Age Dependent Effects of Repeated Cocaine Exposure and Withdrawal on Cortical Activity

Daryn Cass*
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Abstract

Exposure to a drug of abuse during adolescence significantly increases the risk for addiction. However, the mechanisms underlying the increased risk remain unclear. We assessed the impact of repeated cocaine injection on cortical activity in rats, and asked whether the age at which drug exposure takes place plays a role in determining the neuroadaptations observed in the mesocortical reward system, after 3 days and 3 weeks withdrawal from cocaine. We assessed cytochrome oxidase (COI) staining as a measure of metabolic activity. In the adolescent-exposed group we found a significant COI increase in the medial prefrontal, orbitofrontal, cingulate, motor, and somatosensory cortices after 3 weeks withdrawal from cocaine injection. In the adult animals we observed a significant decrease in activity in many of the cortical structures studied. These results suggest that different forms of cortical neuroadaptation occur when cocaine is given during adolescence compared to adulthood.

Introduction

Adolescence

Adolescence in humans

Adolescence is commonly defined as a period of physical, psychological, and social transition between childhood and adulthood (Blakemore et al., 2008). The age range for adolescence in humans is considered to be between 12 and 18 years of age. The boundaries of adolescence are often blurred, and the entire second decade of life is often considered adolescence (Spear, 2000). It is a period of important functional changes in the human brain (Blakemore et al., 2008), as many psychiatric disorders such as schizophrenia, substance abuse and mood disorders emerge during this period (Paus et al., 2008). These could arise from alterations in aspects of neurodevelopment that are still taking place during adolescence.

Functional MRI studies have allowed for the study of brain activity during several tasks which can provide useful information on the development of specifics executive functions (Paus et al., 2005, 2008). These studies have shown that while executive functions such as working memory and response inhibition are fully developed during childhood, other functions such as planning time and delayed gratification do not fully develop until adolescence (Paus et al., 2005). While we know little as to why these certain executive functions develop later than others, their development is dependent on the presence of subpopulations of neurons, the interaction between neurotransmitters and their receptors, and effective neurotransmission between brain structures, which change during adolescence (Tseng and O'Donnell, 2005, 2007c).

Adolescence in primates and rodents

While there are many gaps in our knowledge of changes in brain function during adolescence, there have been data that suggest that prominent changes in brain regions such as the frontal cortex occur during adolescence not only in humans but also in other species ranging from rodents to non human primates (Spear, 2000). Indeed, animal models are used extensively to model human psychopathology, with each model being based on only a few features which are thought to be central to the disorder (Spear, 2000). Work in rodents may provide a cost effective way to examine neuronal development and neurobehavioral characteristics accompanying adolescence (Figure 1; Tseng et al., 2009). It is important to realize however that the frontal cortex systems of rodents are lot less prominent than in humans and other primates (Seamans et al., 2008). Also they do not undergo increases in levels of neuroactive steroids in the same way humans and specific non human primates such as the chimpanzee do, which may also be a source of changes in brain development (Spear 2000). The cost effectiveness of using rodents however has made them a popular category of organisms in which to study brain development and related disorders.

The frontal cortex

The role of the frontal cortex

The role of frontal cortex was demonstrated in one of the most well known case studies in neuroscience in 1848. Phinneus Gage, a railroad worker survived an accident in which a long iron rod was driven through his head, destroying the frontal lobe of his brain. Before his accident Gage was one of the most dependable people on his workforce but could no longer perform his job after the accident. He underwent a change in personality and had become erratic, impulsive, unreliable, and uncompassionate towards others (Ratiu et al., 2004). This was one of the first situations which showed the frontal cortex plays a role in higher brain functions such as working memory, decision making, planning, reasoning, judgment, and impulsivity. It is therefore not surprising that many cognitive disorders such as attention deficit hyperactivity disorder (ADHD), autism, bipolar disorder, depression, and schizophrenia have been associated with dysfunctions of the prefrontal cortex (Moghaddam et al., 2008; Lewis et al., 2005).

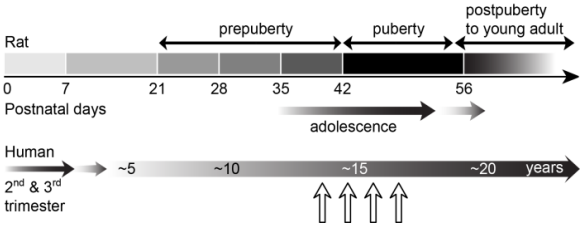


Figure 1: Timeline This timeline depicts the ages at which rats and humans go through adolescence. It can be used to determine the correct rat age to use while performing experiments aimed at modeling adolescence in humans (modified from Tseng et al., 2009

*This author wrote the paper as a senior thesis under the direction of Dr. Shubhik DebBurman.

The prefrontal cortex is also part of a network called the mesocorticolimbic system, also known as the brain reward system (Carlezon and Thomas, 2009). The prefrontal cortex receives dopamine inputs from the ventral tegmental area (VTA) (Figure 2). The dopamine signal in the prefrontal cortex is essential for sustaining higher cognitive functions discussed above (Gabbott et al., 2005). The prefrontal cortex also projects to the nucleus accumbens, amygdale, and hippocampus which are involved in reward, emotional reactions, and memory respectively (Gabbott et al., 2005). Therefore changes in prefrontal dopamine activity are expected to alter these responses.

Changes in the prefrontal cortex during adolescence

The prefrontal cortex undergoes continued development throughout adolescence, whereas maturation of other brain regions such as the sensorimotor system occurs sooner (Paus et al., 1999). Modern MRI studies have allowed for the study of structural changes in the brain taking place during adolescence. These changes include increases in cortical white matter, indicative of increased myelination of neurons, possibly allowing for faster

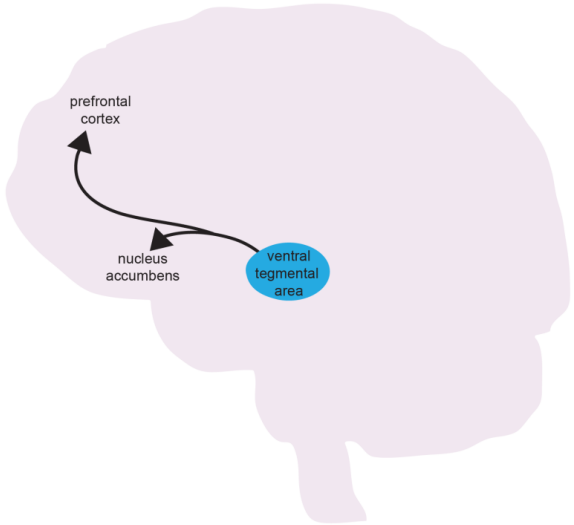


Figure 2: Mesocorticolimbic pathway Saggital section of a human brain depicting a simplified view of the mesocorticolimbic, or brain reward, system. Dopamine is produced in neurons in the ventral tegmental area, located in the midbrain. These neurons project to the nucleus accumbens and the prefrontal cortex, two areas that are heavily reliant on dopamine

synaptic transmission (Paus et al., 1999). Increases in cortical gray matter follow an inversed U shape (Paus et al., 1999). While this occurs in childhood for many brain structures, this process does not take place until adolescence for the frontal cortex. At the beginning of adolescence, increased synaptogenesis takes place, producing many synapses. As groups of neurons begin to function more synchronously and effectively, synaptic reorganization takes place in the frontal cortex during adolescence, allowing for synaptic pruning to occur, causing the amount of synapses and gray matter to decrease by eliminating excess neurons in humans (Giedd et al., 1999; Huttenlocher, 1979; Ulhaas et al., 2009).

The prefrontal cortex is heavily influenced by mesocortical dopamine. Interactions between dopamine and the prefrontal cortex change during adolescence. Dopamine innervation of the prefrontal cortex peaks during adolescence in rats and monkeys (Kalsbeek et al., 1988; Rosenberg et al., 1994; Tarazi and Baldessarini, 2000). Levels of dopamine receptors also increase postnatally in rodents, with D1 receptors reaching mature levels by PD60 (early adulthood) (Tarazi and Baldessarini, 2000). The emergence of more D1 receptors as well as the development of D1 and N-methyl-D-aspartate (NMDA) coactivation in the PFC is necessary for appetitive instrumental learning in adult rats as this coactivation improves memory retrieval and working memory performance (Baldwin et al., 2002). Also interactions between dopamine and NMDA receptors acquire mature characteristics in young adult rats (Tseng and O'Donnell, 2005). Thus morphological and functional changes take place in the prefrontal cortex during adolescence. These changes may allow for mature mechanisms of cortical information processing to develop, which could explain why behaviors associated with prefrontal cortical function change with the transition to adulthood.

Dopamine modulation in the prefrontal cortex

Changes in the frontal cortex during adolescence are mediated by prefrontal cortical circuits, involving the main cell types in the frontal cortex, pyramidal neurons (output neurons) and interneurons Batista-Brito and Fishell, 2009). Cortical interneurons use γ-Aminobutyric acid (GABA) to exert inhibitory effects on pyramidal cells (Batista-Brito and Fishell, 2009). Interactions between dopamine and these cell types change during adolescence (Batista-Brito and Fishell, 2009). Besides D1 effects on prefrontal cortical function, there are D2 effects as well. Evidence indicates a D2 modulation of working memory, however the effects of D2 receptors on prefrontal cortical function are less clear. In the prepuburtal prefrontal cortex D2 receptor activation is shown to increase (Seamans et al 2001; Trantham-Davidson et al., 2004) as well as decrease (Gulledge and Jaffe, 1998; Tseng and O'Donnell, 2004) pyramidal cell excitability and downregulate NMDA mediated responses. This effect was more pronounced in the adult prefrontal cortex, showing that D2 modulation of pyramidal cell excitability is still changing during adolescence (Tseng and O'Donnell, 2007). Figure 3 shows a summary of how cells in the prefrontal cortex respond differently to D1 and D2 receptor stimulation before and after adolescence. Specifically during adolescence the response of prefrontal cortical cells increases in response to dopamine receptor stimulation. Figure 4 is a model of cellular interactions in the frontal cortex showing the effects of ventral tegmental area dopamine on pyramidal cells and interneurons in the frontal cortex after adolescence.

