project solely to the ventral pallidum.[13] Repeated restrained stress and CSDS models showed long-term depression occurred in D1-MSNs, but not in D2-MSNs. When the activity of the neurons was analyzed further, D1-MSNs displayed a reduced frequency of miniature excitatory postsynaptic currents (mEPSCs), while D2- MSNs displayed an enhanced mEPSC frequency in CSDS susceptible mice. If D1-MSNs intrinsic excitability increased, so did the interaction time as D2-MSNs showed no type of intrinsic excitability. Intrinsic properties driving cell firing is a complicated issue by a set of complex interacting mechanisms that need to be considered to truly understand the nature of these neurons.

Through the research applications that we have now, optogenetic stimulation has found how stimulation of D1-MSNs promotes resilient behavior and inhibiting these MSNs induce depression-like behavior when mice are subjugated to the CSDS model.[16] Optogenetic stimulations have also shown that stimulation of D2-MSNs induce depressive-like behaviors, and inhibition of these fibers had no effect.[18] Consequently, researchers have shown that D2-MSNs collaterals connect more strongly to D1- MSNs than the other way around, indicating the interconnectivity of the two neuronal types and how stress may cause an imbalance of firing within these subtypes. While aberrant D2- MSNs may be involved in the expression of a depressive-like behaviors, increased activity of D1-MNSs may provide a reversible switch for these behaviors.[18]

Taking note of the importance NAc has in mediating mood, reward, and motivation, the causes of depression in relation to NAc are unclear due to the diversity of connections and cell types. For example, the ventral pallidal (VP) is an important convergent point within the circuitry of motivation and reward. It is known to receive dense inputs from both D1-MSNs and D2-MSNs; more input from D2-MSNs than D1-MSNs and transmits this information to its downstream targets.[19] In essence, it is a heterogenous region that integrates limbic and cognitive signals centrally positioned to mediate these reward and motivational behaviors. A specific population of VP neurons are parvalbumin-positive (PV) neurons and are reported to be plastic in response to the environment and have a role in stress-induced depressive behaviors. VP PV neurons have two main downstream projections: the VTA and the lateral habenula (LHb). The lateral habenula is a structure responsible for aversive stimuli. These two projections have distinct neuronal properties. The PV^{VP}→LHb solely consists of excitatory glutamate post-synaptic activity and the PV^{VP}→VTA mainly consists of inhibitory GABA projections and few excitatory DA projections. Elevated activity of VTA and LHb in mice have been associated with depression.[19]

A group of researchers created a CSDS model and labeled PV neurons projecting to the LHb and the VTA and observed their activity with whole-cell patch-clamp recordings. Following the stress model, susceptible animals to the stress showed a greater increase in intrinsic excitability of PV^{VP}→LHb, and later elucidated a coupled reduction in inhibitory inputs, compared to the resilient mice that ultimately lead to an increase in glutaminergic activity.[19] Surprisingly, for PV^{VP}→VTA neurons in susceptible mice had no changes in intrinsic excitability compared to the controls. However, there was a significant reduction in excitability in resilient mice indicating that there was some form of compensatory mechanism that increases GABA projection from VP to VTA that maintains a low level of activity. The end result of cellular hyperactivity in the VTA or

LHb through different mechanisms is commonly seen in susceptible animals who undergo CSDS.[19] When the researchers tried to reduce the activity through optogenetic inhibition and then inducing them to stress, they found different behavioral actions depending on what projection they were inhibiting. For instance, reducing PV^{VP}→LHb led to resolving behavioral despair, while reducing PV^{VP}→VTA led to resolving social withdrawal, essentially inducing resilience to CSDS.[19] Neither inhibitions led to resolving anhedonia. It was interesting for them to find that inhibiting these pathways solely had no effect on baseline measures of locomotion, sociability, and anxiety. After stress was applied, artificial reduction in VP PV neuronal activity helped attenuate depressive-like behavior. This may suggest that the connection between VP and VTA is weak when experiencing no stress but strengthens when exposed to stress.[19] Whereas artificial stimulation of PV^{VP}→VTA projection was enough to induce depressive behaviors, stimulation of just PV^{VP}→LHb with or without stress was not sufficient to induce these behaviors. So, this seems that LHb while is involved in the stress mechanisms within the midbrain, is not the sole contributor to dysfunction within these neuronal patterns.

