The Developmental Disruptions of Ventral Hippocampal PV-Positive Interneurons by Early Adolescent Drug Exposure

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Abstract

Adolescence is associated with an amplified susceptibility to such neuropsychological disorders as schizophrenia and drug abuse. In the brain, a ventral hippocampal-prefrontal cortex pathway mediates cortical functioning by specialized GABAergic interneurons, marked by selective expression of calcium binding proteins, Parvalbumin (PV) or Calretinin (CR). A previous study showed that PV and CR interneurons undergo changes throughout development in the prefrontal cortex (Caballero et al., 2012); however, whether such changes occur in the ventral hippocampus is unknown. Thus, we examined the expression of PV and CR in the ventral hippocampus of male rats and found that PV expression undergoes a significant upregulation after postnatal day 35 (PD35) in adolescents (PD45-55) and adults (PD65-75) compared to juveniles (PD25-35). In contrast, there was no change in CR expression. Next, we determined if PV expression is disrupted by early adolescent exposure (PD35-40) to drugs. Results indicated that an NMDA antagonist, MK-801 and cocaine prevented the normative upregulation of PV interneurons in the ventral hippocampus, but not for a CB1 cannabinoid receptor agonist, WIN. Together, these findings allow us to understand the developmental regulation of GABAergic circuits and factors that may impact neurodevelopment.

Introduction

Psychiatric Disorders and the Brain

The history of mental disorders dates back well throughout many ancient civilizations, whose records document disordered states of perception and cognition. The alteration of a person's thoughts, feelings and behaviors in distinct ways usually results in difficulty of normal functioning (Berrios, 1999). In the past, there has been a thin line between what are considered to be brain and psychiatric disorders. However, the amount of evidence that all psychiatric disorders are associated with distinct patterns of neurophysiological alterations and increased or decreased functional connectivity within the brain has blurred this line (Carson, 2012; Glees, 1947; Stearns, 1946). Given the association between behavioral changes and alterations of the brain's physical structure or chemical basis, it is important to study in depth the neurobiological factors associated with psychiatric disorders to understand the underlying mechanisms at work, as well as to establish lines of treatment or therapy. Studies of brain development demonstrate that a disruption of neural circuits at crucial time periods is implicated in the onset and progression of psychiatric disorders (De Bellis et al., 1999; Schore, 1997).

Magnetic Resonance Imaging (MRI) of the human brain has revolutionized the way we can understand structure and function throughout development. MRIs and other neuroimaging techniques allowed us to understand that a subset of psychiatric disorders occur during childhood or adolescence (Paus, 2005; Paus et al., 2008). The brain is often considered to be a malleable machine during the period of adolescence and thus the presence of mental problems can interrupt normal brain development (Giedd, 1999). Research findings push the limits on what we already know about how the brain works in this regard. One of the most unique qualities and properties of the human brain is its plasticity, meaning that it can be manipulated in many different ways. The brain has the ability to be plastic to compensate for dysfunctions throughout the lifetime of an individual. However, plasticity in the brain appears to be increased during the period of adolescence (Dahl, 2004; Romeo et al., 2006). The list of psychiatric disorders that can arise throughout adolescence and later in life is extensive, and thus my thesis will address the underlying mechanisms that are thought to be related to the cognitive and working memory deficits that are characteristic of many neuropsychiatric disorders. Research in schizophrenia will be used as an example to show this relationship.