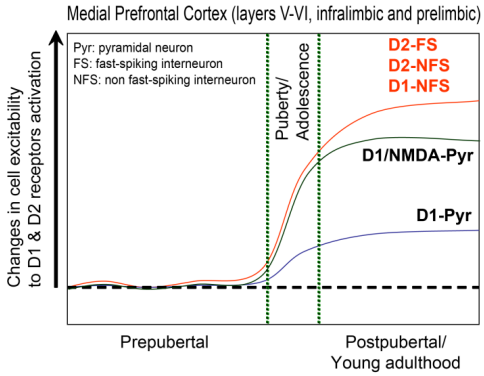


Figure 3: Summary of the response of pyramidal cells and GABAergic interneurons in response to dopamine receptor stimulation throughout development. The response to dopamine in different cell types in the prefrontal cortex is low throughout development and increases during the adolescent transition to adulthood. Psychostimulant use during adolescence could possibly interact with the development of cell excitability in response to dopamine receptor stimulation (modified from Tseng, 2007a)

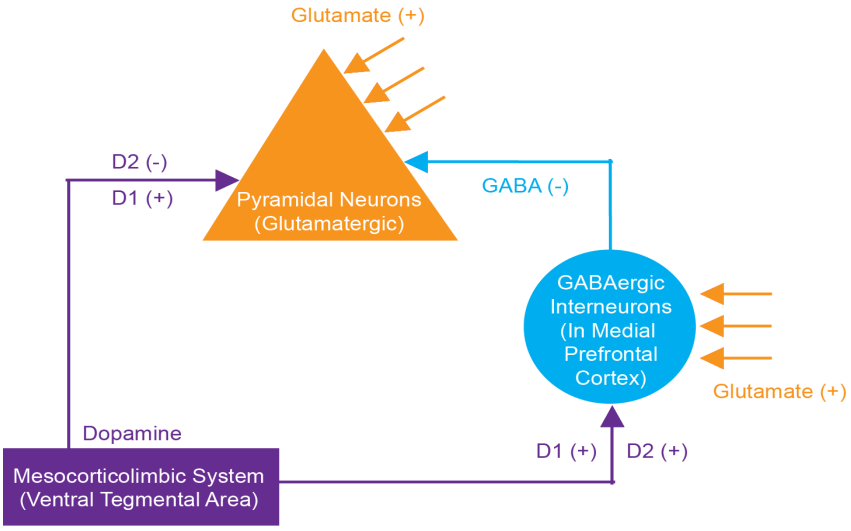


Figure 4: The effects of dopamine on pyramidal cells and GABAergic interneurons in the prefrontal cortex. Dopamine produced in the ventral tegmental area excites pyramidal cells by binding to D1 type receptors, and exerts inhibitory effects by binding to D2 receptors. Dopamine excites GABAergic interneurons through D1 as well as D2 receptors. These cells in turn exert an inhibitory effect on pyramidal cells (modified from Tseng, 2007a).

Cocaine

Addiction

Cocaine is a psychomotorstimulant derived from the coca plant that produces a feeling of euphoria when ingested. Coca leaves have traditionally been chewed by South American natives for thousands of years (Das, 1993). The alkaloid was isolated in the mid 1800's and was then used in beverages and medicine in the U.S. until it was outlawed in 1914 (Das and Laddu, 1993). Cocaine use and addiction is a prominent problem in our society. A person is generally considered to be addicted by the Diagnostic and Statistical Manual of Mental Disorders of 1994 (DSM-IV) standards when they meet more than three of the following symptoms within a 12 month period: progressive increase in dose or time used; persistent desire for; or failure to reduce substance use; increasing efforts made to obtain substance; social, occupational, or recreational activity is replaced by activity associated with substance use; continued substance use despite recognized physical and psychological consequences; withdrawal symptoms; tolerance (increased dose needed to achieve the same effect, or reduced responses to the same dose.

According to the 2007 National Survey on Drug Abuse and Health conducted by the National Institute of Drug Abuse 35.9 million Americans over the age of 12 reportedly used cocaine at least once in the past year, and over 2.1 million were current users (had used cocaine in the past month). Cocaine addiction is associated with dysfunction of the frontal cortex since behaviors that correspond to dysfunction of this part of the brain, such as impulsivity, impaired decision making, and disinhibition, are often observed in chronic cocaine users (Bolla et al., 1999). Cocaine acts by increasing dopamine in the mesocorticolimbic system by blocking dopamine reuptake transporters. When dopamine is released into the synaptic cleft, dopamine binds to D1 and D2 dopamine receptors on postsynaptic cells. Dopamine, which is released into the synaptic cleft, but does not bind to these receptors is taken up into the presynaptic terminsl through dopamine transporters, a process which is referred to as dopamine reuptake (Figure 5). While the blockade of Dopamine reuptake appears to be the most important for cocaine's addictive properties, it is also able to block the reuptake of the other monoamine neurotransmitters norepinephrine and serotonin (Ritz et al., 1990). Blocking of these transporters leads to the increased presence of monoamines in the synaptic cleft which in turn increases transmission at the affected synapses.

When cocaine is administered, acute physical consequences include an increased heart rate, the narrowing of blood vessels, increased blood pressure, and an elevated body temperature (Gray, 1993). While these physical changes are not usually harmful to the individual at low doses, higher doses can lead to potentially fatal effects such as seizures, heart failure, stroke, and brain hemorrhages (Gray, 1993). Use of cocaine can also cause long term neurological changes. In response to repeated cocaine, the brain adapts and becomes less sensitive to natural reinforcers as well as the drug itself. Levels of D2 receptors have been shown to decrease in the brains of cocaine addicts. This becomes problematic since the brain cannot respond the same anymore to normal levels of dopamine due to a depletion of receptors, thereby diminishing the reducing the pleasurable effects of dopamine in response to normal stimuli (Asensio et al., 2010).

It is because of changes that occur in an addicted brain, that while many addicts attempt to undergo withdrawal from cocaine, few succeed. Relapse is the most important clinical problem in treating cocaine addiction (Gawin and Kleber, 1986). There is an increase in sensitization to cocaine related cues which increases throughout the first weeks of withdrawal, which is referred to as the incubation of cocaine craving (Bossert et al., 2005). This leads to increased cocaine cravings during this time which often

result in relapse (Grimm et al., 2001). Levels of certain neurotransmitter receptors and their subunits have been shown to change after withdrawal from cocaine exposure (Conrad et al., 2008; Kalivas, 2008). Neurons in the prefrontal cortex are less excitable after withdrawal from cocaine, indicating there are long lasting neuroadaptations accompanying withdrawal (Nogueira et al., 2006). Despite these findings there is still little known about how withdrawal affects the overall state of the mesocorticolimbic system. Part of my thesis will examine whether there are long term changes in the frontal cortex of animals undergoing exposure to and withdrawal from cocaine.

Cocaine and adolescence

Make the case for the prefrontal cortex and cocaine and adolescence

Adolescents make up a group that is the most vulnerable to drug addiction, specifically cocaine (Crews et al., 2007), and substance abuse in adolescence is a reliable predictor of substance abuse during adulthood (Merline et al., 2004). Adolescents are more likely to experiment with drugs than other age groups (SAMHSA, 1999), possibly due to the fact that they show higher levels of risk taking and novelty seeking and lower levels of harm avoidance which is likely due to a heightened sense for reward during adolescence (Laviola et al., 2003). Consequently substance abuse is likely to arise at this time point in life, and a disproportionate number of adolescents become addicted compared to any other age group. Specifically, individuals who use cocaine during adolescence (12-14) have a four fold higher chance of developing an addiction than individuals who try the drug during young adulthood (21-25) (O'Brien and Anthony, 2005). Moreover it is more difficult to treat individuals who became addicted during adolescence than during (young) adulthood.

Withdrawal

Since drug seeking depends on the motivational circuits in the frontal cortex, and these circuits undergo change in adolescence, this could be a reason why adolescents are at such a higher risk for drug seeking behavior and the development of a drug addiction. Exposure to psychostimulants such as cocaine could disrupt changes taking place in the frontal cortex, leading to long term changes that may persists into adulthood (Andersen, 2003). Rats that receive binge like exposure to cocaine during adolescence displayed increased responsiveness to the stimulant effects of cocaine, and showed difficulty performing attention requiring tasks. These animals also showed altered expression of genes encoding transcription factors in the prefrontal cortex (Black et al., 2006).

Various addiction paradigms, provide evidence indicating that the propensity for addiction to develop in adolescent is higher than adults. Using a measure of drug seeking called conditioned place preference where animals' preference for (or aversion to) a drug associated environment is compared to that of a non drug environment, Brenhouse and Andersen found that adolescent rats exhibited place preference at a lower dose than adult animals, and adolescent animals also took longer to extinguish cocaine place preference compared to adults. Moreover, adolescent animals were also more vulnerable to drug primed reinstatement of cocaine place preference (Brenhouse et al., 2008).

