UNIVERSITY OF CALIFORNIA, SAN DIEGO

Effects of Maternal Immune Activation upon Amphetamine-Facilitated Intracranial Self-Stimulation

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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The Thesis of Boris Il'ich Chobrutskiy is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Co-Chair

Chair

University of California, San Diego

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Dedication

This work is dedicated to my parents, Ilya and Raisa Chobrutskiy.

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I'd like to express my gratitude to Dr. Neil Richtand, who offered me the opportunity to join his lab and participate in this project. As a physician and research advisor, Dr. Richtand has bettered an incalculable number of lives in both the clinic and the lab. The guidance and support he gives his students makes working in his lab an experience of immense value, and has evoked in me the passion to pursue a lifelong career in research.

Thank you to Dr. Gery Schulteis for his advice and expertise, which was relied on daily in nearly every facet of this project. It was truly an honor to work with an individual with so much experience and knowledge.

Last but not least, I'd like to thank the members of the Richtand and Schulteis labs, especially Clay Archer, Robert Tseng, Ben Tam, and Tyler Rigdon for their guidance and contributions to this project.

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ABSTRACT OF THE THESIS

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Professor Neil Richtand, Chair

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Activation of the maternal innate immune response during pregnancy increases risk of schizophrenia, an illness with elevated rates of drug addiction. Measures of drug effects upon brain reward function are provided by intracranial self-stimulation (ICSS) reward thresholds. In order to better understand effects of MIA on an offspring's sensitivity to drugs of abuse, we

examined effects of MIA on amphetamine facilitation of ICSS reward threshold under both acute and withdrawal states.

The objective of this thesis project was to determine MIA effects upon amphetamine facilitated ICSS reward threshold during both the acute and withdrawal phases following investigator-administered amphetamine injection. Pregnant Sprague-Dawley rats were injected with polyinosinic:polycytidylic acid (poly I:C 8mg/kg), lipopolysaccharide (LPS 50μg/kg), or saline (control). Adult offsprings' ICSS reward thresholds were measured acutely, 10 minutes after investigator-administered amphetamine (0.125, 0.25, and 0.5 mg/kg subcutaneous), and during the withdrawal phase 8 and 12 hours following daily amphetamine injections (4mg/kg x 4 days).

MIA was found to significantly attenuate the direct acute effects of amphetamine upon brain reward threshold. Thus, MIA offspring require more drug to achieve similar drug reward effect. Additionally, MIA attenuates the magnitude of ICSS withdrawal response across repeated bouts of intoxication/withdrawal. Higher drug consumption to achieve intoxication, and less aversion to continued drug consumption generated by a blunted withdrawal effect may work in tandem to enhance addiction risk in MIA offspring. Identification of the mechanisms underlying this effect may contribute to prevention, treatment, and understanding of prognosis in human addictions.

Introduction

Environmental and genetic factors play a critical role in determining risk for drug addiction (Enoch 2011, Goldman et al 2005). For instance, developmental stress is an environmental factor that has been implicated in contributing more than half of the attributable risk for drug abuse in adulthood (Andersen & Teicher 2009, Dube et al 2003). Activation of the maternal innate immune response during gestation is a common environmental exposure in early development. In epidemiological studies maternal immune activation has been linked to increased risk of schizophrenia (Brown & Derkits 2010), an illness with a high comorbidity with drug addiction. Effects of this environmental exposure upon offspring have been widely studied in the "maternal immune activation" (MIA) animal model.

MIA animal models employ non-infectious immunogens, including synthetic nucleic acid poly-inosinic:polycytidylic acid (poly I:C) to simulate viral infection and bacterial endotoxin lipopolysaccharide (LPS) to simulate bacterial infection. Poly I:C and LPS activate toll-like receptors TLR3 and TLR4, respectively, which stimulate maternal cytokines - soluble polypeptides that mediate the inflammatory response of the innate immune system.