Previously discussed was the opposing phenotypes shown when mice were exposed to either CMS or CSDS. However, the VTA is a heterogeneous structure and its mechanisms are not so simple. Another set of researchers aimed to understand the nature of stress through CMS models on the pertinent structures in the mesolimbic-cortical system. They questioned whether heightened LHb activity actually drives depressed behaviors. In the process, they revealed the unique heterogenous activity of the VTA.

A main area they focused on was the infralimbic prefrontal cortex (ILPFC). It is homologous with Brodmann area 25 (BA25) in humans, which is widely considered as a critical area in depressive circuitry.[20] Recent rodent studies showed that activating ILPFC reduced

dopamine neuron activity as well as in downstream connections such as the striatum, which contains the NAc, found in the ventral portion of the striatum. This study uses a CMS depressive model to study the nature of this pathway. When they activated ILPFC, through local infusion of NMDA, the number of spontaneously active VTA DA neurons was profoundly reduced in the medial portion of the VTA.[20] It did drive a previously non-bursting population into a high bursting state in the lateral portion of the VTA. There were no changes in the average dopamine firing rate in the VTA. Activation of LHb through local infusion of NMDA decreased population activity of the lateral VTA and slightly increased firing rate of the medial VTA. Bursting activity of the VTA was not affected at all. When mice were subjected to CMS, they found that the medial portion of the VTA was severely impacted, with a greater than 50% decrease in spontaneous active VTA dopamine neurons.[20] There were no changes observed for dopamine neuron firing or bursting activity in the VTA. This highlights the possible connection between the ILPFC and the medial VTA. To prove this connection, they inactivated either ILPFC or LHb and found that ILPFC restoration reduced dopamine neuron population activity in the medial portion of the VTA. LHb inactivation did not affect the population activity in any portion of the VTA.[20]

Therefore, they demonstrated that by restoring VTA neuronal dopamine activity attenuates the effects of CMS. It is important to note that DA neurons must be spontaneously active to respond to behaviorally relevant, phasic inputs. Hence, it is imperative to understand the change in population activity, rather than solely focusing on the changes in the firing rate and bursting as it better reflects the responsiveness of the system.[20] This may help shed light on the conflicting results from CSDS and CMS studies as the cause of differences could be due to the timing differences of interventions or focus of recordings.

As discussed previously, the ventral pallidum has a profound effect on the VTA, as it sends powerful GABAergic projections to the VTA. Afferent GABAergic inputs change the pacemaker firing pattern into a slow, irregular firing.[26] Also, half the neurons in the VTA do not fire due to inhibition from VP, and thus maintains a baseline tone or responsiveness of the dopamine system.[25] Stressors augment this system. The role of dopamine can differ in the induction phase of stress that produces varying expression of depression related behaviors. The duration of stressor affects the magnitude and duration of the negative affective state. For example, acute restraint stress activates the DA system by increasing DA population activity shortly after the stressor. Then, there is a reduction in activity after 24 hours. Even if the stressor is continually present, the consequent depression-like behavior is shown, but rather than constantly having an increased DA population, VTA DA neuron attenuation occurs long after the removal of the stressor.[25] This phenomenon has created the framework that initial increase in DA during and immediately after stress drives downstream plasticity that ultimately promotes depressive-like behavior.[25] However, mice exposed to CSDS exhibit a significant increase in VTA firing rates the day after the last defeat and this up regulated firing can persist for up to two weeks. On the other hand, stimulating DA neurons long after CSDS exposure have anti-depressive effects, indicating that VTA hypoactivity may be present or otherwise demonstrates that there are intrinsic mechanisms at play here.

