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 cortical pathway mediates cortical function 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 (Cabakalo 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 (PD45-65) 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 administration to male rats on 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 administration to male rats on day 35 (PD35) in adolescents (PD45-55) and adults (PD65-75) compared to juveniles (PD25-35).

Introduction
Psychiatric Disorders and the Brain
Neuropsychiatric disorders are associated with differences in neuronal networks, which include the prefrontal cortex and hippocampus and amygdala. These areas of the brain are known to undergo neurodevelopmental 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 (Simons et al., 1989; Green, 2006). Multiple hypotheses exist to explain the dysfunctions associated with neuropsychiatric disorders. For example, 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 brain structures such as the prefrontal cortex that receive dopaminergic innervations from the substantia nigra and ventral tegmental area (YTA; Seeman et al., 2005; Fig. 1 & 2). More recently, the majority of evidence points to neuropsychological hypotheses that are based on disruptions in the normal development of neural circuitry (Paton 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: dysfunctional immune system, genetic susceptibility, excessive stress, endogenous factors, developmental factors, and others. The brain, specifically the prefrontal cortex, is responsible for a number of functions such as decision making, emotional regulation, and executive function. The dopamine hypothesis attributes dysfunction to the dysregulation of dopaminergic neurons within brain structures such as the prefrontal cortex that receive dopaminergic innervations from the substantia nigra and ventral tegmental area (YTA; Seeman et al., 2005; Fig. 1 & 2). More recently, the majority of evidence points to neuropsychological hypotheses that are based on disruptions in the normal development of neural circuitry (Paton et al., 2009; Owen et al., 2011; Fig. 2).

These hypotheses may or may not be mutually exclusive. However, my study will explore the neuropsychological hypotheses of neuropsychiatric disorders. In addition, I will examine the role of early adolescent drug exposure and its impact on the prefrontal cortex and the ventral tegmental area, which are associated with neuropsychiatric disorders.

Addiction
Addiction is a condition where 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 towards obtaining recreational, illicit and medicinal drugs used to 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 chemical substances 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 impulse control (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 ‘wanting’ of a 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 affect 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 and excites its activity. Dopamine receptors 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. Excitatory drugs, which bring about the release 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 (Steinberg, 2000; Cabakalo & 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). Without exposure to these drugs can lead to alterations in synaptic firing, regulation, and maturation within the brain.
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(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 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-late teens and is impacted by an interplay between genes and the environment (Giudet et al., 2008; Lentino et al., 2009). 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, 2008). The risk of neuropsychological dysfunctions increases during adolescence, but it is still unclear at what point during this development that the brain begins to mature. It 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 a way to address the gaps in knowledge that arise due to the ethical concerns and 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, 2008; 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 (Glasgow, 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 validity of any human disease model has to be developed; in order to understand the mechanisms that are responsible for the display of behaviors in an adolescent. 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 of important findings. The aim of this research project is not to model a specific neuropsychiatric disorder, rather 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

Development of various psychiatric disorders in humans is challenging due to the subjectivity of symptoms and lack of uniformity in the pathology of a disorder as outlined by the extensive literature (Gould et al., 2006; Lipka et al., 2003; Lodge et al., 2009; Neve et al., 2009; Wilner, 1984). To highlight the importance of animal models we will focus on what has been found in schizophrenia.

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 in a number of studies of hippocampal damage (Yonelinas et al., 2000). Mouse models that have a mutant or deleted CA1 synapse have a pronounced decrease in spatial memory functioning (Tsien et al., 1996). The hippocampus houses a neatly organized layer of various neuronal cell types. These intrinsic neurons develop in a manner similar to what is seen in age, egressing excitatory or inhibitory patterns on other cell types such as the pyramidal cells of the PFC (Tsai et al., 2009). In 1997, 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, 1983; Verret 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 consequence 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 (Harrodson, 2014). Since the hippocampus is interconnected within the ventral hippocampus-prefrontal cortex pathway, its progression and interneuronal connectivity along this pathway could also be affected. The pathway originates in the ventral hippocampus (subicular 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 (Glu). GABAergic 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 neuron dendrites (e.g., GABAAR, 2005). The hippocampus and neocortex GABA displays excitatory effects and suggests that GABAergic activity is critical in maintaining the disambiguity 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; Mann et al., 2007). Perturbation in early development causes interference in the normal GABAergic signaling as well as changes in neural oscillations.
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, 2009). Addiction triggers many of the brain regions that are involved in 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 suggests, there is indeed an association between addiction and psychiatric disorders (Rounsaville et al., 1991; Schuckit, 2006).

The CB1 Cannabinoid Receptor Antagonist, 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 development of psychiatric disorders (Garrett et al., 2001). 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). Kuczewski 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 motor 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 interneurons, interrupting the development of 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

Glutamate is the primary excitatory neurotransmitter in the central nervous system (Furgiuele et al., 2000). The evidence suggests that the well amplified susceptibility of adolescence to the onset of schizophrenia or drug addiction may, in part, be due to the neural plasticity associated with cognition, could be due to a developmental disruption of GABAergic mechanisms in cortical development. Animal studies have shown that a reduction in the amount of GABAergic signaling within the brain leads to deficits in neuronal coordinated tasks that it is a reduction of the GABAergic parvalbumin-positive interneurons, that is associated with deficits in neuronal coordinated tasks 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 slow-threshold spiking) and expression patterns of calbindin 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, calbindin and calbindin (Freund et al., 1989), 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 inhibition of calcium-binding proteins in order to control pyramidal cells in the prefrontal cortex (Lewis et al., 2012). During development, non-PV calcium hypofunction at PV-positive, fast-spiking interneurons produces schizophrenia like effects (Taenig 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 that 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 into early adolescence (Eggermont et al., 2006). The evidence suggests that the well amplified susceptibility of adolescence to the onset of schizophrenia or drug addiction may, in part, be due to the neural plasticity associated with cognition, could be due to a developmental disruption of GABAergic mechanisms in cortical development. Animal studies have shown that a reduction in the amount of GABAergic signaling within the brain leads to deficits in neuronal coordinated tasks that it is a reduction of the GABAergic parvalbumin-positive interneurons, that is associated with deficits in neuronal coordinated tasks related to an animal model of schizophrenia (Lodge & Grace, 2009).

Calcium binding proteins (CBPs) are a family of calcium-binding proteins that have an essential role in the regulation of calcium homeostasis in various mammalian tissues and the nervous system. CBPs have a wide range of functions, including neurotransmitter release, synaptic plasticity, and cell proliferation. In the nervous system, CBPs play a critical role in the regulation of neurotransmitter release and synaptic plasticity. Calcium binding proteins are a family of calcium-binding proteins that have an essential role in the regulation of calcium homeostasis in various mammalian tissues and the nervous system. CBPs have a wide range of functions, including neurotransmitter release, synaptic plasticity, and cell proliferation. In the nervous system, CBPs play a critical role in the regulation of neurotransmitter release and synaptic plasticity.
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