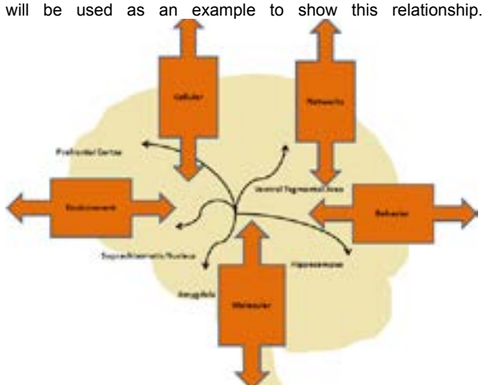

Figure 1: Brain Regions implicated in Neuropsychiatric Disorders: A simplified drawing of the human brain depicting the areas associated with higher cognitive functions, memory function, emotion control and hormone balance, that include the prefrontal cortex, hippocampus and amygdala. These areas of the brain are known to undergo neuropathological changes influenced by a number of factors (cellular, environment, molecular, behavioral, neuronal networks) in neuropsychiatric disorders. Other areas of the brain implicated, include pathway systems that are interconnected between multiple brain regions such as the ventral tegmental area, hippocampus and prefrontal cortex.

According to the National Institute of Mental Health, an estimated 26.2% of Americans from ages 18 to mid-adulthood suffer from at least one neuropsychiatric disorder, which translates to about 57.7 million people (Kessler et al., 2005). Neuropsychiatric disorders are associated with differences in brain activity that include changes in the subcortical networks of multiple regions of the brain (Simpson et al., 1989; Green, 2006). Multiple hypotheses exist to explain the dysfunctions associated with neuropsychiatric disorders. For example, in schizophrenia there have been three hypotheses put forward to understand the full complexity of the disorder. The glutamate hypothesis suggests that dysfunction is directly linked to glutamatergic abnormalities (Javitt et al., 1991; Krystal et al., 1994; Fig. 2). The dopamine hypothesis attributes dysfunction to the dysregulation of dopaminergic neurons within many brain structures such as the prefrontal cortex that receive dopaminergic innervations

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from the substantia nigra and ventral tegmental area (VTA; Seeman et al., 2005; Fig. 1 & 2). More recently, the majority of evidence points to neurodevelopmental hypotheses that are based on disruptions in the normal development of neural circuitry (Fatemi et al., 2009; Owen et al., 2011; Fig. 2).

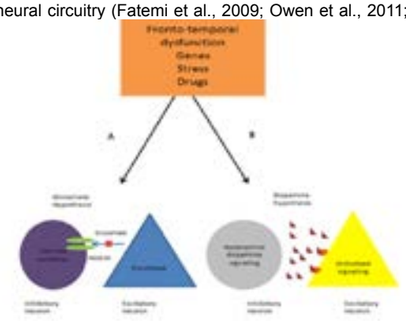

Figure 2: Hypotheses of Schizophrenia: There are a number of factors that affect the onset of neuropsychiatric disorders such as schizophrenia; dysfunctions of the frontal-temporal lobe, susceptible genes, environmental stress and drugs. (A) In the glutamate hypothesis, the glutamatergic signaling is diminished due to the disruption of NMDA receptors. (B) In the dopaminergic hypothesis, there is a hyperactivation of dopaminergic signaling. (C) In the neurodevelopmental hypothesis, depicting the process by which neurons in the cerebral cortex are made, GABA is needed for the development of pyramidal cells (dendrites and synaptic inputs) as it prohibits these cells from migrating to the other regions. These cells thus stay behind to develop the cortex (Wang et al., 2009). Any disruption of GABA signaling will interfere with this process.

These hypotheses may or may not be mutually exclusive. However, my study will explore the neurodevelopmental hypothesis of neuropsychiatric disorders, the external factors that can trigger the onset of these disorders, the implicated brain regions and the specific brain mechanisms that orchestrate deficits associated with neuropsychiatric disorders.

Addiction

The onset of deficits associated with neuropsychiatric disorders can be triggered by different environmental factors. One of these factors is the extended use of harmful and illicit drugs that lead to addiction. Addiction is specifically concerned with compulsive behaviors geared at obtaining recreational, illicit and medicinal drugs to use beyond the limits that are considered safe and that leads to an inability to stop drug consumption (Robinson et al., 2000). The insults that can occur during development may be due to a combination of stressors, of an environmental nature, and exogenous chemicals. When a person takes a drug, whether it is marijuana, cocaine or even alcohol, it can often lead to a dependence on the drug; which disrupts the normal distribution and regulation of chemicals in the brain (Angres et al., 2008). The brain often remembers that one surge of extreme excitability, and is then focused on recreating that 'high,' which results in addiction (Angres et al., 2008). Repeated drug use leads to a number of changes at the structural and cellular levels of the brain, which alter the ability of brain regions to control functions such as judgment and decision-making (Blum et al., 2012). Neurotransmitters, chemicals that transmit signals from a neuron to specific cells, are most affected by illicit drug use (Blum et al., 2012).

