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The Effects of SNAP-25 Deficits and Pre-Natal Nicotine in a Mouse and ADHD Model

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It has been recognized that the foundation of most, if not all, complex disease processes, particularly neuropsychiatric disorders, is due to the interactions between subtle genetic deficits and environmental insults. One of the most studied neuropsychiatric disorders shown to contribute from both heritable-related factors and environmental stressor sensitivity is Attention Deficit Hyperactivity Disorder (ADHD). Of the heritable issues involved, meta-analysis of genetic linkage studies has shown that the SNARE protein SNAP-25, is one of many possible proteins that may provide answers to the genetic component. In contrast to this genetic factor, studies have shown that prenatal nicotine exposure causes a direct effect to the fetus and predisposes them to nicotine and substance abuse behavior. Finally, preliminary studies have shown that heterozygote SNAP-25 deficient mice following i*n-utero* exposure to nicotine are, in fact, hyperactive compared to their wild type littermates. To study the relationship between SNAP-25 and prenatal nicotine exposure with locomotor activity, we compared wild type mice with and without nicotine exposure to SNAP-25 wild type mice with and without nicotine exposure. Additionally, this study also examined prenatal nicotine exposed SNAP-25 heterozygous mice with their wild type counterpart with intraperitoneal cocaine to see if the combination of factors increased the likelihood of substance abuse potential. The results demonstrated that prenatal nicotine exposed mice had greater locomoter activity and substance abuse behavior. Moreover the SNAP-25 heterozygous genotype compounded both of these affects and demonstrated greater results.

INTRODUCTION

It has been recognized that the foundation of most, if not all, complex disease processes, particularly neuropsychiatric disorders, is due to the interactions between subtle genetic deficits and environmental insults. ADHD is defined as a child exhibiting behaviors such as hyperactivity, inattentiveness, and compulsive-like attitude. ADHD is a common childhood disorder that has been shown to be influenced by both genetic and environmental factors, the latter of which is a current research interest. Many studies have shown an association exists between prenatal tobacco smoke exposure and the eventual diagnosis of ADHD. Of note, it is estimated that 11% of U.S. pregnant women smoke during their pregnancy (11). Furthermore, many clinical and epidemiological studies have shown that children exposed to nicotine in utero are at increased risk of developing ADHD, major depressive disorder (MDD), substance abuse, conduct disorder, and antisocial behavior (4,10,12,13,16). This has thought to result from nicotine interacting with neuronal nicotinic receptors consequently stimulating them and turning on genes that control cell replication, differentiation, growth, and death of involved limibic and frontal cortex cells.

Of the genetic factors, SNAP-25 is a synaptosomal-associated protein of 25kDa, located on chromosome 20p12, that is involved in the release of

presynaptic neurotransmitters. It encodes for a presynaptic membrane protein that is part of the SNARE complex (along with synaptobrevin and syntaxin) which is essential for synaptic neuroexocytosis. Several single nucleotide polymorphisms (SNPs) in this gene have been implicated to have an association with ADHD (8). Interestingly, Coloboma mice (which is a proposed animal model for ADHD because of their locomotive hyperactivity), are hemizygously lacking the chromosomal area for SNAP-25. Their locomotor hyperactivity decreases when provided either transgenic SNAP-25 or amphetamines (7,17).

Whether these syndromes are caused by nicotine exposure, genetic and/or psychosocial adversities associated with maternal smoking is not completely clear. Animal models suggest a direct impact of PNE. However, the fact that nicotine is forcefully administrated in these paradigms raises some questions about the specificity of these findings. The goal of this study is to further investigate the association of genetic and environmental factors on ADHD by looking at the genetic susceptibility of heterozygote SNAP-25 mice and the addictive behavior associated with an often-used drug, cocaine.

METHODS

Animals

All behavior tests were conducted on experimentally naive adult (60-100 days of age offspring of C57BI/6J dams prenatally exposed to saccharin (.066%) sweetened nicotine (0.05 mg/ml) or saccharine (.066%) only solutions (as shown in Figure 1). Additionally, mice were provided standard laboratory mouse chow along with fresh water at their will. Offspring were housed in a temperature-controlled vivarium with a 12 hr light/dark cycle. All animal procedures were approved by the Institutional Animal Care and Use Committee.

