Sex differences in cognitive development following adolescent amphetamine exposure

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Teenagers are vulnerable. Illicit drug use during adolescence significantly increases the risk of developing and struggling with addiction throughout life.[1,2,3] Addiction is a chronic, relapsing brain disease associated with deficits in cognitive functions mediated by the prefrontal cortex (PFC).[4] The PFC is amongst the last regions in the brain to fully mature – its development persists through adolescence and into early adulthood.[5,6]

Sex specific hormones play a critical role in shaping the neural circuitry underlying behavior.[7,8,9] Ongoing neurodevelopmental processes in adolescence coincide with puberty, the period of peak sexual maturation.[10] At the onset of puberty in female mice, ovarian hormones regulate circuit development in the frontal cortex.[11] Neuronal and synaptic development in the PFC progress at different rates in male and female rodents.[12,13] Similarly, adolescent human MRI studies report differences between sexes in the timing of both gray and white matter development.[5,14,15,16]

Drug use during adolescence might disturb PFC development and lead to cognitive impairments that increase vulnerability to addiction. Previous research from our lab suggests that exposure to amphetamine during development leads to neuroanatomical and behavioral changes in male mice.[17,18] Specifically, repeated high dose amphetamine exposure during early adolescence disrupts both the maturation of medial PFC circuitry and relevant cognitive functions.[17] At the preclinical level, there is a paucity of investigation on how drug use affects females. Given known sex differences in numerous neurodevelopmental processes during puberty, drugs likely affect the developing PFC differently between males and females. However, the role of sex in the effects exerted by adolescent drug exposure remains unreasonably ambiguous.

Here, we sought to identify sex differences in the long-term cognitive effects of repeated exposure to high doses of amphetamine during early adolescent development. We compared differences in behavioral inhibition, cognitive flexibility, and motivation between adult C57BL/6 male and female mice following repeated exposure to the equivalent of recreational amphetamine doses in early adolescence.

Beginning postnatal day 21, mice were injected with a sensitizing regimen of amphetamine (4 mg/kg) or volume-matched 0.9% NaCl saline control, every other day for a period of ten days (PND 22±1 - 31±1) (Figure 1a). Locomotor activity, defined as distance traveled in a given period of time, was measured for 90 minutes following each saline or amphetamine injection. We found that both male and female mice exhibit robust sensitization of drug-induced locomotor activity over the 90 minutes following treatment (Figure 1b). Amphetamine seemed to acutely affect both sexes similarly. Following this ten-day regimen, mice were left undisturbed for six weeks in order for adolescents to reach adulthood.

In adulthood, we administered a Go/No-Go Task adapted to mice in operant conditioning chambers.[19] For a reward (i.e., chocolate pellet), mice were required to nose poke in response to a light ('go' cue), and to withhold response when a tone ('no-go' cue) was simultaneously presented with the light. Erroneous responses during the 'no-go' trials were recorded as commission errors – a metric traditionally employed to assess behavioral inhibitory performance.[19] Previously, we have found that adult male mice exposed to amphetamine in early adolescence make more commission errors in a Go/No-Go task,[17] indicating a deficit in behavioral inhibition (Figure 1c). Here, our findings indicate that, in contrast to males, female mice repeatedly exposed to amphetamine during early adolescence make the *same* amount of commission errors as saline-treated controls (Figure 1c). Therefore, behavioral inhibition appears to remain intact in adult female mice exposed to amphetamine during early adolescence.

Motivation to receive rewards comprises the basis of behavioral task performance. To investigate whether sex differences in previous experiments could be explained by mouse motivational level, we administered a progressive ratio (PR) task to male and female mice.[20] Mice were trained to nose poke in exchange for a reward, whereby the number of nose pokes required to earn a reward increased exponentially after each reward trial. The breakpoint ratio of the PR task indicated the maximum number of actions (i.e., nose pokes) a mouse is willing to make in exchange for a single reward. We found that neither male nor female mice treated with amphetamine during adolescence showed differences in breakpoint ratio, compared to their saline-treated counterparts (Figure 1d). Therefore, motivation level did not appear to account for the differences observed in Go/No-Go performance.