Current gaps in knowledge

While we know that more adolescents become addicted to cocaine than any other age group, it is still largely unclear

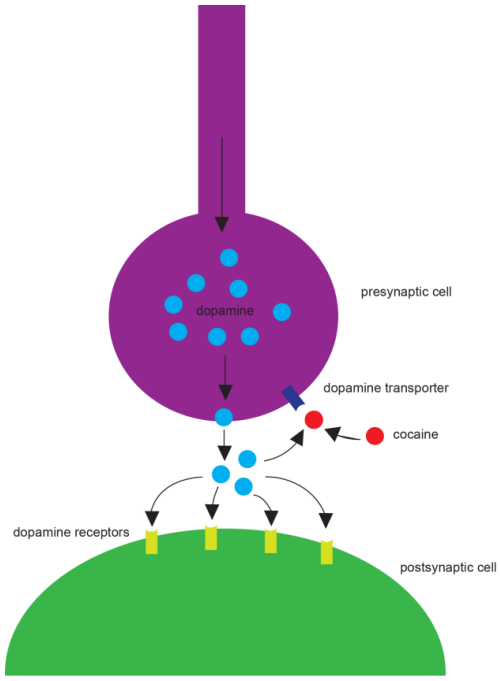


Figure 5: synapse depicting cocaine's mechanism of action. Dopamine (blue) is released by the presynaptic cell (purple) into the synaptic cleft where it binds to dopamine receptors (lime) on the postsynaptic cell (green). Dopamine that does not bind to the receptors is taken back up into the presynaptic cell through dopamine transporters (dark blue) in a process called reuptake. Cocaine (red) blocks the reuptake of dopamine, thereby increase the amount of dopamine in the synaptic cleft.

what mechanisms account for this difference. We also do not know how cocaine affects the adolescent mesocorticolimbic system differently than that of adults. Exposure to cocaine could possibly interfere with developmental processes still taking place in the adolescent brain. Interference with these processes could potentially lead to changes persisting far into adulthood. In this study we examined activity in different regions in the frontal cortex in adolescent and adult rats following cocaine exposure and withdrawal. We used Sprague-Dawley rats which is the most commonly used strain for anatomical, physiological and behavioral studies aimed at understanding the neural bases of brain function. Thus the data collected in this study can be compared with findings already reported.

Hypothesis and Aims

The present study is aimed at investigating how cocaine exposure and withdrawal impacts the overall state of the frontal cortex, and whether the postnatal day (PD) at which the drug exposure is given plays an important role. We hypothesize that a distinctive pattern of cocaine induced cortical neuroadaptation is expected to emerge when cocaine exposure and withdrawal occur during adolescence compared to adulthood. In order to test this hypothesis we performed the following aims:

Aim 1: To determine how repeated cocaine exposure and withdrawal affects the state of the frontal cortex.

Aim 2: To determine how repeated whether the age at which the drug is given plays a role in determining the state of the frontal plays a role in repeated cocaine exposure and withdrawal.

Methods

All experimental procedures were carried out according to the USPHS Guide for Care and Use of Laboratory Animals and were approved by the Rosalind Franklin Institutional Animal Care and Use Committee.

Animals
Male Sprague Dawley rats were obtained from Harlan. Animals were housed in groups of 2-4 and with food and water pellets available ad libitum. The animal room was maintained at a constant temperature and humidity on a 12 hour light dark cycle with the light cycle taking place during the day and the dark cycle at night.

Cocaine injection protocol
Adolescent and adult animals were randomly assigned to receive either saline or cocaine injections. Rats received one injection per day of cocaine (15-25 mg/kg i.p.) or saline for 5 days. Animals then underwent a 3 or 3 week withdrawal time before being sacrificed.

Tissue Processing
Rats were deeply anesthetized using 8% chloral hydrate (in 0.9% NaCl saline solution). Animals were killed by decapitation, and brains were removed and fixed in 4% paraformaldehyde (4% paraformaldehyde in 0.1M phosphate buffer) for 2 hours and stored in 30% sucrose for 48 hours. Brains were then cut into 50µm coronal sections using a freezing microtome (SM-2000R; Leica Microsystems, Wetzlar, Germany) and stored in 0.1M phosphate buffered saline (PBS) until further processing.
Cytochrome oxidase histochemistry
Fifty µm coronal sections between levels bregma 2.7 and 1.2 were mounted on double gelled slides, using one section every 150µm and allowed to dry for 30 minutes. Slides were then incubated in a 38°C bath with a filtered solution containing 0.1M PB (phosphate buffer) 0.33 g/L horse heart cytochrome C (Sigma), 0.2 g/L catalase from bovine liver (Sigma), 44g/L sucrose (Sigma), and 3,3'-diaminobenzidine (DAB; Sigma) for 90 minutes. Following the 90 minute incubation slides were washed twice for 10 minutes in cold 0.1M PBS before being dehydrated 2 times for 3 minutes in each of the following solutions; 95% ethanol, 100% ethanol, and xylene. Slides were then coverslipped with permount and dried under the fume hood.

Densitometry measurements
Relative optical density (ROD) measures for different cortical structures were obtained using a slide scanner (Nikon coolscan V ED) and scanning software (Nikon Scan 4.0). Structures analyzed included the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), cingulate cortex (CG), motor cortex (MC), and somatosensory cortex (SSC). ROD values were obtained by subtracting density values of the desired structure from the density value of the anterior commisure (white matter) and dividing them by the optical density of the slide background (always 255). Mean values from each animal were averaged to attain a ROD value per structure for each group of animals. The ROD values of each experimental group were normalized to the ROD values of the accompanying saline group.

Statistical analysis
We analyzed our data to determine whether our results were statistically significant using the appropriate software (Statistica 6). We determined whether there was an interaction between the experimental variables. If this was the case we performed a Tukey post hoc test to determine the level of significance.

Results

Experimental set up
Forty four adolescent male rats (PD35) were included in the present study. 10 saline and 12 cocaine treated animals were sacrificed after 3 days withdrawal (PD43) and 10 saline and 12 cocaine treated animals were sacrificed after 3 weeks withdrawal (PD61). The adult (PD75) group is composed of 10 saline and 25 cocaine treated rats. 5 saline and 12 cocaine treated animals were sacrificed after a 3 day withdrawal period (PD83) and 5 saline and 12 cocaine treated animals were sacrificed after a 3 week withdrawal period (PD101). Figure 6A shows the injection timeline, and figure 6B shows a table summarizing the number of animals used per group.

Optimization of cytochrome oxidase histochemistry
We first examined the effects of repeated cocaine administration and withdrawal on cortical metabolic activity as measured by cytochrome oxidase (COI) histochemistry in adolescent and adult rats as described by Hevner et al. in 1995 and Wong-Riley in 1989. We measured activity in 5 frontal cortical areas, the medial prefrontal cortex, orbitofrontal cortex, cingulate cortex, motor cortex, and somatosensory cortex. Activity was measured in coronal sections between stereotaxic planes 3.0 to 1.2 mm from bregma (Figure 7). Before we could start experiments we needed to optimize COI histochemistry and determine the appropriate incubation time. All sections were incubated for 90 minutes in order to obtain the optimal COI signal. This incubation time was chosen because it shows the least amount of variability in COI signal (Figure 8).

Changes in activity across cortical structures
We first compared COI activity in the cocaine exposed animals after 3 days versus 3 weeks withdrawal by determining the average COI value of all cortical structures combined for each rostro-caudal level. In the adolescent exposed animals, COI staining at level 2.7 revealed an increase in metabolic activity after 3 weeks compared to 3 days withdrawal from cocaine (Figure 9A). Our results did not indicate changes in activity at level 1.7 and 1.2 (Figure 9 A). In the adult group, COI staining revealed different results to that observed in adolescent animals. In adult animals cocaine exposure following a 3 week withdrawal period elicited a decrease in activity compared to the 3 day withdrawal period (Figure 9 B). This was not level specific as our analyses indicated there was no interaction between withdrawal time and rostro-caudal level. There is however a withdrawal effect which can be generalized to all 3 levels.

COI staining in the medial prefrontal cortex and orbitofrontal cortex
The first structure we analyzed was the medial prefrontal cortex. In the adolescent group, COI staining did not reveal any changes in activity at 3 days withdrawal. At 3 weeks withdrawal, activity in the cocaine treated animals was higher compared to the saline treated group (Figure 10 A). In the cocaine exposed animals COI staining also indicated an increase activity at 3 weeks compared to 3 days withdrawal (Figure 10 A). In contrast, no changes in activity were observed in the medial prefrontal cortex of the adult animals (Figure 10 B).
In the orbitofrontal cortex of the adolescent group, COI staining revealed a relative increase in activity in the cocaine treated animals at 3 weeks withdrawal compared to three days withdrawal (Figure 10 C). In the adult group we measured a different effect. In this case there is a relative

decrease in activity in the cocaine treated rats after 3 weeks withdrawal compared to three days withdrawal (Figure 10 D).

Activity in the cingulate, motor, and somatosensory cortices at level 2.7

The remaining three cortical structures, the cingulate , motor, and somatosensory cortices were measured at all 3 rostro-caudal levels. In the cingulate cortex of adolescent rats, COI staining revealed an increase in activity in the cocaine exposed animals at 3 weeks withdrawal compared to 3 days withdrawal (Figure 11 A). In the cingulate cortex of the adults exposed to cocaine, we observed a decrease in activity after 3 weeks withdrawal compared to 3 days (Figure 11 B).

In the motor cortex our results also indicated an increase in activity after 3 weeks compared to 3 days withdrawal from cocaine in the adolescent group (Figure 11 C). We observed different results in the adults. COI staining indicated a decrease in activity after 3 weeks compared to 3 days withdrawal from cocaine as well as a decrease in activity in the cocaine exposed animals compared to the saline animals at 3 weeks (Figure 11 D).