Mice that undergo stress are split into two categories, resilient or susceptible to stress. A group of researchers set out to understand workings of the molecular differences between these two groups using the CSDS model. First, they confirmed that this model produced higher hyperactive VTA-DA neurons in susceptible mice than in resilient mice through fluorescent probes and confirmed their state through their behaviors. It was known that these increased firing patterns were associated with increased hyperpolarization-activated cation channel-mediate current (I_h) in

susceptible mice.[27] So, they hypothesized that I_h activity in resilient mice would be lower since VTA DA firing rate was lower. To their surprise, through whole-cell voltage-clamp recordings in VTA-DA neurons of brain slices, they saw that I_h levels were greater in resilient mice, not lower.[27] This was unanticipated since I_h was viewed as a stress-inducing ion mechanism. To understand why this was happening, they focused on K+ channels.

To see the relationship between I_h and K+ channels, the researchers pharmacologically increased I_h through an infusion of lamotrigine, a potentiator known to increase I_h, into the VTA of susceptible mice repeatedly for 5 days. After the infusions, they noticed that the susceptible mice were in the social zone more than before, suggesting that their depressive symptoms were reversed. They also saw I_h and K+ levels increased in the lamotrigine group compared to the control group.[27] The excitability of the DA neuron in the lamotrigine group was lower than in the control group. This phenomenon was confirmed through another experiment that increased I_h current through overexpression of HCN-2 channels, a channel that mediates I_h current, in DA specific neurons of susceptible mice. This led them to believe that excessively potentiating I_h resulted in the homeostatic upregulation of K+ channels current. To see if the upregulation of K+ channels current was a natural mechanism of action, the researchers focused primarily on creating a firing pattern through optogenetic stimulation of the VTA-DA neurons in susceptible mice that simulated hyperactivity in that area of the brain. This experiment confirmed the increase in K+ currents without altering the I_h currents, as well as exhibited behavioral changes within the susceptible mice. This suggested that the K+ current compensation may be directly caused by the hyperactivity induced by I_h potentiation .[27] In other words, stimulating VTA DA neurons that was previously thought to induce depressive-like behaviors in a CSDS model was actually beneficial that is the stimulation had to occur long after the stress. This highlights a natural homeostatic

mechanism occurring in resilient mice when they undergo stress. This compensatory measure is not present in susceptible mice.

VTA-DA neurons have different projections to the brain. The researchers wanted to make sure where the homeostatic plasticity was seen. Recent studies have shown that VTA-DA neurons projection onto the nucleus accumbens (NAc) exhibited larger I_h, whereas medial prefrontal cortex (mPFC) projection onto the VTA-DA had smaller I_h.[27] To see if homeostatic plasticity occurred in different neural projections, the researchers overexpressed HCN2 in VTA-mPFC and VTA-NAc neurons. They found that VTA-mPFC neurons did not show a change in K+ current, whereas in VTA-NAc neurons did, suggesting that the VTA-NAc pathway is susceptible to homeostatic plasticity.

Upstream brain regions controlling pathway specific DA neurons were not understood completely until now. An important nucleus in the brainstem is the locus coeruleus (LC) which produces norepinephrine (NE) and modulates vigilance, arousal, cognition, sleep/awake transitions, and addictions. Researchers became interested in the LC due to its neuronal connection to the VTA and its implication in stress resilience in which resilient mice had a greater release of NE that project to the VTA. The next question was whether or not LC was responsible for the homeostatic plasticity mechanics we see in the mesolimbic DA neurons. When researchers analyzed this question further, they found that resilient mice had a natural higher firing rate and bursting frequency of LC neurons compared to the control and susceptible mice, particularly the LC-VTA projecting neurons.[28] When they optically repeatedly stimulated this pathway in susceptible mice, they found reversal of depressive behaviors, promoting resilience. These repeated bouts of stimulations increased the in vivo firing rate and bursting frequency of LC neurons similarly seen in naturally resilient mice. When they looked at the downstream changes

in the VTA, they saw similar intrinsic current balances of I_h channels and K+ currents that were observed in resilient mice in the previously described study. They found that α_1 and β_3 adrenergic receptors were highly expressed in the VTA-NAc pathway and were necessary for mediating the resilient behavior and intrinsic current balance induced by the LC-VTA pathway in the VTA-NAc circuit, elucidating potential therapeutic targets for treating depression.[28]

