

Amphetamine and the Adolescent Brain – Consequences for the Prefrontal Cortex

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Illicit substance use during adolescence has dramatic and significant consequences for lifetime addiction vulnerability. Adolescent exposure to drugs of abuse is the single largest predictor of substance abuse problems in adulthood.¹ Alarming, the 2012 National Survey on Drug Use and Health indicates that this is a growing problem, as they report that the percentage of adolescents using illicit substances is increasing every year.² Recent studies have demonstrated that the earlier drug use is initiated in adolescence, the greater the risk for lifetime drug abuse, indicating that drugs may interfere with developmental processes which continue until adulthood.^{1,3,4}

Due to the ongoing development of the brain during this time, adolescence marks a period of enhanced vulnerability to the development of psychiatric disorders. This is thought to be due in part to the profound influence environmental factors have on the adolescent brain. In particular, the prefrontal cortex (PFC) is one of the last brain regions to mature in the human brain, and plays a crucial role in higher-level cognitive processing.⁵ Addicts of illicit substances have been shown to have both significant cognitive deficits, as well as altered prefrontal cortical function.⁶ The PFC receives dense innervation from midbrain dopamine neurons, and dopamine in this area is essential for normal cognitive function.^{7,8} Dopamine innervation to the prefrontal cortex is known to develop throughout adolescence in both rodents and primates, and this protracted development may render the PFC dopamine circuitry particularly vulnerable to drug-induced alterations.^{9,10}

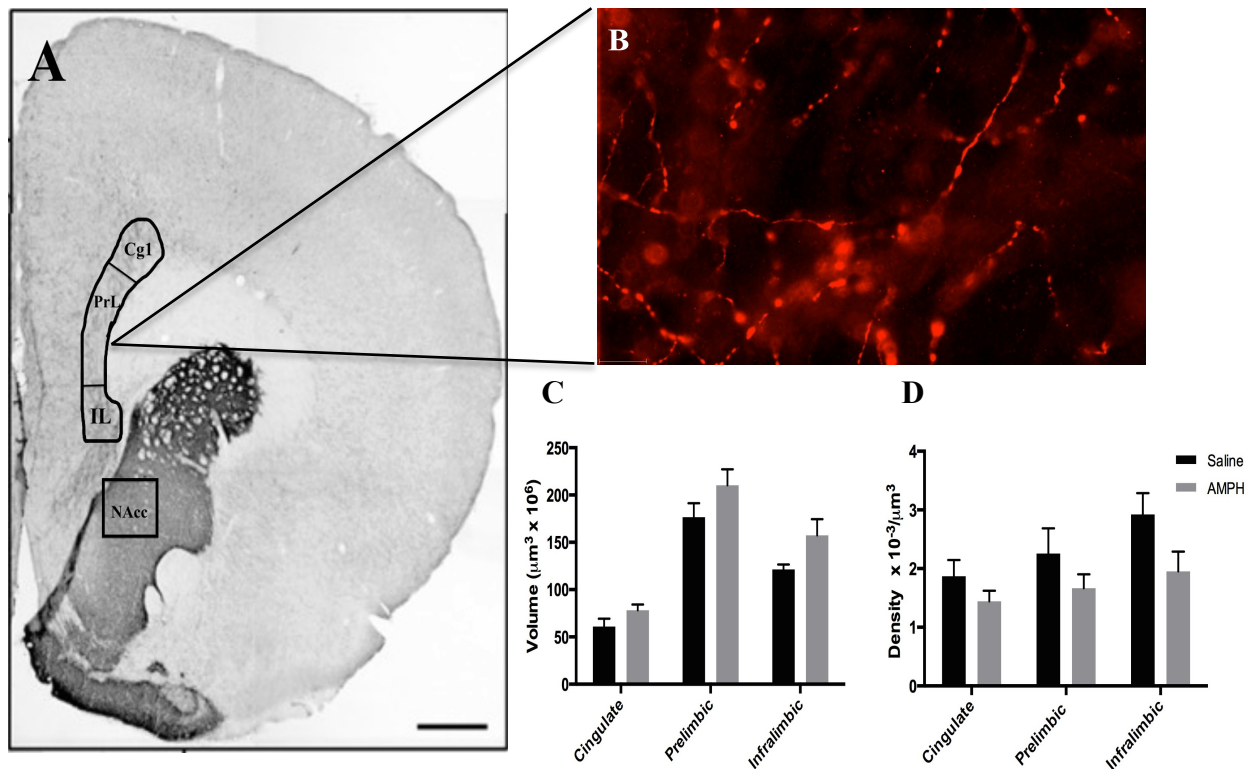
To elucidate the consequences of adolescent drug exposure on the development of dopamine innervation to the prefrontal cortex, I used unbiased stereology, a specific method of brain tissue analysis using a Leica DM400B microscope, where I was blind to the experimental conditions of each specimen analyzed. Ultimately, my goal was to assess whether repeated exposure to amphetamine in adolescent, but not adult, mice would alter the organization of dopamine connectivity in adulthood. Early adolescent (post-natal day 21±1) and adult (post-natal day 75±15) male C57/BL6 mice received amphetamine or saline injections intraperitoneally over five sessions. Dopaminergic innervation was assessed using an antibody for tyrosine hydroxylase (TH), an enzymatic marker of dopamine synthesis. Stereological analysis of TH staining, using the microscope software *Stereoinvestigator* (MicroBrightField), was assessed in three regions of the prefrontal cortex: the infralimbic (IL), prelimbic (PrL) and cingulate (Cg1) cortices (Fig. 1A). We were particularly interested in the IL cortex, as this area has been previously shown to receive the densest projections of dopaminergic neurons from the VTA and is implicated in drug use.^{11,12} In fact, the IL cortex is thought to be the rodent analog of the ventromedial PFC in humans, a region heavily involved in decision-making and inhibition of inappropriate responses.¹³