In the somatosensory cortex of adolescents COI staining revealed an increase in activity after 3 weeks compared to 3 days withdrawal from repeated cocaine (Figure 11 E). We did not find any changes in activity in the somatosensoty cortex of the adult group (Figure 11 F).

Activity in the cingulate, motor, and somatosensory cortices at levels 1.7 and 1.2

The increases and decreases in COI activity we observed in the cocaine treated adolescent and adult animals were localized to the most anterior rostro-caudal level, 2.7. At level 1.7 no changes in activity were observed after either of the withdrawal times in neither adolescent nor adult rats (Figure 12 A-F).

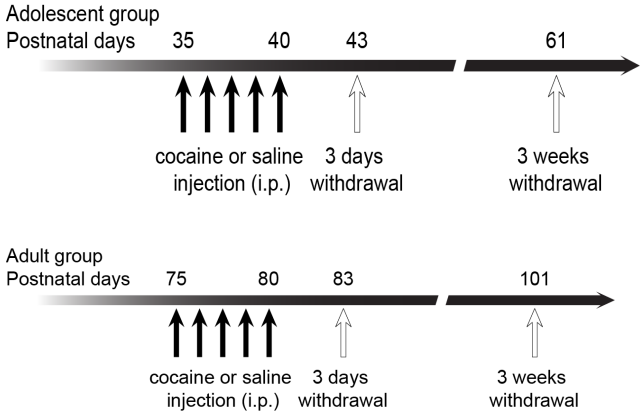
At level 1.2, the most posterior level at which we measured COI activity, we did not see any changes in the cingulate (Figure 13 A and B) or somatosensory cortices (Figure 13 E and F). COI staining did not indicate any changes in activity in the motor cortex of the adolescent group (Figure 13 C). However it did reveal a relative change in activity in the adult group. Activity in the motor cortex of rats exposed to cocaine undergoing a 3 week withdrawal time was significantly lower than that observed after 3 days withdrawal from cocaine (Figure 13 D).

Summary of COI activity: comparing activity in adolescent versus adult animals

For our last set of analyses we compared activity in adolescent versus adult animals in order to determine whether changes in activity in rats exposed to cocaine follow the same trajectories in adolescents compared to adults. Figure 14 A through C shows relative changes in activity in adolescent compared to adult rats after 3 days withdrawal. At level 2.7 COI staining indicated an increase in activity in adult compared to adolescent rats in the orbitofrontal and cingulate cortices (Figure 14 A). No changes in activity were found after 3 days withdrawal at level 1.7 or 1.2 (Figure 14 B and C).

After 3 weeks withdrawal from cocaine at level 2.7 COI staining revealed an opposite effect from the 3 day withdrawal period. In all cortical structures COI staining indicated an increase in activity in the adolescent group compared to a decrease in the adult group (Figure 14 D). At level 1.7 COI staining did not indicate any changes in activity in adolescent compared to adult rats at 3 weeks withdrawal (Figure 14 E). Our results did not reveal any changes in activity at level 1.2 after 3 weeks withdrawal, except for in the motor cortex. COI staining in the motor cortex showed an increase in activity in the adolescent animals compared to a decrease in the adults (Figure 14 F).

A.



B.

number of animals per treatment		adolescent	adult
3 day withdrawal	saline	10	5
	cocaine	12	12
3 week withdrawal	saline	10	5
	cocaine	12	12

Figure 6: Injection timeline and table. Adolescent and adult rats underwent 5 days of repeated cocaine exposure and were then terminated after a 3 day or 3 week withdrawal period (A). Each group contains saline treated and cocaine treated rats, this table (B) indicates the number of animals in each group.

Discussion

Adolescents are more prone to drug addiction than any other age group (Crews et al., 2007). We do not know however what the underlying neural mechanisms are that mediate this increase in susceptibility. So far limited research has been performed comparing the outcome of cocaine addiction in adolescents versus adults, and the results of this research are ambiguous. This may be due to the fact that in experiments studying addiction in rodents, the age of the animals is often not taken into consideration (Spear, 2000).

The goal of my thesis was to compare metabolic activity using COI staining in several regions of the frontal cortex, an area of the brain which is a part of the mesocorticolimbic, or brain reward system, and is therefore implicated in addiction. We compared activity in adolescent and adults rats and within these age groups we also compared activity at different withdrawal times from repeated cocaine exposure.

We hypothesized that a distinctive pattern of cocaine induced cortical neuroadaptation will emerge when cocaine exposure and withdrawal occur during adolescence compared to adulthood. After analyzing cortical activity in adolescents and adults at three days and three weeks withdrawal we came to 2 major findings that are worth further discussion: 1) cocaine exposure and withdrawal elicited a pattern of cortical activation across all structures in the adolescent rats which follows a rostrocaudal gradient and takes longer than three days to develop; 2) The age at which cocaine exposure takes place determines the pattern of activity. In the adult group we found a relative decrease in activity compared to adolescent animals after 3 weeks withdrawal. Moreover, the pattern of inactivation in the adults

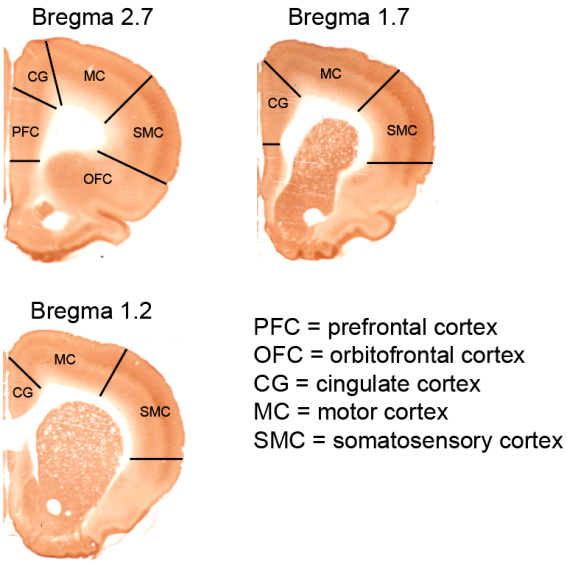


Figure 7: Cortical regions and rostrocaudal levels at which COI activity was measured. Metabolic activity was measured in 5 different cortical regions, and at three different rostrocaudal levels from Bregma, levels 2.7, 1.7, and 1.2

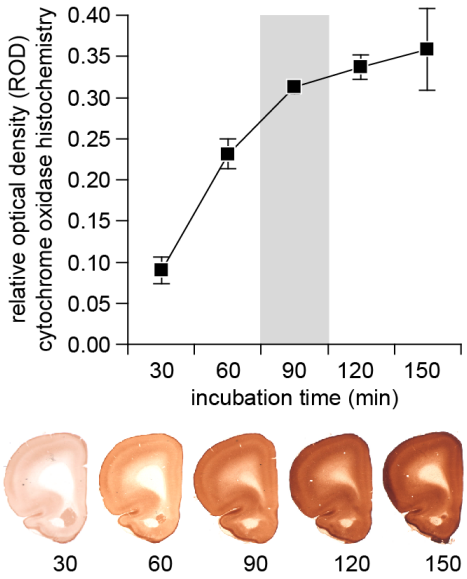


Figure 8: Incubation timecourse: We tried several COI incubation times and determined that 90 minutes was the optimal incubation time. Variability in relative optical density is the lowest at this incubation time.

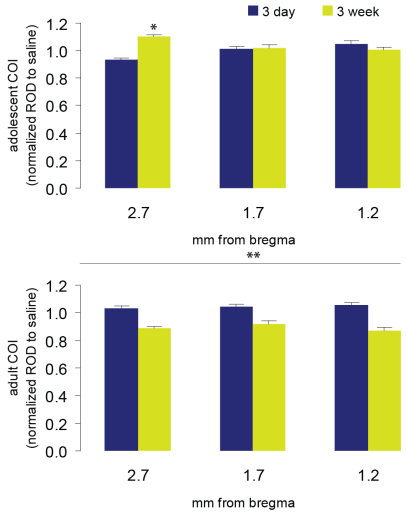


Figure 9: COI activity at 3 different rostrocaudal levels. (A) In the adolescent group of animals exposed to cocaine COI revealed a withdrawal x rostro-caudal interaction ($p < 0.05$) as well as a withdrawal effect ($p < 0.05$ Tukey post hoc test after significant ANOVA) at level 2.7. No effects were observed at levels 1.7 or 1.2. (B) In the adult group we did not find a withdrawal x rostro-caudal interaction. However there is significant withdrawal effect ($p < 0.005$).

