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Research Report

Increased BOLD activation to predator stressor in subiculum and midbrain of amphetamine-sensitized maternal rats☆

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ABSTRACT

Amphetamine, which is known to cause sensitization, potentiates the hormonal and neurobiological signatures of stress and may also increase sensitivity to stress-inducing stimuli in limbic areas. Trimethylthiazoline (5 μL TMT) is a chemical constituent of fox feces that evokes innate fear and activates the neuronal and hormonal signatures of stress in rats. We used blood oxygen level dependent (BOLD) MRI to test whether amphetamine sensitization (1 mg/kg, i.p. ×3 days) in female rats has a lasting effect on the neural response to a stress-evoking stimulus, the scent of a predator, during the postpartum period. The subiculum and dopamine-enriched midbrain VTA/SN of amphetamine-sensitized but not control mothers showed a greater BOLD signal response to predator odor than a control putrid scent. The greater responsiveness of these two brain regions following stimulant sensitization might impact neural processing in response to stressors in the maternal brain.

1. Introduction

Although the rates of illicit drug use among females 12 and older has declined, they still remain high according to the 2008 National Survey on Drug Use and Health (SAMHSA, 2009). This is worrisome in light of that fact that drug use and abuse, especially of stimulant drugs, can exert long lasting effects on neurobiology and behavior that can lead to addiction during critical reproductive epochs such as lactation (Febo and Ferris, 2007; Grimm et al., 2003; Hyman et al., 2006). Our previous

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work shows that pre-pregnancy cocaine sensitization can have lasting effects on maternal behavior during the postpartum period and on maternal prefrontal cortical responses to suckling stimulation from pups (Febo and Ferris, 2007; Nephew and Febo, 2010). Addiction during early motherhood has been associated with problems with the ability to cope with stressors (Arevalo et al., 2008; Harmer et al., 1999). Amphetamine sensitization is associated with heightening of stress responses through cross-sensitization with hypothalamic–pituitary–adrenal (HPA) axis function (Antelman et al., 1980; Barr et al., 2002). Amphetamine-sensitized rats show alterations in glucocorticoid receptor levels and elevated plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) in response to a variety of widely used experimental stressors in rodents (Schmidt et al., 2001; Shilling et al., 1996). Even a single injection of amphetamine can increase CORT and ACTH responses to stressors for as long as 22 but not 46 days (Schmidt et al., 2001). Heightened sensitivity to stressful stimuli itself is a vulnerability factor for reinstatement of stimulant self-administration (Ahmed and Koob, 1997). Based on these data, it is possible that development of amphetamine sensitization alters the neural processing of stimuli that evoke stress and anxiety in the maternal brain. Modeling this phenomenon in rodents is especially difficult since one needs to model the day-to-day stressors for humans using day-to-day stressors that a maternal rodent might encounter in the wild. One such stressor in rodents is that of a predator. The chemical 2,4,5,-trimethylthiazoline (or TMT) is a synthetic analog of the chemical constituent of fox feces that is widely known to evoke unconditioned fear responses to rodents (Blanchard et al., 2003; Endres and Fendt, 2008; Morrow et al., 2000; Rosen et al., 2005; Staples et al., 2008; Wallace and Rosen, 2001). TMT emits a putrid smell that has been shown to elevate plasma CORT levels, autonomic activation, defensive postures, and prefrontal DA release in rats much like other stressors (Hamamura and Fibiger, 1993; Morrow et al., 2000; Staples et al., 2008). Therefore, TMT may be viewed as an ecologically valid model as a stressor that may also cause strong distress in rodents. Here, we test whether amphetamine sensitization before pregnancy alters how the maternal rat brain processes a stressful stimulus such as the scent of the predator.

2. Results

2.1. Amphetamine sensitization

An interaction between drug treatment (saline × amphetamine) and day of treatment was observed for horizontal activity ($F_{1,17}=14.8$, $p=0.001$), stereotyped activity ($F_{1,17}=19.4$, $p=0.0004$), and vertical activity ($F_{1,17}=11.6$, $p=0.003$). Administration of 1 mg/kg amphetamine on the 3rd day of injections resulted in a greater motor reactivity in comparison to first exposure (Fig. 1A). Females showed a greater horizontal, vertical, and repetitive infrared beam breaks ($p<0.01$, Bonferroni multiple comparisons test). Females showed evidence of sensitization after about 35 days of withdrawal, with saline controls showing a faster decline in the motor response to amphetamine challenge (Fig. 1B).

2.2. Light–dark box results

There was no effect of amphetamine pretreatment on time spent in the dark chamber of a light–dark box. Furthermore, no difference in baseline anxiety level was observed before pregnancy or the postpartum period.

2.3. BOLD signal response to TMT vs. SB

Postpartum days 2–4 rats were presented with SB and TMT during MR scanning. Fifty-eight ROI were segmented and analyzed. We first analyzed the effects of TMT vs. SB in 12 rats collapsed across saline ($n=5$) and amphetamine ($n=7$) groups (Fig. 2A–B). The composite maps show that TMT evokes BOLD activity across limbic prefrontal regions such as the medial-to-infralimbic prefrontal cortex, the anterior cingulate, the lateral orbital regions, and the insular cortex (Fig. 2A). TMT produced almost twice the magnitude BOLD signal response of SB in several areas (Fig. 2A time course below 2D maps). Significant differences were also observed in the number of activated voxels (Fig. 2B). Statistically significant differences in BOLD signal changes between SB and TMT were observed in the anterior cingulate cortex ($p=0.03$, two-tailed Mann–Whitney U test for independent samples), retrosplenial cortex ($p=0.001$), subiculum ($p=0.02$), somatosensory cortex ($p=0.02$), lateral amygdala ($p=0.02$), and motor cortex ($p=0.02$) (Fig. 2C). Fig. 2D shows additional ROI previously associated with the neural response to TMT or the expression of anxiety-related behavior that did not show significant differences between SB and TMT in the present study.

2.4. Effect of amphetamine sensitization on the BOLD signal response to TMT

We next analyzed the interaction between odor presentation (TMT vs. SB) and drug treatment (saline vs. amphetamine pretreatment) and observed that the subiculum and the midbrain showed a significant interaction between odor and drug (Kruskall–Wallis ANOVA, $p<0.05$). Amphetamine but not saline pretreated animals showed a greater BOLD activation in response to TMT than SB in the subiculum ($t_{11}=3.7$, $p=0.0032$, unpaired two-tailed t-test for homoscedastic variances) and midbrain ($t_{11}=3.8$, $p=0.0029$) (Fig. 3A–C).

2.5. Physiological alterations with TMT

No differences in baseline respiratory rates were observed between SB and TMT. However, TMT elicited a slight but significant increase in respiratory rate above baseline and above that observed for SB (Fig. 4). No significant differences were observed between saline and amphetamine treatment groups.

2.6. TMT-induced freezing in saline and amphetamine treated dams

The behavioral effects of the TMT and SB were confirmed post-imaging (Fig. 5), with greater levels of freezing induced by TMT ($p<0.000,1$ two-tailed Mann–Whitney U test for independent samples). However, no differences were observed between saline and amphetamine treatment groups.