MIA offspring exhibit abnormalities in dopamine system function within the nucleus accumbens, the site of integration of drug-associated memories and modulation of drug reward and reward-enhancement. Baseline dopamine levels in the nucleus accumbens were found to be reduced in adult offspring of female rats treated with LPS at the end of gestation (Bakos et al 2004), but

increased in adult offspring of female rats treated with LPS daily throughout gestation (Romero et al 2010). Additionally, Increased levels of both dopamine production (Vuillermot et al 2010) and turnover (Ozawa et al 2006) were observed in adult MIA offspring mice. Opposite results have been observed in adolescent MIA offspring, including decreased dopamine production, dopamine transporter expression, and dopamine D1 and D2 receptor expression (Romero et al 2010, Vuillermot et al 2010).

Drug-associated behavioral abnormalities have also been observed in MIA offspring. Reinstatement of drug-induced conditioned place preference to amphetamine (AMPH) is enhanced in adult MIA offspring in a rat MIA model (Richtand et al 2012). Acquisition of conditioned place preference to both AMPH (Borcoi et al 2015) and cocaine (Labouesse et al 2015) are enhanced in MIA offspring in mouse MIA models. In combination, these observations suggest evidence of a biological process in MIA offspring altering behavioral response to stimulant drugs. Direct measures of drug effects upon brain reward function are provided by intracranial self-stimulation (ICSS) reward thresholds. ICSS measures the amplitude of electrical current (or frequency) thresholds experienced as rewarding, as evidenced by willingness to work for response-dependent stimulation (Schulteis 2010). ICSS thresholds are lowered in animals under the acute effects of drugs of abuse including amphetamine (Kornetsky & Esposito 1979), methamphetamine (Bauer et al 2013), cocaine (Kornetsky & Esposito 1979), morphine (Esposito & Kornetsky

1977), and alcohol (Dewitte & Bada 1983). ICSS thresholds are elevated during the withdrawal state (Lin 1999). We therefore used ICSS to directly ascertain the effects of MIA on amphetamine (AMPH) facilitation of brain reward threshold.

Here, we report highly significant effects of MIA exerted through attenuation of an offspring's reward sensitivity to acute AMPH intoxication. Moreover, MIA offspring also experience a significantly attenuated withdrawal from repeated intermittent bouts of AMPH intoxication. As discussed below, the need for more drug to achieve the same intoxication reward level, and less aversion to repeated intoxication produced by an attenuated withdrawal state mirrors elevated genetic risk for addictions observed in both human and animal models (de Wit & Phillips 2012, Kamens et al 2005, Schuckit et al 2004, Schuckit & Sweeney 1987, Shabani et al 2012, Wheeler et al 2009). These data demonstrate effects of MIA on the experience of drug reward, a factor directly impacting the potential for drug abuse.

Materials and Methods

2.1. Maternal Immune Activation and Research Design

The experimental design is summarized in Fig. 1. Eight week-old male and nulliparous female Sprague Dawley rats (Harlan Laboratories, Indianapolis, IN) were housed two per cage in a temperature- and humidity-controlled room with a 12-h light/dark cycle and allowed food and water ad libitum. Following a two-week acclimation males and females were co-housed overnight, then separated and single housed on the following day, defined as gestational day 0. On gestational day 14 pregnant dams (identified by weight gain of ≥40 g) were injected with polyinosinic: poly-cytidylic acid (polyI:C) (Sigma, St. Louis, MO, P1530; 8 mg/kg, i.p.), lipopolysaccharide (LPS) (Sigma, St. Louis, MO, P1530; 50μg/kg, i.p.), or saline (control).

Litters were culled to 8 offspring on postnatal day 1, weaned on postnatal day 21, and housed 2 rats per cage. Male offspring were implanted with stainless steel bipolar electrodes (Plastics One® Inc) into the lateral hypothalamus at 9 weeks of age (Fig. 1). ICSS was performed according to a modified procedure of the Kornetsky and Esposito discrete-trial current-threshold procedure (Kornetsky and Esposito 1979). Animals undergoing surgery were housed two per cage postoperatively. All procedures were in strict adherence to the National Institutes of Health guidelines and approved by the VA San Diego Healthcare System Institutional Animal Care and Use Committee.