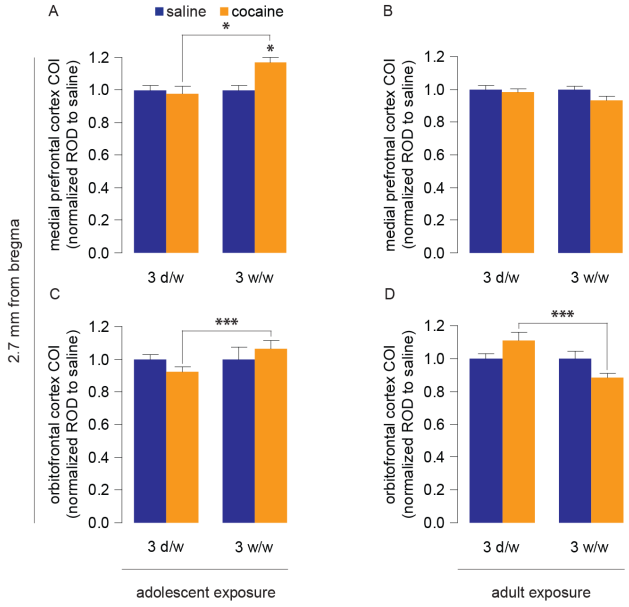


Figure 10: COI activity in the mPFC and OFC. (A) In the mPFC of the adolescent animals COI staining revealed a treatment x withdrawal interaction ($p < 0.05$). There was a treatment effect between the saline and cocaine treated animals at 3 weeks withdrawal ($p < 0.05$, Tukey post hoc test after significant ANOVA) as well as a withdrawal effect between the cocaine treated animals at 3 days and 3 weeks withdrawal ($p < 0.05$). (B) No effects were observed in the mPFC of adult animals. (C) In the OFC of the adolescent exposed animals COI staining revealed a treatment x withdrawal interaction ($p < 0.005$) as well as a withdrawal effect between the cocaine treated animals at 3 days and 3 weeks withdrawal ($p < 0.0005$, Tukey post hoc test after significant ANOVA). (D) COI staining revealed a treatment x withdrawal interaction ($p < 0.05$) in the OFC of adult animals as well as a withdrawal effect between the cocaine treated animals at 3 days and 3 weeks withdrawal ($p < 0.0005$, Tukey post hoc test after significant ANOVA).

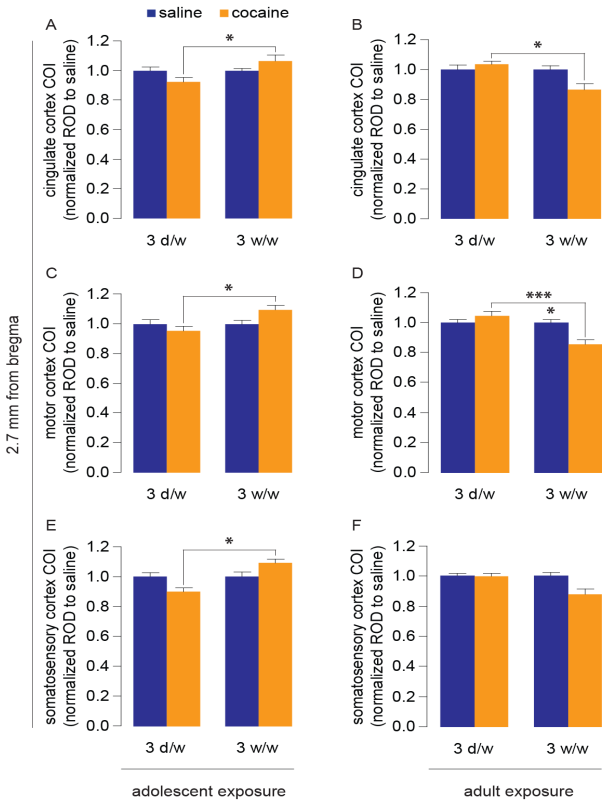


Figure 11: COI activity in the CG, MC, and SSC at level 2.7. (A) COI staining revealed a treatment x withdrawal interaction in the CG of adolescent animals ($p < 0.05$). There was a withdrawal effect between the cocaine treated rats at the three day and three week withdrawal time points ($p < 0.05$ Tukey post hoc test after significant ANOVA). (B) In the CG of adult animals COI staining indicated a treatment x withdrawal interaction ($p < 0.05$) as well as a withdrawal effect between the 3 day and 3 week withdrawal time points for the cocaine treated group ($p < 0.05$ Tukey post hoc test after significant ANOVA). (C) In the MC of adolescent animals COI indicated a treatment x withdrawal interaction ($p < 0.05$) as well as a withdrawal effect amongst the cocaine exposed animals ($p < 0.05$ Tukey post hoc test after significant ANOVA). (D) COI staining in the MC of adult animals revealed a treatment x withdrawal interaction ($p < 0.05$). It also revealed a treatment effect ($p < 0.05$ Tukey post hoc test after significant ANOVA) between the saline and cocaine treated animals at 3 weeks withdrawal as well as a withdrawal effects amongst the cocaine exposed animals ($p < 0.0005$ Tukey post hoc test after significant ANOVA) (E) In the SSC of adolescent rats COI staining indicated a treatment x withdrawal interaction ($p < 0.005$) as well as a withdrawal effect amongst the cocaine treated rats between three days and three weeks withdrawal ($p < 0.0005$ Tukey post hoc test after significant ANOVA). (F) No effects were observed in the SSC of adult rats.

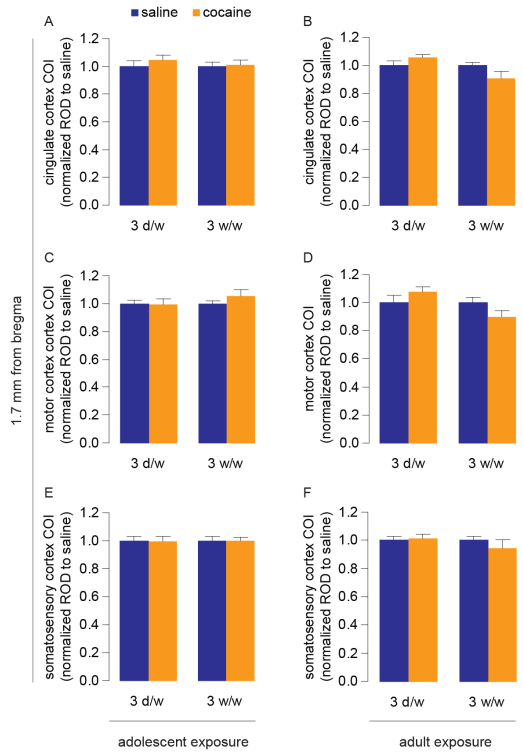


Figure 12: COI activity in the CG, MC, and SSC at level 1.7. (A-F) No changes in activity were observed in the CG, MC, and SSC of adolescent or adult rats.

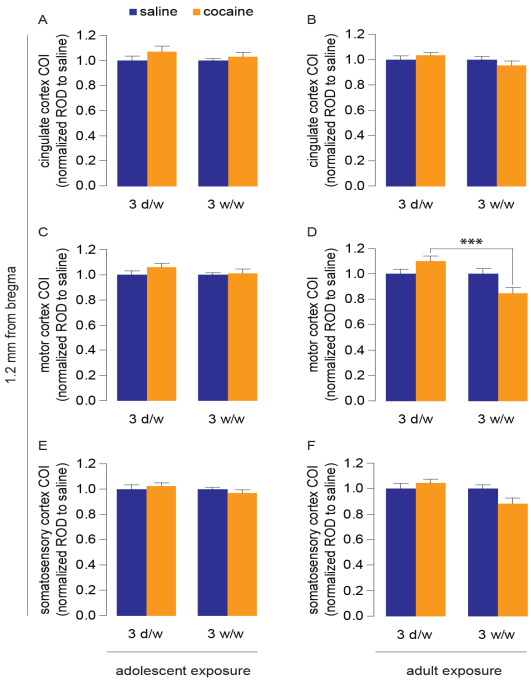


Figure 13: COI activity in the CG, MC, and SSC at level 1.2. (A and B) No changes in COI activity were observed in the CG of adolescent or adult rats. (C) COI staining did not reveal any changes in activity in the MC of the adolescent group. (D) In the MC of adult animals COI staining revealed a treatment x withdrawal interaction ($p < 0.05$) as well as a withdrawal effect between the animals exposed to cocaine undergoing a 3 day and 3 week withdrawal period ($p < 0.05$ Tukey post hoc test after significant ANOVA). (E and F) There were no changes in activity in the SSC of adolescent or adult rats.