The psychological process of addiction is mediated by the dopamine system, which functions as a reward pathway (Gardner et al., 1993). In animal models, if the neurotransmission of dopamine is disrupted by an antagonist, the pleasure of positive reinforcement will lose their effects, suggesting that dopamine plays a key role in the 'liking' or the pleasure aspect of addiction (Wise, 1982; Gardner et al., 1993). However, when substances such as cocaine enhance dopamine function by blocking its re-uptake into the presynaptic neuron the animal will experience high levels of pleasure that leads to a 'wanting' behavior or motivation to consume the drug, which becomes known as addiction (Volkow et al., 1997; Fig. 3). If external substances interfere with the normal balance of brain circuits during a crucial period of neurodevelopment, then the likely outcome is a host of cognitive and perceptual deficits. According to the National Institute of Health, 34.7% of adolescents have admitted to trying an illicit drug and approximately 20% reported repeated use of an illicit drug (Schulenberg et al., 2012).

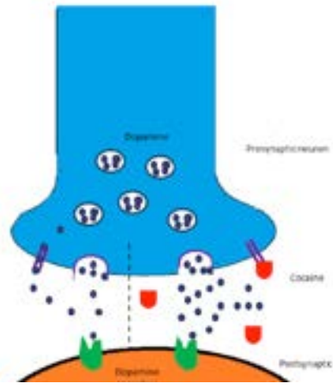

Figure 3: The effect of drugs on neuronal activity. A neurotransmitter, such as dopamine (purple) is released by the presynaptic neuron (blue) and binds to its receptors (green) on the postsynaptic neuron and provides a feeling of pleasure and excitability. Excess neurotransmitters that do not bind to the receptors are taken back up into the presynaptic cell through a reuptake process. At the same time inhibitory neurotransmitters (e.g. GABA) are released. Exogenous drugs, such as cocaine (red) increase the amount of neurotransmitters in the synapses as they block the reuptake of neurotransmitters, which increases the amount of dopamine in the synaptic cleft. This results in an elevation of the feelings of pleasure and at the same time blocks the release of inhibitory neurotransmitters.

Adolescence

Adolescence is a period marked by a number of physical and psychological changes (Steinberg, 2011). The rapid period of development creates a window of opportunity for external factors to influence the normal progression of brain circuits, which can lead to an increased risk of developing psychiatric disorders during this time (Andersen, 2003; Caballero & Tseng, 2012). The period of adolescence is so vital to the natural trajectory of human life and function that even the slightest disruption within this timeline can lead to long-lasting adverse effects, which change our experiences as adults. Adolescents who display high risk seeking behaviors often experiment with a number of illicit drugs (Steinberg, 2008). Continued exposure to these drugs can lead to alterations in synaptic firing, regulation, and maturation within the brain

(Anderson, 2003). Behaviorally, these alterations can affect cognitive abilities such as perception, attention, memory, motor control, and executive functioning (Green, 2006; Krystal 1994). Particularly, in adolescence the cognitive abilities that are refined involve one's ability to think systematically or rather to think logically about all aspects of a problem or situation (Boyd et al., 2003). The underlying mechanisms that cause these dysfunctional changes in the brain still need to be studied in greater detail.