Though both sexes demonstrate the same immediate effects of amphetamine during adolescence, and though both sexes show unaltered overall motivation level to acquire rewards, female mice appear to retain better the ability for behavioral inhibition in adulthood. Our findings show, for the first time, that early exposure to amphetamine affects cognitive development differently in females than in males. These results provide a preliminary insight toward how sex-specific developmental trajectories may be impacted dissimilarly by adolescent drug use. Follow-up studies in the lab are examining relevant neuroanatomy and cognitive function.

Traits like impulsivity, self-control, and the inability to inhibit certain behaviors are associated with vulnerability to addiction and other psychopathologies.[21,22] Research in humans show that women have both higher rates of mental illness, and progress from initial drug use to addiction faster than men.[23,24,25] Altogether, our findings demonstrate a role for sex in disruptions to developing cognition. Investigating the sex-specific nature of adolescent vulnerability to addiction will be critical to guide the development of substance abuse prevention and intervention strategies appropriate for both men and women.

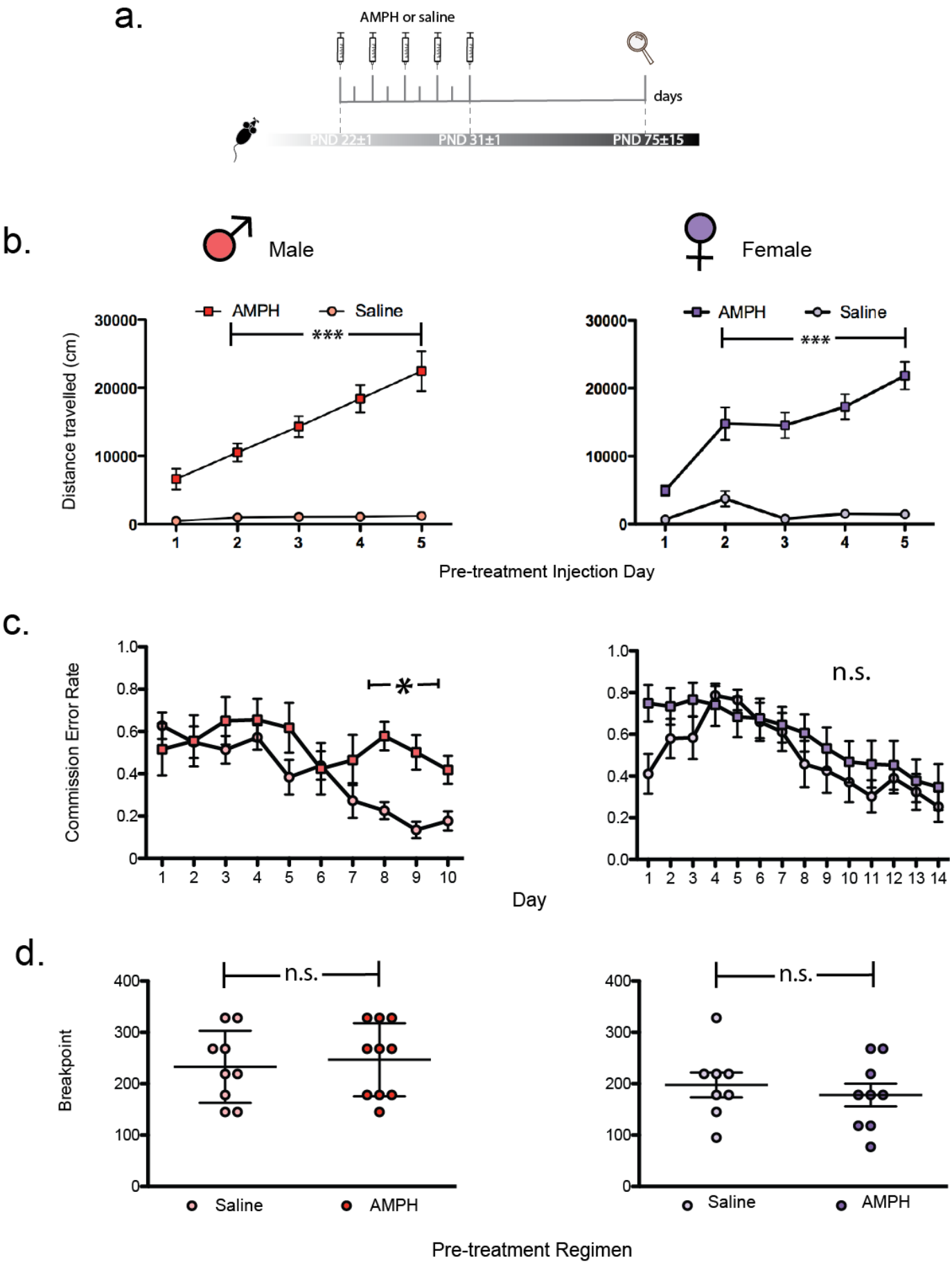
a.
AMPH or saline
days
PND 22±1
PND 31±1
PND 75±15
b.
Male
Female
AMPH
Saline
***
Distance travelled (cm)
30000
20000
10000
0
1
2
3
4
5
Pre-treatment Injection Day
c.
Commission Error Rate
*
n.s.
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day
d.
Breakpoint
n.s.
400
300
200
100
0
Saline
AMPH
Pre-treatment Regimen