Locomotor activity measurement

Apparatus: Locomotor activity was tested using an automated system (San Diego Instruments, San Diego, CA) following the method described by Hess et al. 1996. Locomotion was defined as actual beam interruptions recorded during a 10 minute interval with a testing period of 180 minutes. Data from each of the 10 minute intervals was obtained and the sum from the 18 intervals comprised the accumulated locomotion score.

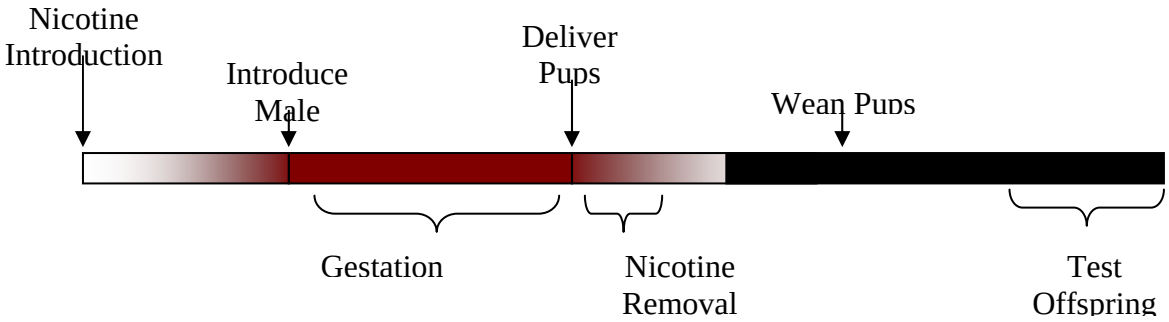
Conditioned place preference

Apparatus: Conditioned place preference boxes were constructed of Plexiglas with 2 distinctly different chambers (one black, one white) connected by an anteroom with a removable door. Assignment of cocaine paired side was alternated, such that an equal number of mice were assigned the white side for cocaine as were assigned to the black side for cocaine.

Training: Each mouse received either an i.p. dose of cocaine (4 mg/ kg, or saline) and confined to either the white or the black side of the box for 15 minutes. Mice were returned to their home cage for 4 hours and then given a second injection of either drug or saline which ever they did not receive in the first injection event, and then placed in the alternate chamber. Again, the mouse remained confined in the alternate side of the box for a 15 minute increment. Pairing of chamber side (black or white) with injection condition (cocaine or saline) was counterbalanced across groups (PNE and

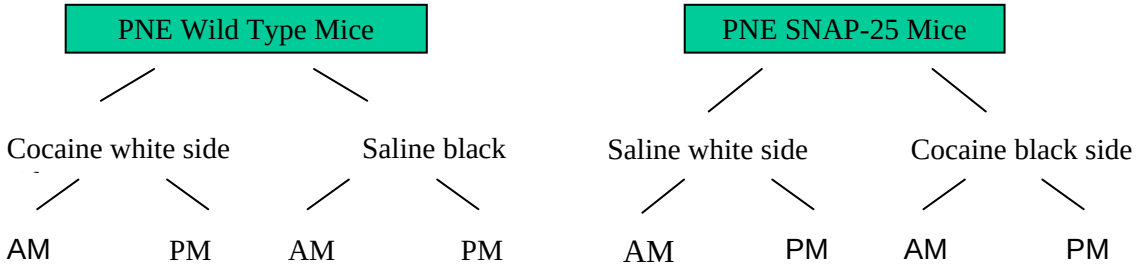
control) and alternated for time of day (am or pm) of which is outlined in Figure 2. This process of morning and afternoon injections was repeated for 3 consecutive days. Finally, on day 4 mice were placed in the anteroom with the door subsequently removed and thus giving them access to either side of the box. The time spent in each chamber was recorded for 10 minutes total with the mice having free access to either chamber via the anteroom. A total of 3-6 mice per group were tested for cocaine conditioned place preference. All conditioning and testing was done between 0900-1530 hr under dim illumination in a sound attenuated room.

Figure 1
Nicotine exposure paradigm



- Voluntary drinking paradigm, 2-bottle choice
- Nicotine 0.05 mg/ml in a 0.066% saccharin solution
- Average daily nicotine consumption 3.5 mg/kg/day, roughly equivalent to 10 cigarettes per day in a 60 kg human.