Functional MRI studies show that the brain continues to grow and develop well into the mid-twenties and is impacted by an interplay between genes and the environment (Giedd et al., 2009; Lenroot et al., 2008). A critical region of development is the frontal lobe, which governs planning, working memory, and impulse control. However, it has been noted that some functions develop fully by adolescence, while others still undergo changes during this period (Spear, 2000). The risk of neurodevelopmental dysfunctions increase during adolescence, but it is still unclear at what point during this development that the brain is most vulnerable. Thus, answers may lie within the underlying mechanisms of brain function, specifically with the expression and migration of populations of neurons. To carry out these investigations we used animal models.

Adolescence in Non-Human Models

The use of animal models is crucial to the understanding of diseases as well as characterizing changes in brain function or behavior. Non-human models, such as rodents and primates, provide an effective way to address the gaps in knowledge that arise due to structural or functional changes in the brain throughout development. These animals are biologically similar to an extent and also display an adolescent stage similar to that of humans (Spear, 2000; Fig. 4). Rodents undergo changes over developmental periods in the anatomical and structural design of the brain. The majority of research in animals that is focused on modeling a human phenotypic pathology, disease treatment, and prevention methods provides a cost-effective way of studying physiological and anatomical changes in humans (Glaswow, 1975). Although differences are not to be ignored, they are outweighed by important similarities. Animal models are beneficial due to the ethical concern that surrounds the study of any human disease. A model has to be developed in order to understand the mechanisms that are responsible for the display of behaviors in humans. Successful modeling, over the past decades shows that animal models often give a close approximation to what is seen in humans and has also led to the discovery and production of many treatments. The aim of this research project is not to model a specific neuropsychiatric disorder in rats, but it is the aim to show how the use of animal models can allow us to understand the underlying mechanisms that may be implicated in neuropsychiatric disorders.

Modeling Psychiatric Disorders in Animal Models

Developing a model for human neuropsychiatric disorders is challenging due to the subjectiveness of symptoms and lack of objective diagnostic tests. However, animals prove to be effective treatment models for many neuropsychiatric disorders and provide us with deeper understandings of the pathophysiology of a disorder, as outlined by the extensive literature (Gould et al., 2006; Lipska et al., 2003; Lodge et al., 2009; Nestler et al., 2010; Tseng, 2009; Willner, 1984). To highlight the importance of animal models we will focus on what has been found in schizophrenia research. In a neonatal ventral hippocampal lesion model (NVHL), rats that suffered a legion to the ventral hippocampus shortly after birth developed many of the positive, negative and cognitive symptoms associated with a diagnosis of schizophrenia, during adolescence. According to the Diagnostic and Statistical Manual of Mental Disorders (4th ed., text revision, American Psychiatric Association, 2000), schizophrenia is characterized as the presence of positive and negative symptoms; including delusions and hallucinations, loss of emotion or speech during a one-month period. The disorder exists on a spectrum ranging from mild to severe with a number of different subtypes. Schizophrenia is also associated with impairments of cognitive functions (Elvevag et al, 2000). Keeping in mind that schizophrenia is highly concerned with human intelligence and disorganized thinking, the 'positive-like symptoms' in the NVHL rats manifested as hyper-locomotion, hyper-responsivity to stress and overall deficits in sensorimotor gating (Lipska et al., 2003; Tseng, 2009). 'Negative-like symptoms' in NVHL rats showed deficits in motivation, social interaction and deficits in grooming; while 'cognitive-like symptoms' showed deficits in working memory, spatial memory, and learning in maze-type tasks that is comparable to a diagnosis of schizophrenia in humans during mid-late adolescence (Lipska et al., 2003; Tseng, 2009; Fig. 4). Many of these behavioral changes observed in rats are not exclusive to a diagnosis of schizophrenia, but rather are indicative of almost all forms of neuropsychiatric disorders.