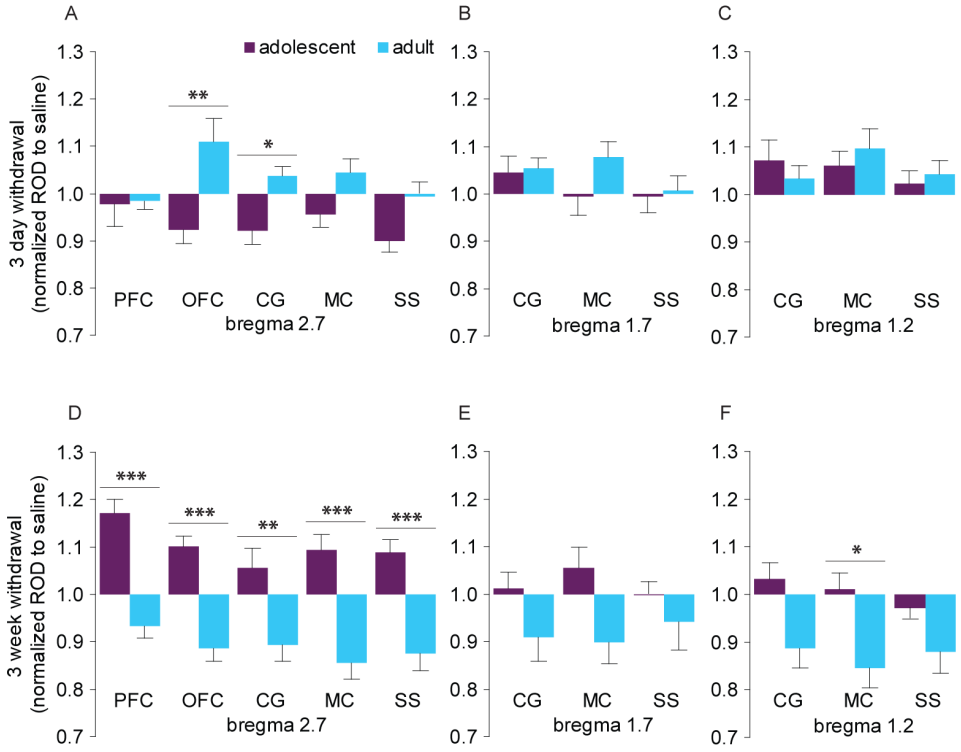


Figure 14: Summary comparing COI activity in adolescent and adult animals at 3 days and 3 weeks withdrawal from cocaine. (A) After a 3 day withdrawal period from cocaine COI staining revealed age x treatment interaction ($p < 0.05$) as well as an age dependent effect in the OFC ($p < 0.005$ Tukey post hoc test after significant ANOVA) as well as the CG ($p < 0.05$ Tukey post hoc test after significant ANOVA) at level 2.7. (B and C) No changes in activity were between adolescent and adult animals were identified at levels 1.7 or 1.2 after a 3 day withdrawal period. (D) After a 3 week withdrawal period from cocaine COI staining revealed a age x treatment interaction ($p < 0.0005$) as well as an age dependent change in activity in the PFC ($p < 0.0005$ Tukey post hoc test after significant ANOVA). It also revealed an age x treatment interaction in the OFC ($p< 0.005$) as well as an age effect ($p < 0.0005$ Tukey post hoc test after significant ANOVA). In the CG COI also indicated an age x treatment interaction ($p < 0.05$) as well as an age dependent effect ($p < 0.005$ Tukey post hoc test after significant ANOVA). In the MC COI staining also revealed an age x treatment interaction ($p < 0.05$) and an age effect ($p < 0.0005$ Tukey post hoc test after significant ANOVA). Lastly COI in the SSC also showed an age x treatment interaction ($p < 0.005$) and an age dependent effect ($p < 0.0005$ Tukey post hoc test after significant ANOVA). (E) COI staining did not reveal any age dependent changes in activity at level 1.7 after a 3 week withdrawal period. (F) At level 1.2 COI staining indicated and an age x treatment interaction after a 3 week withdrawal period from cocaine in the MC ($p < 0.05$) as well as an dependent change in activity ($p < 0.05$ Tukey post hoc test after significant ANOVA).

did not follow a rostro-caudal pattern, and was not restricted to the most frontal part of the brain.

Possible mechanism mediating the increased cortical activation in adolescent animals

One of the possibilities is that cocaine administration during adolescence interferes with the development of neurons in the frontal cortex. The two major cell types in the frontal cortex are pyramidal cells and interneurons. GABAergic inhibitory interneurons make up approximately 20% of the total cell population in the frontal cortex (Markram et al., 2004). These neurons are a part of the mesocortical system as they respond to and inhibit dopamine producing cells pyramidal cells in the frontal cortex (Tseng and O'Donnell 2004, 2007b; Tseng et al., 2006b). Thus, they are important for the refinement of brain functions, for example they are necessary for the proper tuning of activity of pyramidal cells (Kawaguchi, 2001; Tseng et al., 2006b).

Mesocortical dopamine can stimulate these interneurons and enhance GABAergic neurotransmission to pyramidal cells of the prefrontal cortex. This effect is only observed during adulthood as GABAergic interneurons are not functionally present in the prepubertal brain (Tseng and O'Donnell, 2007d). In the juvenile brain, D1 receptor stimulation increases cell excitability, while D2 receptor stimulation does not have a major effect on cell excitability until adulthood (Tseng and O'Donnell, 2007b). Tseng and O'Donnell showed in 2007 that the enhancement of the GABAergic tone in adult animals is due to D2 receptor dependent increase of inhibitory interneuron excitability. This effect was only observed in animals 45 days and older. The direct pathway may initiate the attenuation of excitatory inputs to pyramidal cells, while the inhibition of synaptic transmission could be maintained through the indirect inhibitory interneuron involving pathway (Figure 15 A). It is important that these changes occur during the period of adolescence. Levels of markers of GABA transmission such as the calcium binding protein parvalbumin (PV) which is used by interneurons change during adolescence in rodents (Cass et al, 2010). Similarly, the amount of PV containing terminals shows a rapid rise during adolescence before being pruned to adult levels in primates (Lewis et al., 2005). If cocaine interfered with the development of interneurons during adolescence, it could explain the increase in activity

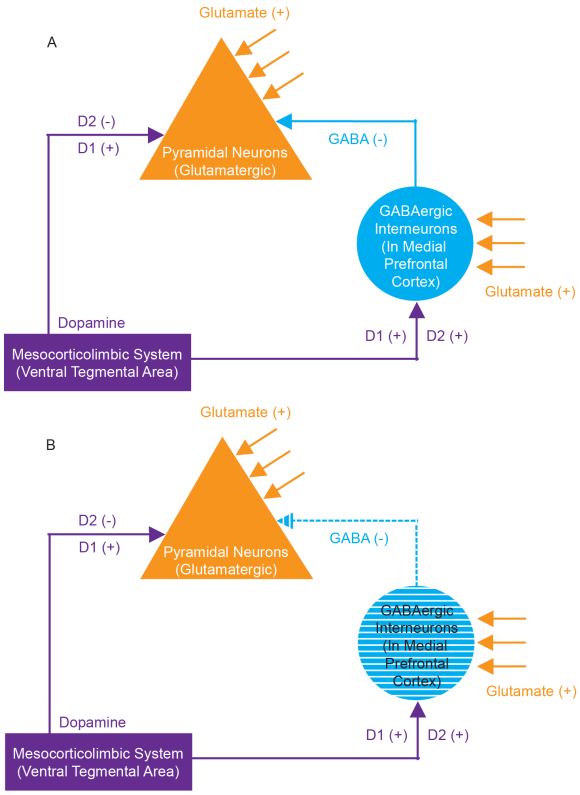


Figure 15: Proposed mechanism of action (A) Pyramidal cells in the mPFC receive innervation from the VTA and GABAergic interneurons after normal development. (B) Model after cocaine administration during adolescence where the functional development of GABAergic interneurons is prevented.

observed in the frontal cortex of these animals since there would be a lack in inhibitory GABAergic tone normally provided by interneurons (Figure 15 B).

This mechanism may be involved in the mediated increase in activity we observed after cocaine exposure because in a developmental rodent model of schizophrenia, the neonatal ventral hippocampal lesion, PV interneuron function becomes lower in the prefrontal cortex compared to control animals (Tseng et al., 2006a). Schizophrenia is a disorder which is also associated with dysfunction of the prefrontal cortex and emerges during adolescence. A similar mechanism may be mediating the improper maturation of interneurons as a consequence to cocaine administration. Moreover, PV levels are reduced in the prefrontal cortex of adolescent animals in response to repeated amphetamine exposure (Morshedi and Meredith, 2007). This is of relevance since amphetamine is a psychostimulant that impacts the mesocorticolimbic system in a similar manner as cocaine.

In the adolescent exposed animals, we also observed that the pattern of cortical activation follows a rostro-caudal gradient. This pattern of activation is similar to the pattern of dopamine innervation observed in the frontal cortex which is strongest at the most rostral part. Therefore, we speculate that the increase in activity at the most frontal part of the brain is due to the fact that dopamine innervation in this part of the brain is higher than the more caudal regions of the frontal cortex.

Possible mechanism underlying cortical inactivation in the adult animals

In the adult rats our results indicated a pattern of cortical inactivation after 3 weeks withdrawal from cocaine exposure when compared to the adolescent animals. In the adolescent animals we proposed that repeated cocaine exposure prevents the development of GABAergic interneurons in the frontal cortex, and that the inhibitory signal to the pyramidal cells in the frontal cortex does not develop. However, the interneurons in question complete development by adulthood and are responsive to D2 receptor mediated activation (Tseng and O'Donnell, 2007d). A possible explanation for the cortical inactivation observed in the adult rats is that the inhibitory GABAergic tone produced by interneurons is enhanced after cocaine exposure. Because cocaine enhances the dopaminergic signal produced by mesocortical neurons, dopamine could enhance the excitatory effects of D1 and D2 receptor stimulation on prefrontal interneurons and therefore decrease pyramidal cell output.

On the other hand, the pattern of cortical inactivation observed in adult animals after 3 weeks withdrawal from repeated cocaine exposure does not follow a rostro-caudal gradient as the pattern of activation does in the adolescent animals. We therefore suspect that a different mechanism of cortical adaptation may be occurring when cocaine is given during adulthood. In addition to the mesocortical dopamine system, the pattern of cortical inactivation may be due to effects of other neurotransmitters in the frontal cortex such as serotonin and norepinephrine, which are also altered by cocaine (Yano and Steiner, 2007).

The patterns of cortical neuroadaptation take longer than 3 days to develop

In both the adolescent and adult rats the observed patterns of cortical neuroadaptation take longer than 3 days to develop, as the changes in activity are only observed after 3 weeks withdrawal from repeated cocaine exposure. In the adolescent group of animals this suggests that repeated cocaine exposure during adolescence can produce neurological changes that persist into adulthood.

In adult animals there is sufficient evidence that the prefrontal cortex plays a role in reinforcement learning and acquisition of drug self administration as well as the frequently observed relapse after a period of withdrawal (Tzschentke, 2000; Van den Oever et al., 2009). Changes in specific synapse proteins in the prefrontal cortex persist up to 100 days after withdrawal from repeated cocaine exposure in rats (Lull et al., 2009). Also, attenuation of the dopamine signal in the prefrontal cortex lowers the propensity for relapse after a period of withdrawal in a rodent model of addiction (Sun and Rebec, 2005). Lesions of the prefrontal cortex in rodents, which inactivate it, facilitate the acquisition of cocaine self administration (Schenk at al., 1991). Inactivation of the ventral region of the prefrontal cortex is associated with increased reinstatement of drug seeking after withdrawal (Peters et al., 2008). Therefore, a decrease in activity in the frontal cortex as we observe in the adult animals may play a role in mediating the relapse to cocaine after a period of withdrawal.