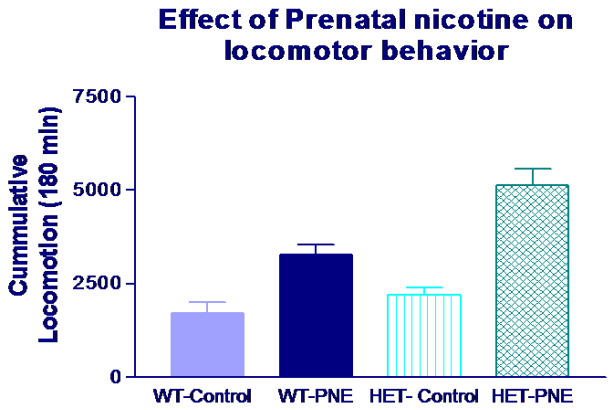
Figure 2
Experimental Design for cocaine placed preference



Mice were injected with either 4 mg/kg cocaine or saline and confined to the side of the box for 15 minutes twice a day for 3 consecutive days. On Day 4, mice are offered access to both sides of the box for 10 minutes and the time spent on the black and the white side are recorded.

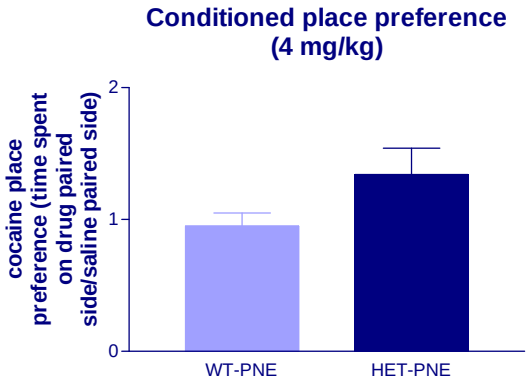
RESULTS

Figure 3
Locomotor Activity Measurement



The effect of prenatal treatment on spontaneous locomotion in male and female adult offspring. Wild type offspring (blue bars) were those from saccharin drinking mothers (light blue) and prenatal nicotine exposed (dark blue mice are presented in the solid bars. SNAP-25 HET mice are presented in the turquoise bars with the offspring from saccharin drinking mothers in the vertical striped bar and nicotine exposed offspring in the hatched bar. The data are from n = 4-6 mice per group and are mean (+/-SEM) cumulative locomotion in a 180 minute continuous test period.

Figure 4
Conditioned Placed Preference



Cocaine place preference in Wild type PNE mice (light blue bars) or SNAP-25 HET PNE mice (dark blue bars). Cocaine preference is given as time spent on the drug paired side divided by the time spent on the saline paired side of the box. Thus, scores of 1 indicate no preference while scores greater than one express preference for the drug. The data are from n = 3-6 mice per group.

DISCUSSION

In order to evaluate our hypothesis that diminished expression of Snap25, encoded from a single functional allele, may serve as a susceptibility locus acted upon by environmental factors, we performed an initial limited study exposing pregnant Snap25 heterozygous dams to consume nicotine through a two-bottle choice paradigm and assayed the locomotor activity and cocaine conditioned place preference of their progeny after reaching adulthood As expected, prenatal nicotine exposure led to increased activity compared to saccharine treated mice in wild type mice. Importantly, this increase was significantly exacerbated in HET mice ($p<0.01$), demonstrating that offspring of heterozygote dams that have consumed nicotine are more hyperactive. Further testing demonstrated that HET mice prenatally exposed to nicotine were also more susceptible to cocaine preference as seen in the CPP studies. These findings suggest that SNAP-25 mice may pose a genetic risk for environmental triggers, like nicotine, to induce behaviors similar to those seen in ADHD. Additional studies are needed to see if the increased hyperactivity and drug preference is accompanied by other behavioral deficits. These studies are needed to further validate this model of gene X environment interaction in ADHD. Since it's been discussed in previous studies that nicotine potentially plays a role in psychological disorders, especially in the prenatal period, it was our objective to determine whether a SNAP-25 allele deletion would act synergistically with PNE to increase the likelihood of addictive behavior. Our study shows that the mice who contained the SNAP-25 deletion, in addition to PNE, exhibited a greater degree of addictive behavior. This supports the idea that having a known environmental risk factor for developing ADHD (PNE) along with having a genetic risk factor (SNAP-25 HET), affects the severity of addictive behavior. This information was supported in the observation that the mice subjected to cocaine placed i.p. injections preferred to reside longer in the environment during the time of preference selection of which they were previously exposed to during their dosing periods.

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