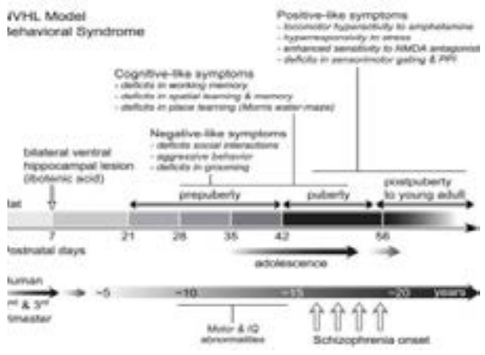

Figure 4: Timeline comparison of human and rat development: (A) The developmental period of rats and humans are similar, as both humans and rats develop with the different stages of life although at different paces. This allows us to perform neurodevelopment studies of humans on rat subjects that will capture the scope of a given developmental period. (B) In the neonatal ventral hippocampal lesion model, the emergence of behavioral changes associated with schizophrenia-like symptoms is similar for both rats and humans, from the emergence of symptoms to the actual onset of the disease. (Obtained/ modified from Tseng, 2009).

Postmortem studies of neuropsychiatric disorders, for example schizophrenia, show alterations in multiple neurotransmitter systems such as γ-aminobutyric acid (GABA), glutamate, dopamine and serotonin (Harrison, 2000; Powchick et al., 1998). The most promising evidence for the pathology of schizophrenia is a decrease in GABA-related signaling (Lodge et al., 2009). Inducing a developmental disruption of prenatal rats with a mitotoxin, methylazoxymethanol acetate (MAM), led to a viable animal model of schizophrenia-like phenotypes that showed both anatomical and behavioral deficits (Grace et al., 1998). Lodge et al. (2009) showed that MAM-treated rats showed a decrease in parvalbumin (PV) positive interneurons in the medial prefrontal cortex and ventral region of the hippocampus

and that these rats performed poorly on a task performance test. Using an animal model that implicated abnormally low prefrontal and hippocampal functioning, we are able to characterize the underlying mechanisms, such as interneurons, which are deregulated during development to determine the behavioral deficits we see in neuropsychiatric disorders.

Innervations of the Prefrontal Cortex (PFC)

The prefrontal cortex (PFC) has expanded considerably throughout human evolution compared to other areas of the brain (Rilling, 2006); and so one of the most crucial structures involved in neurodevelopment during periadolescent transition is the prefrontal cortex (Rilling, 2006). Damage to the PFC often results in a loss of higher-order cognitive functions such as problem solving, working memory, abstract thinking & inhibitory control (Anderson et al., 1999). Adolescents will often engage in risk-seeking behaviors due to the unrefined nature of cortical activity within the PFC to mediate rational judgments and delay gratification (Steinberg, 2008). The PFC is an important structure in understanding the progression of neurodevelopment as it continues to develop and change late into adolescent maturation (Caballero & Tseng, 2012).

Psychiatric disorders such as schizophrenia, which arise in late adolescence or early adulthood years, suggest that insults during the very crucial time period of early adolescence confers a risk in the development of psychiatric disorders. Impairments of cognitive functions that are observed in schizophrenia can be attributed to changes in the prefrontal cortex (Hashimoto et al. 2003). As neuronal connections grow and increase across the brain, neuronal pathways that are not being used are lost in order to maximize the efficiency of other pathways.

The PFC is interconnected with a number of other brain regions and thus changes in one area may cause changes in another (Fig. 5). The PFC obtains glutamatergic inputs from the hippocampus, and sends inputs to multiple regions of the brain (Ishikawa et al., 2003). The transmission of information throughout the brain suggests that the hippocampus, prefrontal cortex and associated neural circuitry could be responsible for cognitive and behavioral processes that are functionally associated.