Stress Exposure in Adolescence

Adolescence is a critical period for physiological, behavioral, and neurobiological development preparing the child for adulthood.[5] This is the time point where they are most susceptible to many stress-related diseases such as anxiety and depression. The areas of the brain involved with stress regulation have distinct maturational processes between early life and adolescence within humans.[6] But one needs to consider the different types of stressors. For example, abused infants acquire a highly negative affect while neglected infants acquire a blunted affect. Accounting for these distinct maturational periods and knowing there are different variations of stress exposure lead to conflicting findings.[6] For example, early studies of natural disasters have reported that adolescents can be both more and less vulnerable to trauma. Two years after exposure to a dam collapse, children of ages 8-15 had PTSD symptoms greater than children of ages 2-7. Whereas children exposed to a bushfire, their PTSD symptoms were inversely correlated with their age 8 months after the fire, but positively correlated with both age and cumulative stress after a little over 2 years.[6]

Animal studies have attempted to address chronic stress in adolescence to see what impacts it has on adulthood. A group of researchers looked at 8 rhesus monkeys exposed to chronic stress during adolescence to see its effects in adulthood.[17] First, they separated 4 baby monkeys from their mother and placed them in an incubator for the first month of birth, while the other half were

with their respective mothers for 7 months. Those separated during the initial month of birth were paired together for the next 6 months. After the full 7 months, they were all placed together and lived with each other for the next four years. After those four years, they were separated again and exposed to unpredictable chronic stressors for two months. Chronic stressors included space restriction, intimidation, long-illumination, and fasting. The parameters used to detect depressive-like symptoms were locomotion and huddling behavior. They also recorded hair-cortisol levels before and after chronic stressors were induced. They found that separated monkeys had decreased locomotion and increased huddling behavior when exposed to chronic stress.[17] Their cortisol levels were significantly elevated after the two months compared to monkeys who were reared in their early adolescence. Changes in behavior may indicate that early adversity can cause structural changes within the brain that when exposed to stress in the future can lead to more depressive-like symptoms.

Other animal studies continue to show age-related vulnerabilities to stress. For example, one group wanted to see how similar stress models affected different cohorts of rats during adolescence versus adulthood.[5] Both groups received chronic variable stress (CVS) respective to their stage in life for 2 weeks to serve as prior traumatic experience and then were left alone for 50 days. After, they administered behavioral challenges and measured their hypothalamo-pituitary-adrenocortical (HPA) axis response. HPA dysregulation contributes to many neuropsychiatric disorders, such as depression. They found that adolescent mice exposed to CVS had lasting behavioral changes they viewed as depression-like symptoms; consistent with other rat studies conducted previosuly.[5] The HPA reaction times were more sensitive in adults exposed to stress than adolescent mice. Relating the HPA reaction times to the behavior of the mice in response to stress indicates that stress-related re-organizations are less pronounced in adulthood

and further explains that exposure to early life stress increases the risk of exposure to subsequent trauma due to changes in stress systems. Seeing these impacts in animals can give us perspective into how this may affect humans.

Emotional regulation is not developed in a growing child. Experiencing stressful situations may interfere with this process of regulation over time and cause heightened emotional reactivity at the neural, psychophysiological, and behavioral measures.[21] Examples of negative emotional regulation consist of rumination or inability to distinguish between negative emotional states. Unable to distinguish emotional states is known as negative emotion differentiation (NED) which can be associated with a broad range of negative outcomes. Multiple studies have shown a link between a low NED to depression and depressive symptom severity.[22] But these studies have not shown that the effects are longitudinal. NED is negatively related to depression. A low NED means a higher chance for depression. This higher risk for depression depends on the severity of the stressful life events and environmental conditions they encounter.[22] Meaning, if the adolescent does not experience a severely stressful live event but has a low NED, it does not mean they will have depressive-like symptoms. The associations between stressful life events and the internalizing psychopathology have bidirectional influences and cascading effects over time, making this a complex issue. It has been stated before that higher intensity of stressful life events increases risk for depression symptoms. Multilevel modeling showed that adolescents reported a greater number of dependent interpersonal stressful life events after experiencing a time of depression at a previous time point, illustrating this two way relationship between stress and symptoms of depression.[21] This type of finding supports the stress generation model, which hypothesizes that depressed individuals behave in ways that add to the occurrence of additional

stressors which in turn maintain depression-like symptoms or increase the risk of relapse for depression.[23]