There are very few studies which examined withdrawal in adolescents. In a rodent model of addiction and withdrawal adolescent were self administered more cocaine during the addiction stage, and displayed higher levels of cocaine seeking in the withdrawal stage. In this study adolescent rats displayed higher responses to cocaine during the reinstatement phase after a period of withdrawal whereas adult rats showed heightened responses to cocaine associated cues during this phase (Anker and Carroll, 2010). Also, Anxiety increases in adolescent rats as withdrawal time from cocaine increases (Santucci and Rosario). These studies do not specifically link the prefrontal cortex to withdrawal in adolescence, but since changes in activity take place in this region after a period of withdrawal we suspect that it might play a role in mediating the heightened drug seeking during withdrawal in adolescents like it does in adults.

Future directions

Now knowing that cocaine exposure elicits a different pattern of cortical activation in adolescent and adult animals, we can work towards further elucidating the mechanisms mediating the increase in activity in adolescents. We hypothesize that the functional presence or absence of GABAergic interneurons in the frontal cortex may in part be mediating the increase in activity observed in the frontal cortex of adolescent animals. In order to test this we can: 1) perform immunohistochemical parvalbumin staining to assess the impact of cocaine on prefrontal interneuron function. Parvalbumin is one of the calcium binding proteins interneurons use. This protein is necessary for their function. An increase or decrease in parvalbumin immunoreactivity will indicate an increase or decrease in interneuron function respectively; 2) perform electrophysiological recordings in the frontal cortex and determine the patterns of activity in pyramidal cells as well as interneurons after cocaine exposure.

Conclusion

The factors that contribute the prevalence of addiction in adolescents remain unsolved, but our findings specifically implicate changes in the frontal cortex in response to cocaine as at least a partial reason for this. Together, these results suggest that a different form of neuroadaptation occurs in the adolescent brain after cocaine exposure, in particular at the rostral frontal cortex, a cortical region that is heavily influenced by the mesocortical dopamine system. If we are able to identify the underlying mechanisms mediating this increase in activity, these could be possible targets for attenuating the elevated incidence of addiction in adolescents.

Acknowledgements

I would first like to thank Dr. Kuei Yuan Tseng for giving me the opportunity to work in his research lab for the past two years, and make this project my own. I would also like to thank my senior thesis advisor Dr. Shubhik DebBurman for helping me make my thesis a reality. I am not only thankful for the support, advice, and encouragement they have each provided for me throughout this project, but also for their efforts in starting up the RFUMS-LFC internship program. Thanks to their efforts this program has benefited me greatly, as I hope it will for many future students to come. I would also like to thank the other members of my senior thesis committee Dr. Ann B. Maine and Dr. Benjamin Goluboff for their time and commitment. I would also like to acknowledge my former and current lab members Shannon Blume, Natalie Simak '11, Gregorio Galinanes, and Li-Jun Heng for all of their help as well as their friendship which has made time in the lab more than enjoyable. I would like to recognize Kendall McKinven '10, Alison Schneider '10, David Ehrenreich '10, Lindsay Jauch '07, and Derek Atchley '10 for their unconditional friendship and support (as well as comedic relief from Derek especially) which has been invaluable to me. I would also like to acknowledge my fellow senior thesis students Michael Fiske '10, Jaime Perez '10, and Paige Keasler '10 as well as Emily Shanahan '09, Alexandra Ayala '09, Quincy Roberts '09, and Martina Kolb '10. Lastly, a huge thanks to my parents Ray and Holly Cass as well as Holly Jauch for all of their love and support throughout the years, even when I "have way too much on my plate".

References

Andersen, S.L. (2003). Trajectories of brain development: point of vulnerability or window of opportunity? Neuroscience Biobehavior Reviews, 27, 3-18.

Anker, J.J., Carroll, M.E. (2010). Reinstatement of cocaine seeking induced by drugs, cues, and stress in adolescent and adult rats. Psychopharmacology, 208(2), 211-222.

Asensio, S., Romero, M.J., Romero, F.J., Wong, C., Alia-Klein, N., Tomasi, D., Wang. G.J., Telang, F., Volkow, N.D., Goldstein, R.Z. (2010). Striatal dopamine D2 receptor availability predicts the thalamic and medial prefrontal responses to reward in cocaine abusers 3 years later. Synapse, 64, 397-402.

Baldwin, A.E., Sadeghian, K., Kelley, A.E. (2002). Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. Journal of Neuroscience, 22, 1063-1071.

Batisto-Brito, R., Fishell, G. (2009). The developmental integration of cortical interneurons into a functional network. Current topics in developmental biology,

97, 82-104.

Black, Y.D., Maclaren, F.R., Naydenov, A.V., Carlezon Jr, W.A., Baxter, M.G., Konradi, C. (2006). Altered attention and prefrontal cortex gene expression in rats after binge-like exposure to cocaine during adolescence. Journal of Neuroscience, 26(38), 9656-9665.

Blakemore, S.J. (2008). The social brain during adolescence. Nature Reviews Neuroscience, 9, 267-277.

Bolla, K.I., Rothman, R., Cadet, J.L. (1999). Dose-related neurobehavioral effects of chronic cocaine use. Journal of Neuropsychiatry and clinical neuroscience, 11, 361-369.

Bossert, J.M., Ghitza, U.E., Epstein, D.H., Shaham, Y. (2005). Neurobiology of relapse to heroin and cocaine seeking: An update and clinical implications. European Journal of Pharmacology, 526, 36-50.

Brenhouse, H.C., Andersen, S.L. (2008). Delayed extinction and stronger reinstatement of cocaine conditioned place preference in adolescent rats, compared to adults. Behavioral Neuroscience, 122, 460-465.

Brenhouse, H.C., Sonntag, K.C., Andersen, S.L. (2008). Transient D1 dopamine receptor expression on prefrontal cortex projection neurons: relationship to enhanced motivational salience of drug cues in adolescence. Journal of Neuroscience, 28(10), 2375-2382.Carlezon, W.A. Jr., Thomas, M.J. (2009). Behavioral substrates of reward and aversion. Neuropharmacology, 56(1), 122-132

Casey, B.J., Giedd, J.N., Thomas, K.M. (2000). Structural and functional brain development and its relation to cognitive development. Biological Psychiatry, 54(1-3), 241-257.

Cass D.K., Simak N., Blume S.R., Tseng K.Y. Repeated cocaine exposure and withdrawal elicits opposite patterns of cortical metabolic activation in adolescent vs. adult rats. (In submission, The Journal of Neuroscience)

Chambers, A., Taylor, J.R., Potenza, M.N. (2003). Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. American Journal of Psycchiatry, 160, 1041-1052.

Conrad, K.L., Tseng, K.Y., Uejima, J.L., Reimers, J.M., Heng, L., Shaham, Y., Marinelli, M., Wolf, M.E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature

Crews, F., He, J., Hodge, C. (2007). Adolescent cortical development: A critical period of vulnerability for addiction. Journal of Pharmacology, Biochemistry, and Behavior, 86, 189-199.

Das, G. (1993). Cocaine abuse in North America: a milestone in history. Journal of Clinical Pharmacology, 33, 296-310.

Das, G., Laddu, A. (1993). Cocaine: friend or foe? (part 1). Int Journal of Clinical Pharmacology Ther Toxicol, 31, 499-555.

Ernst, M., Romeo, R.D., Andersen, S.L. (2009). Neurobiology of the development of motivated behaviors in adolescence: a window into a neural systems model.
Journal of Pharmacology, Biochemistry, and Behavior, 93, 199-211

Gabbott, P.L.A., Wanrer, T.A., Jays, P.R.L, Salway, P., Busby, S. (2005). Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. The Journal of Comparative Neurology, 492, 125-177.

Gabbott, P.L.A., Dickie, B.G.M, Vaid, R.R., Headlam, A.J.N., Bacon, S.J. (1997). Local circuit neurones in the medial prefrontal cortex (areas 25, 32, and 24b) in the rat:
morphology and quantitative distribution. The Journal of Comparative Neurology, 377, 465-499.

Gawin, F.H., Kleber H.D. (1986). Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. Archives of General Psychiatry, 43, 107-113.

Giedd, J.N., Blumenthal, J., Jeffries, N.O., Castellanos, F.X., Liu, H., Zijdenbos, A., Paus., T., Evans., A.C., Rapoport, J.L. (1999). Brain development during adolescence: a longitudinal MRI study. Nature Neuroscience, 2(10), 861-863.

Gray, J.D. (1993). Medical consequences of cocaine. Canadian Family Physician, 39, 1975-1976, 1979-1981

Grimm, J.W., Hope, B.T., Wise, R.A., Shaham, Y. (2001). Incubation of cocaine craving after withdrawal. Nature, 412, 141.

Gulledge, A.T., Jaffe, D.B. (1998). Dopamine decreases the excitability of layer V pyramidal cells in the rat prefrontal cortex. Journal of Neuroscience, 18, 9139-9151.

Hevner, R.F., Liu, S., Wong-Riley, M.T.T. (1995). A metabolic map of cytochrome
oxidase in the rat brain: histochemical, densitometric, and biochemical studies. Neuroscience, 65(2), 313-342.

Huttenlocher, P.R. (1979) Synaptic density in human frontal cortex – developmental
changes and effects of aging. Brain Research, 163(2), 195-205.