Functions in the Ventral Hippocampus

The hippocampus is a crescent shaped structure that is divided into four zones: CA1 to CA4. It is a vital brain structure for learning in terms of its role in memory and spatial manipulation. The role of the hippocampus in memory has been demonstrated from studies of hippocampal damage (Giap et al, 2000). Mouse models that have a mutant or deleted CA1 pyramidal cell glutamate receptor showed a deficit in spatial memory functioning (Tsien et al., 1996). The hippocampus houses a neatly organized layer of various neuronal cell types. These interneurons continue to develop as we age, exerting excitatory or inhibitory patterns on other

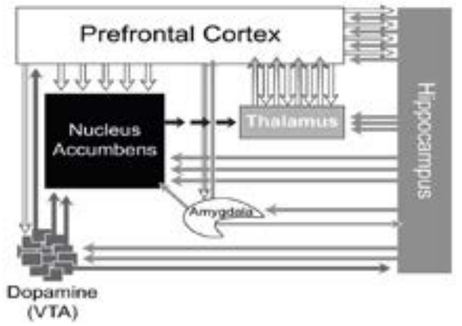

Figure 5: Anatomic and functional interconnections of major brain circuits. Although the functions and areas of the brain remain separate, there is a great deal of connectivity between multiple brains regions. There is a particularly important relationship between the prefrontal cortex and the hippocampus in terms of neuronal signaling. (Modified from Tseng, 2009)

cell types such as the pyramidal cells of the PFC (Tseng et al., 2006). In 1977, a study using monkeys first documented a direct projection from the hippocampus to the prefrontal cortex (Thierry et al., 2000). In a rat model, a direct pathway exists from the temporal field CA1 of the hippocampus to an area of the PFC (Swanson, 1981; Verwer et al., 1997).

The role and importance of the ventral hippocampus was demonstrated in a neonatal ventral hippocampal lesion, which led to functional deficits in cortical interneurons and abnormalities in behavior (Tseng et al., 2008). The most significant correlation of cognitive deficits associated with psychiatric disorders seems to be a distinct reduction in the size of the hippocampus related to a disruption of development rather than tissue deterioration (Harrison, 2004). Since the hippocampus is interconnected within the ventral hippocampus-prefrontal cortex pathway, this suggests that interneuronal connectivity along this pathway could also be affected. The pathway originates in the ventral hippocampus (subiculum) fields that are rich with interneurons innervating primary cells in the prefrontal cortex (Thierry et al., 2000). GABAergic cortical interneurons within the ventral hippocampus have been implicated in hippocampal function and dysfunction. As mentioned previously, animals that are given a neonatal ventral hippocampal lesion (NVHL) show deficits in motor and cognitive functioning late in adolescence or early adulthood, suggesting the importance of this region (Tseng et al., 2009). The hippocampus also contains a number of interneurons that are thought to mediate GABAergic signaling.

GABAergic Signaling

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and is synthesized from glutamate (Glutamatergic anomalies have been noted in neuropsychiatric disorder pathology; Sampson et al, 2003). GABA inhibits synapses by binding to specific transmembrane receptors in the membrane of postsynaptic neuronal processes (Streeter et al., 2005). The hippocampus and neocortex GABA displays excitatory effects and so GABAergic interneurons provide both a disinhibitory and an inhibitory regulation of hippocampal circuits (Benes et al., 2001; Mann et al., 2007). Perturbation in early development causes interference in the normal GABAergic signaling as well as changes in neural oscillations (Benes

et al., 2001; Caballero & Tseng, 2012). GABAergic signaling has been implicated in the maturation process of many adult neurons since the inhibitory property is needed for the refinement of cortical circuitry during the transition to adulthood (Markwardt et al., 2008). As mentioned previously, a decrease in the amount of GABAergic signaling within the brain leads to phenotypes associated with neuropsychiatric disorders, such as schizophrenia (Lodge et al., 2009). Thus it is hypothesized that it is a reduction of the GABAergic parvalbumin-positive interneurons that cause deficits in neuronal coordinated tasks in rats related to an animal model of schizophrenia (Lodge & Grace, 2009).

GABAergic signaling is mediated by highly specialized interneurons that have distinct electrophysiological properties (i.e. fast-spiking or low threshold spiking) and expression patterns of calcium binding protein (Somogyi et al., 2005; Nakazawa, 2010). Among these, GABAergic interneurons have been located in the prefrontal cortex and the hippocampus based on the calcium binding proteins parvalbumin, calretinin and calbindin (Freund et al., 1996), as discussed next.