While a low NED has been linked to depression, another neural vulnerability is blunted reward sensitivity. Studies have shown that life stress disrupts the neural processing of reward and loss. These changes, in turn, affect future behaviors. Considering the stress generation model, looking at how neural processing of reward and loss influences future live stresses is imperative. Using the RewP index as a neural marker to examine the neural sensitivity to reward and loss through event-related potential measuring would help understand this relationship.[23] Specifically, RewP will help illustrate what kind of life events, dependent or independent, have an effect on subsequent depressive symptoms. A blunted RewP can predict the generation of dependent, not independent, life stress in adolescents.[23] In connection with RewP and the neural response to loss is indirectly associated with future depression symptoms based on the effects of the dependent life stress. Though it seems that stress is involved with the progression of depression, not everyone who experiences early life stress in their childhood has mental disorders in their adulthood. This can be explained as resilience.

Treatment Options for Depression

Treatments for depression require a specific evaluation and treatment plan. The following treatments include medications, brain stimulation therapies, psychotherapy, and alternative approaches. Common medications include SSRIs, which treat depression by increasing levels of serotonin in the brain by blocking reuptake into the neurons. The problem with the next generation of medications for depression is that it takes a couple of months to feel any effects and may not work overall. These people would be considered treatment resistant. Treatment for adults and adolescents are quite different. The two lines of treatment are psychotherapeutic and/or

psychopharmacologic approaches. For children under 18, there are two main types of medications used. The first line of treatment is fluoxetine.[29] If it is not effective, other medications that can be used are escitalopram or sertraline. Unfortunately, the second line of treatment is lacking as there is no clear systemized method for treatment resistant patients. Options are either to increase the dosage or include cognitive behavioral therapy (CBT) with the treatment regimen. CBT is a type of therapy that attempts to change the way you think and behave, a type of top down neuronal processing approach. Studies have been performed to see how usage of both medications and therapy may be better than just medication. But this is not the case. In some patients, it worked, but in others the combination had no effect.[29] Nearly 40% of adolescents remain depressed after initial treatment, and over half of that population remain depressed despite switching medications or adding psychotherapy.[29] Interpersonal factors seem to have an effect in pharmacological treatment. Studies have shown that placebo groups for treating depression had a positive effect than with treatment alone. This suggests that "how" prescribers give medication matters more than what type of drug is given.[30] Readiness for change was found to be the single most powerful determinant of treatment effectiveness.[30] If these two main types therapies are ineffective, other forms of therapies deserve some form of attention.

Stress is deemed as a negative potentiator for depression. However, as stated before, there are many forms of stress and some may be beneficial to the human psyche. There is a person nicknamed the 'Iceman' due to his feats in exposing himself in extreme cold situations for long periods of time based on his self-developed techniques of forced deep breathing and breath retention, cold exposure, and meditation. This practice is coined as the Wim Hof Method (WHM). Many baffled and in awe for his many world records, few scientists have decided to look further

into this method through scientific inquiry and neuroimaging to better understand why he is able to withstand extreme cold for an extended period of time, while others are not.