Kalivas. P.W. (2008). Addiction as a pathology in prefrontal cortical regulation of corticostriatal habit circuitry. Neurotoxicity Research, 14(2-3), 185-189.

Kalsbeek, A., Voorn, P., Buijs, R.M., Pool, C.W., Uylings, H.B. (1988). Development of
the dopaminergic innervation in the prefrontal cortex of the rat. Journal of Comparative Neurology, 269, 58-72.

Kawaguchi, Y. (2001). Distinct firing patterns of neuronal subtypes in cortical synchronized activities. Journal of Neuroscience, 21(18), 7261-7272.

Laviola, G., Macri, S., Morley-Fletcher, S., Adriani, W. (2003). Risk taking behavior in adolelescent mice: psychobiological determinants and early epigenetic influence. Neuroscience and behavioral reviews, 27, 19-32.

Leslie, F.M., Loughlin, S.E., Wang, R., Perez, L., Loftipour, S., Belluzzi, J.D. (2004). Adolescent development of forebrain stimulant responsiveness. Insights from animal studies. Annals of the New York Academy of Sciences, 1021, 148-159

Lewis, D.A. (1997). Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. Neuropsychopharmacology, 16:6, 385-398.

Lewis, D.A., Hashimoto, T., Volk, D.W. (2005). Cortical inhibitory neurons and schizophrenia. Nature Reviews Neuroscience, 6(4), 312-324.

Lull, M., Erwin, M.S., Morgan., D., Roberts, D.C.S., Vrana, K.E., Freeman, W.M. (2009). Persistent proteomic alterations in the medial prefrontal cortex with abstinence from cocaine self administration. Proteomics Clinical Applications, 3(4), 462-472.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., Wu, C. (2004). Interneurons of the neocortical inhibitory system. Nature Reviews Neuroscience, 5, 793-807.

Merline, A.C., O'Malley, P.M., Schulenberg, J.E., Bachman, J.G., Johnston, L.D. (2004). Substance use among adults 35 years of age: prevalence, adulthood predictors, and impact of adolescent substance use. Journal of Pharmacology, Biochemistry, and Behavior, 94, 96-102.

Moghaddam, B., Homayoun., H. (2008). Divergent plasticity of prefrontal cortex networks. Neuropsychopharmacology Reviews, 33, 42-55.

Morshedi, M.M., Meredith, G.E. (2007). Differential effects of amphetamine on prefrontal parvalbumin interneurons. Neuroscience, 149, 617-624.

National Institute of Drug Abuse (2007). National Survey on Drug Abuse and Health

Nogueira, L., Kalivas, P.W., Lavin, A. (2006). Long term neuroadaptations produced by withdrawal from cocaine treatment: role of dopaminergic receptors in modulating cortical excitability. Journal of Neuroscience, 26(47), 12308-12313.

O'Brien, M.S., & Anthony J.C. (2005). Risk of becoming cocaine dependent: Epidemiological estimates for the United States, 2000-2001. Neuropsychopharmacology, 30, 1006-1018.

O'Donnell P., Tseng K.Y. (2009) Postnatal maturation of dopamine actions in the prefrontal cortex, "Dopamine Handbook", Section 4, Chapter 4.3, pages 177-186; Editors: Anders Björklund, Stephen B. Dunnett, Leslie L. Iversen, and Susan D. Iversen, Oxford University Press.

Paus, T., Zijdenbos, A., Worsley, K., Collins, D.L., Blumenthal, J., Giedd, J.N., Rapoport, J.L., Evans, A.C. (1999). Structural maturation of neural pathways in children and adolescents: in vivo study. Science, 283(5409), 1908-1911.

Paus, T. (2005). Mapping brain maturation and cognitive development during adolescence. Trends in Cognitive Sciences, 9(2), 60-68.

Paus, T., Keshavan, M., Giedd, J.N. (2008). Why do so many psychiatric disorders emerge during adolescence? Nature Reviews Neuroscience, 9, 947-956.

Paz, R.D., Tardito, S., Atzori, M., Tseng, K.Y. (2008). Glutamatergic dysfunction in schizophrenia: from basic neuroscience to clinical psychopharmacology

Peters, J., LaLumiere, R.T., Kalivas, P.W. (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. Journal of Neuroscience, 28, 6046-6053.
Ratiu, P., Talos, I.F., Haker, S., Lieberman, D., Everett, P. (2004). Tale of Phineas Gage,
digitally remastered. Journal of Neurotrauma, 21(5), 637-643.

Ritz, M.C., Cone, E.J., Kuhar, M.J. (1990). Cocaine inhibition of ligand binding at dopamine, norepinephrine, and serotonin transporters: a structure-activity study. Life Science, 46(9), 635-645.

Rebec, G.V., Sun, W. (2005). Neuronal substrates of relapse to cocaine seeking behavior: role of the prefrontal cortex. Journal of the Experimental Analysis of Behavior, 84, 653-666.

Rosenberg, D.R., Lewis, D.A. (1995). Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: a tyrosine hydroxylase immunohistochemical analysis. Journal of Computational Neuroscience, 358(3), 383-400.

SAMSHA (1999). Summary of findings from the 1998 national household survey on drug abuse.

Santucci, A.C., Rosario, A. (2010). Anxiety like responses in adolescent rats following a 10-11 day withdrawal period from repeated cocaine administration. Brain Research Bulletin, 81(4-5), 441-444.

Schenk, S., Horger, B.A., Peltier, R., Shelton, K. (1991). Supersensitivity to the reinforcing effects of cocaine following 6-hydroxydopamine lesions to the medial prefrontal cortex in rats. Brain Research, 543(2), 227-235.

Seamans, J.K., Durstewitz, D., Christie, B.R., Stevens, C.F., Sejnowski, T.J. (2001). Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. Proclamations of the National Academy of Science, 98(1), 301-306.

Seamans, J.K., Lapish, C.C., Durstewitz, D. (2008). Comparing the prefrontal cortex of rats and primates: insights from electrophysiology. Neurotoxicity Research, 14(2,3), 249-262.

Spear, L.P. (2000). The adolescent brain and age related behavioral manifestations. Neuroscience and behavioral reviews, 24, 417-463.

Sun, W., Rebec, G.V. (2005). The role of prefrontal cortex D1 and D2 like receptors in cocaine seeking behavior in rats. Psychopharmaology, 177:3, 315-323.

Tarazi, F.I., Baldessarini, R.J. (2000). Comparative postnatal development of dopamine
D(1), D(2), and D(4) receptors in the rat forebrain. International Journal of Developmental Neuroscience, 18(1), 29-37.

Trantham-Davidson, H., Neely, A.C., Lavin, A., Seamans, J.K. (2004). Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in the prefrontal cortex. Journal of Neuroscience, 24(47), 10652-10659.

Tseng, K.Y. (2009). The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Journal of Behavioral Brain Research, 391(2), 295-305.

Tseng K.Y. (2007a) Acquiring the excitatory and the inhibitory action of dopamine in the prefrontal cortex during postnatal development. "Monoaminergic modulation of cortical excitability", Section II, Chapter 11, pages 175-188; Edited by Tseng KY and Atzori M, Springer, NY, USA.

Tseng, K.Y., O'Donnell, P. (2007b). D2 dopamine receptors recruit a GABA component for their attenuation of excitatory synaptic transmission in the adult rat prefrontal cortex. Synapse, 61, 843-850.

Tseng, K.Y., Lewis, B.L., Lipska, B.K., O'Donnell, P. (2007c). Post-pubertal distruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. Biological Psychiatry, 62, 730-738.

Tseng, K.Y., O'Donnell, P. (2007d). Dopamine modulation of prefrontal cortical interneurons changes during adolescence. Cerebral Cortex, 17, 1235-1240.

Tseng, K.Y., Armin, F., Lewis, B.L., & O'Donnell, P. (2006a). Altered prefrontal cortical metabolic response to mesocortical activation in adult animals with a neonatal ventral hippocampal lesion. Biological Psychiatry, 60, 585-590.

Tseng, K.Y., Mallet, N., Toreson, K., Le Moine, C., Gonon, F., O'Donnell, P. (2006b). Excitatory response of prefrontal cortical fast-spiking interneurons to ventral tegmental area stimulation in vivo. Synapse, 59, 412-417.

Tseng, K.Y., O'Donnell, P., (2005). Post-pubertal emergence of prefrontal cortical up states induced by D1-NMDA co-activation. Cerebral Cortex, 15, 49-57.

Tseng, K.Y., O'Donnell, P. (2004). Dopamine-glutamate interactions controlling prefrontal cortical cell excitability involve multiple signaling mechanisms. The Journal of Neuroscience, 24(22), 5131-5139.

Tzschentke, T.M., Schmidt, W.J. (2000). Differential effects of discrete subarea specific lesions of the rat medial prefrontal cortex on amphetamine and cocaine induced behavioral sensitization. Cerebral Cortex, 10(5), 488-98.

Ulhaas, P.J., Roux, F., Singer, W., Haenschel, C., Sireteanu, R., Rodriguez, E. (2009). The development of neural synchrony reflects late maturation and restructuring of functional networks in humans. Proceedings of National Academy of Sciences Neuroscience, 106(24), 9866-9871.

Van den Oever, M.C., Spijker, S., Smit, A.B., De Vries, T.J. (2009). Prefrontal cortical plasticity mechanisms in drug seeking and relapse. Neuroscience and Behavioral Reviews, 11, 1-9.

Wong-Riley, M.T.T. (1989). Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends in Neuroscience, 12(3), 94-101.

Yano, M., Steiner, H. (2007). Methylphenidate and cocaine, the same effects on gene regulation? Trends in Pharmacological Science, 28(11), 588-596.