Calcium Binding Proteins

Parvalbumin

Parvalbumin is a calcium-binding albumin protein present in GABAergic interneurons expressed into basket, axo-axonic, bistratified and oriens-lacunosum molecular cells (Klausberger et al., 2005). At least 90% of all PV-positive cells are fast spiking, meaning that they have fast firing rates, which is important for sensory response. Many cortical circuits require inhibitory inputs from parvalbumin cells in order to control pyramidal cells in the prefrontal cortex (Lewis et al., 2012). During development, it appears that ventral hippocampus hypofunction at PV-positive, fast-spiking interneurons produces schizophrenia like effects (Tseng et al., 2009). Findings suggest that a reduction in the level of calcium binding protein parvalbumin (PV) particularly in the medial prefrontal cortex and ventral subiculum of the hippocampus is associated with schizophrenia (Lodge et al., 2009). The expression of parvalbumin is sensitive to the developmental period of adolescence with high levels being expressed (Fitzgerald et al., 2011). The evidence suggests that the well amplified susceptibility of adolescence to the onset of schizophrenia or drug abuse, that severely impacts perception and cognition, could be due to a developmental disruption of GABAergic responses of calcium binding proteins within the ventral hippocampal-prefrontal cortex pathway.

Calretinin

Calretinin is also a calcium-binding albumin protein involved in calcium signaling and expressed as long hair-like spines that form mossy fibers (Freund et al., 1996). While there are other calcium binding proteins, calretinin is of particular importance because of its prevalence in early developmental stages of postnatal life (Conde et al., 1994; Fung et al., 2010). Unlike parvalbumin, calretinin is associated with non-fast spiking neuronal activity (Fung et al., 2010). In relation to the broader implications of this study, the purpose of using calcium-binding proteins to gauge developmental effects is the ability to utilize the presence of these GABAergic responses as an indicator of normal cortical functioning. A recent study showed that the expression pattern of parvalbumin and calretinin positive interneurons follow opposing trajectories during periadolescence in the prefrontal cortex of male rats (Caballero et. al, 2012).

Impact of Drugs on the Adolescent Brain

Addictive drugs stimulate the reward pathway within the brain. The circuitry involved in this pathway elicits the release of neurochemical properties that tend to reinforce the behavior leading to a cycle of addiction (Koob et al., 2009). Addiction targets many of the brain regions that are responsible for learning, memory, impulsivity control and executive functions (Young, et al., 1999). Two regions that are known to be associated with these functions are the PFC and the hippocampus. As the literature suggest, there is indeed an association between addiction and psychiatric disorders (Rounsaville et al., 1991; Schuckit, 2006).

The CB1 Cannabinoid Receptor Agonist, WIN

The risk of neuropsychiatric disorders such as schizophrenia increases with adolescent exposure to ∆9-Tetrahydrocannabinol (∆9-THC), which is found in cannabis (Malone et al., 2010). Cannabinoid receptors are regulated by three ligands: endocannabinoids, THC and synthetic cannabinoids (Garrett et al, 1974). There are two types of cannabinoid receptors, CB1 and CB2. CB1 receptors play a key role in the developmental period from prenatal to early postnatal development. CB1 receptors are highly localized in the brain and are most responsible for the effects observed from THC use (Kano et al., 2009). It is expressed in pyramidal neurons, but more so in GABAergic interneurons, indicating a role in GABAergic signaling. (Hill et al, 2007). Kucewicz et al. (2011) showed that a CB1 receptor agonist reduced the electrophysiological properties of the prefrontal cortex and of the hippocampus that was associated with reduced function in memory based tasks, thus reducing GABAergic transmission. The synthetic cannabinoid receptor (CB1) agonist, WIN, elicits effects similar to those of ∆9-Tetrahydrocannabinol; in theory, WIN will reduce the maturation of prefrontal GABAergic interneurons as well as hippocampal GABAergic interneurons. Animal models of brain dysfunction that have been treated with WIN indicate the role of the CB1 receptor in glutamate transmission (Ferraro et al., 2001).