The main component to the WHM is several rounds of deep forced inhalation and shallow exhalations, then retaining the breath for as long as possible while being mindful of the body. The other component to the method is exposure to ice-cold water. This particular study focused on the relationship between conscious and autonomic aspects of CNS function and their responses during an oscillatory cold-warm water exposure using a whole-body garment.[31] They used fMRIs and PET imaging to compare differences between the control group and the Iceman, Wim Hof. The first thing they found was that during the oscillatory changes of the temperature of the garment, the control group had similar skin temperature changes to Wim Hof before he did his breathing method. As in when the temperature of the garment got colder, so did their skin temperatures and when it got warmer so did their skin temperatures. However, a second pass was done in which Wim Hof performed his breathing technique. As the garment changed temperatures, his skin temperature remained constant the whole time.[31] The brain scans showed substantially different functional responses during cold exposure, specifically regions in the brainstem and the interoceptive limbic regions. Specifically, they observed a significant increase in BOLD signal in the periaqueductal grey and the pons when Wim performed the method. No such activation was seen in the control group and when Wim did not perform the method. Also, it is important to note that the signal in the pons are found in the mid-level deep pontine nuclei that contains the parabrachial nucleus and the locus coeruleus.[31] They also found that the left anterior and right middle insula BOLD signal was decreased in Wim when he performed the method compared to the controls. This higher cortical area is associated with the salience network, which is a large-scale network anchored in the anterior insula and dorsal anterior cingulate cortex.[32] The three

subordinate subcortical structures in the salience network contains the amygdala, the ventral striatum, and the substantia nigra/VTA all contributing to complex brain functions that are reacting to stimuli that is pleasurable and rewarding, self-relevant, or emotionally engaging.[32]

It was particularly interesting to find that one specific portion of the brainstem activated was the locus coeruleus. It is thought that the unnatural respiratory pattern causes a decrease in CO_2 levels in the blood and respiratory alkalosis which is likely to initiate the activation of the sympathetic stress response system.[31] During the breath retention phase, the relationship between blood O_2 and CO_2 levels as oxygen saturation decreases. This mimics asphyxia, which likely activated the sympathetic autonomics centers in the brainstem triggering a systemic release of norepinephrine.[31] Since the locus coeruleus does not have one single projection site, for example, it is known to also project to the mPFC, it is uncertain if the LC-VTA is activated in this breathing technique. If, on the other hand, the LC production of NE activates the firing of the VTA, it may be possible to prime the system to acquire resilience-like behavior and may provide a second line of treatment for depression in adolescents and even adults. Currently, clinical trials at University of California San Francisco are being conducted on deep breathing and breath retention techniques to see whether short-term stressors can accumulate to long-term effects on psychological resilience.[33] Through evidence-based research this will support the need for preventative and behavioral treatments that are desperately needed in today's age in dealing with depression.

Conclusion & Future Directions

Major Depression is a complicated condition and many factors play into the progression of the disease such as environment, genetics, and psychological factors. Adolescence is a developmentally critical period of brain development for plasticity and maturation of stress systems. Animal studies try to show how experiencing stress-related trauma in adolescence may

cause structural changes within the brain that ultimately affects them behaviorally in adulthood. Gene x Environmental explanations have been proposed in many studies that try to connect certain polymorphisms to depression and try to explain how having these certain polymorphisms can make them more susceptible to the disease when exposed to stress. But these studies only focus on one gene at a time, rather than a cohort of them, making an understanding of the different genetic relationships difficult. Also, experiencing stress and then developing depressive-like symptoms is not unidirectional. It has been found that people who have had depressive episodes before can be more vulnerable to more stressful life events. People who experience stress in adolescence but do not acquire depressive episodes are resilient. Understanding the molecular mechanisms of resilience may help with creating treatments or approaches that we can take to help adolescent children who experience stress stray away from depression. The first line of treatment for depression includes first treating with an SSRI or combining it with cognitive behavioral therapy. The second line of treatment is not properly outlined for professionals, which allows for inquiry with what methods can be done for treatment. For example, the WHM is currently being studied in terms of how it may provide us in developing psychological resilience through exposure of short-term stressors. What needs to be further examined is whether or not this method activates the LC-VTA pathway that leads to beneficial homeostatic plasticity changes we see in mice. By continuing to dig into the complex etiology of this disorder in terms of resilience and stress can we hope to look out for certain things that may help with raising our children to be strong and resilient functioning adults in society.

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