We found an increase in the volume of TH-positive fiber innervation in the prefrontal cortex in mice exposed to amphetamine during adolescence, in comparison to saline-treated controls (Fig. 1C). In addition, this volume change was not found in the nucleus accumbens (NAcc), found directly ventral to the IL cortex, suggesting the observed drug-induced alterations are specific to the prefrontal cortex. Intriguingly, this increase in volume is coupled with a decrease in TH-positive varicosity counts and density in the prefrontal cortex of mice exposed to amphetamine during adolescence (Fig. 1D). Both aforementioned effects of density and volume are absent in adult-treated mice.

The increase in volume and sparse dopaminergic innervation only in adolescent-treated mice suggests that amphetamine disrupts the development of these fibers, causing aberrant synaptic organization in the prefrontal cortex, which in turn may affect cognitive functioning.

It is important to note that adolescent pre-treated mice were allowed to reach adulthood before analysis. The fact that these alterations we observe in prefrontal neural circuitry persist into adulthood suggests that illicit drug exposure during adolescence can lead to enduring changes in the brain. This has significant implications as aberrant connectivity within the prefrontal cortex has been suggested to underlie the deficits in executive functioning seen in chronic drug users. One of the diagnostic hallmarks of addiction is persistent use of the drug, despite clear negative consequences, which has been reliably linked to hypoactivity within the prefrontal cortex.⁶ Our findings of significant alterations of dopaminergic circuitry within the prefrontal cortex confirm the importance of studying this higher cortical region in the context of addiction, and suggests that atypical neural connectivity may underlie the enhanced vulnerability to later substance abuse in individuals that are exposed to illicit drugs early in life. As altered dopaminergic neurotransmission in adolescence confers enhanced vulnerability to many psychiatric disorders, it is especially compelling to understand the means by which drugs may alter the development of this system. Current work in the Flores Laboratory is extending these findings to link them with known regulators of cortical and dopaminergic development. We hope to uncover the specific biological markers underlying these morphological brain changes we have identified following adolescent exposure to drugs of abuse, as well as their role in mediating abuse vulnerability. Such knowledge of underlying molecular mechanisms will help inform clinical practice, aid in the development of effective treatments, and emphasize the importance of establishing early intervention programs.

Figure 1.



- Micrograph of a coronal section through the wild-type mouse forebrain stained with TH peroxidase immunoreactivity, depicting the three regions of the medial prefrontal cortex (IL, PrL, Cg1) and the nucleus accumbens (NAcc). Scale Bar 500 μm .
- Micrograph of TH-positive immunofluorescence in the mPFC taken with a 100x objective. Scale Bar 10 μm .
- Volume of TH+ innervation was higher in adolescents that were treated with amphetamine. (Two-way ANOVA, main effects of region and treatment. No significant interaction effect. No significant differences found in adults [not shown]).
- Density of TH+ varicosity counts was lower in adolescents treated with amphetamine. (Two-way ANOVA, significant main effect of treatment. The same was not found in adults [not shown]).

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