Figure 1. (a) Experimental timeline of pre-treatment and behavioural assessments in female and male mice (AMPH: amphetamine). **(b) (Left panel)** Female mice treated with amphetamine showed robust drug-induced locomotor activity (two-way ANOVA; main effect of treatment, $F_{1,68} = 77.97$, $p < 0.0001$; main effect of time, $F_{4,68} = 17.62$, $p < 0.0001$; interaction effect, $F_{4,68} = 14.41$, $p < 0.0001$). **(Right panel)** Male mice treated with amphetamine showed robust drug-induced locomotor activity (two-way ANOVA; main effect of treatment, $F_{1,72} = 80.73$, $p < 0.0001$; main effect of time, $F_{4,72} = 21.28$, $p < 0.0001$; interaction effect, $F_{4,72} = 18.18$, $p < 0.0001$). **(c)** Behavioral inhibition is not impaired in adult female mice treated with amphetamine in early adolescence. **(Left panel)** Adult male mice that received amphetamine during early adolescence make more commission errors across the 10 days of the Go/No-Go (two way mixed-design ANOVA, significant main effect of treatment, $F_{(1, 18)} = 4.839$, $p = 0.0411$; significant main effect of time, $F_{(9, 162)} = 5.491$, $p < 0.0001$; significant interaction, $F_{(9, 162)} = 2.584$, $p = 0.0083$. Amphetamine n = 8, Saline n = 12). **(Right panel)** Go/No-Go task commission error rate in female mice. Both AMPH- and saline-treated females improve in commission error rate over time, but there was no significant difference in inhibitory performance between treatment groups (two-way ANOVA; main effect of time, $F_{13,221} = 9.716$, $p < 0.0001$; no effect of treatment, $F_{1,221} = 0.9903$, $p = 0.3336$; no interaction, $F_{13,221} = 1.159$, $p = 0.3116$). **(d)** Motivation for food reward remains unaltered in both male and female mice treated with amphetamine in early adolescence. **(Left panel)** Progressive ratio (PR) task breakpoint ratio in adult male mice. No significant difference between treatment groups in breakpoint over 60-minute task (unpaired two-tailed Student's t test, t = 0.4180, df = 17, p = 0.6812). **(Right panel)** Progressive Ratio (PR) task breakpoint ratio in adult female mice. No significant difference between treatment groups in breakpoint over 60-minute PR task (unpaired two-tailed Student's t test, t = 0.6011, df = 15, p = 0.5568).

* : $p < 0.05$; *** : $p < 0.0001$. (Mixed two-way ANOVA on days and treatment, stars represent effect of treatment)

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