The NMDA Antagonist, MK-801(Dizocilpine)

Glutamate is the primary excitatory neurotransmitter in the central nervous system. MK-801 is a non-competitive antagonist of the N-methyl-d-aspartate (NMDA) receptor (or glutamate receptor) that binds to the ion channels of the glutamate receptor and prevents the flow of ions, which leads to an excess of calcium ions (Wong, 1986). Through animal studies, it is known that MK-801 is a potent anticonvulsant and could possibly be a dissociative anesthetic (Williamson et al., 1989). However, a number of neurotoxic/cytosolic changes as well as cognitive dysfunctions occur in the brain following the administration of NMDA antagonists. MK-801 provides an excellent model system in mimicking psychiatric disorders as research shows that injections of MK-801 in rat models were successful in capturing both the positive and negative phenotypes of schizophrenia, as previously explained (Rung et al., 2005). More specifically, these rats responded to MK-801 with increased social withdrawal and motor activity.

Psychomotor Stimulation of Cocaine

Cocaine is derived from the coca plant and acts as a psychomotor stimulant, a neurotransmitter (particularly with serotonin-norepinephrine-dopamine) reuptake inhibitor and an appetite suppressor that has addictive effects due to its action on the mesolimbic reward pathway (Barnett, 1981). This drug elicits a feeling of euphoria, hyperactivity and feelings of grandeur (Barnett, 1981). Cocaine has a particular effect on dopaminergic signaling in that it binds to the dopamine transporter, which

blocks the normal reuptake function of the transporter and results in an influx of dopamine in the synaptic cleft (Bolla et al., 1999).

Current Gaps in Knowledge

While we know that adolescence is an extremely vulnerable time period in the emergence of neuropsychiatric disorders and substance abuse compared to other age groups, the underlying mechanisms that account for these differences are still unclear. We do not know the specific contribution of the GABAergic mechanisms in cortical development, distribution and regulation, and so the specific classes of GABAergic interneurons still need to be further studied to understand the range of their distinguishing features. We know that GABAergic interneurons are found throughout the brain in regions where cortical circuitry mediates normal functioning, however, we do not know whether these interneurons are being expressed or are following a migratory pattern/projection from interconnected brain regions. We also do not know the effect specific drugs will have on the expression of GABAergic interneurons in the ventral hippocampus. Previous work highlights that exposure and withdrawal has a direct impact on the prefrontal cortex and that the time period of exposure is crucial to this impact (Cass, senior thesis 2010: not yet published). In this study we examined the expression of GABAergic interneurons in different regions of the ventral hippocampus of male rats.

Hypothesis and Aims

This study aims to investigate the normal expression patterns of calcium-binding proteins in the ventral hippocampus throughout postnatal days of male rats and determine what chemical properties can disrupt the protein expression. Therefore, we hypothesize that the expression of Parvalbumin-positive interneurons within the ventral hippocampus is developmentally regulated and disrupted by exogenous drugs. In order to test our hypothesis we performed the following aims:

1. To determine whether expression of calcium-binding proteins (Parvalbumin and Calretinin) is age dependent, we assessed control male rat brains through double labeling immunohistochemistry using two antibodies of two different species to detect the corresponding protein. These tissue samples were then assessed through immunofluorescence microscopy to visualize the location of the protein. We found that there was increase of PV-positive interneurons compared to no change in CR-positive interneurons in the ventral hippocampus across development.
2. To determine whether repeated non-contingent drug exposure (i.p.) during early adolescence (PD35-40) to MK-801, cocaine and WIN interrupts the expression of Parvalbumin-positive interneurons within the ventral hippocampus, subiculum brain sections of rats that were treated under the conditions of WIN, MK-801 or cocaine were assessed for Parvalbumin expression relative to saline treated rats, using immunohistochemistry and immunofluorescence microscopy. We found that PV-expression is developmentally disruptive only for MK-801 and cocaine.

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