2.2 Drug Treatment

D-amphetamine sulfate (Sigma, St. Louis,MO) was dissolved in 0.9% saline. AMPH concentration is described as hemisulfate salt. All injections were given subcutaneously in a final volume of 1 ml/kg.

2.3 Acute Amphetamine

MIA and control offspring were tested for reward thresholds under baseline conditions and 10 minutes following acute experimenter-administered AMPH injections (Fig. 1, acute amphetamine ICSS, 14 weeks). Satisfactory baseline values were defined as 4 consecutive reward thresholds with averaged variance of less than 10%. A dose-response curve was generated using three AMPH doses (0.125, 0.25, 0.5 mg/kg) administered in a Latin square design, with 1 week gaps between each dose for re-acquiring baseline ICSS measurements.

2.4 Chronic Amphetamine

Repetitive AMPH injections (4mg/kg) were administered 24 hours apart for 4 days. ICSS reward thresholds were measured 8 and 12 hours after each injection and compared to reward thresholds measured under baseline conditions at the same time points (Fig. 1, chronic amphetamine ICSS, 17

weeks). Satisfactory baseline values were defined as 4 averaged reward thresholds for each time point with variance of less than 10%.

2.4. Statistical Analysis

Statistical analyses were performed by two-way analysis of variance (ANOVA) for the acute, and three-way ANOVA for the chronic data using Prism version 6 (GraphPad, La Jolla, CA) and SPSS version 22 (IBM, Armonk, NY) with experiment-wise error rate set at $p<0.05$. Baseline measurements were set as four averaged values for every time point in each trial. For acute data, maternal treatment (poly I:C, LPS, or control) and AMPH dose were used as main factors and ICSS values as the dependent measure. For the chronic data, treatment (poly I:C, LPS, or control), day of injection, and time post injection were used as main factors and ICSS values as the dependent measure.

Results

3.1 Baseline Determination

ANOVA did not identify significant group differences in the baseline reward threshold current of Control, poly I:C, and LPS offspring (Fig 2; $F=0.2848$, $p=0.7539$). Similarly, there were no significant group differences in ICSS training time, or time required to acquire stable reward threshold baseline values between MIA and control offspring (data not shown).

3.2 Acute AMPH

Control, poly I:C, and LPS offspring received subcutaneous AMPH administered once weekly in a Latin square design. To test the effects of MIA on AMPH facilitation of ICSS reward threshold, ICSS measurements were determined 10 minutes following AMPH injection dosages of 0.125, 0.25, or 0.5 mg/kg. As previously observed by others (Wise 1999), acute AMPH injection facilitates brain stimulation reward, measured as a reduction in reward threshold current following acute AMPH exposure (Fig. 3). Two-way ANOVA identified highly significant overall effects of MIA treatment ($F=5.383$, $p=0.0092$) and AMPH dose ($F=33.69$, $p<0.0001$). Post hoc comparisons identified attenuation of AMPH effects upon reward threshold in both poly I:C and LPS compared to control offspring [LPS v. control ($F=11.21$, $p=0.0029$); poly I:C v. control ($F=4.2827$, $p=0.0375$)]. Post hoc comparisons of individual amphetamine doses identified a significant attenuation of reward threshold

facilitation at the 0.25mg/kg dose for both poly I:C ($p=0.0231$) and LPS ($p=0.0099$) compared to control offspring. ANOVA did not identify a significant treatment x dose interaction ($F=0.7445$, $p=0.5648$).

As expected (Lin et al 2000), ICSS response latencies decreased after acute AMPH administration (Fig. 4). ANOVA identified a significant effect of dose ($F=5.280$, $p=0.0072$), but no significant effect of MIA treatment in this outcome measure ($F=0.4083$, $p=0.6678$).

3.3 Chronic AMPH

To determine effects of MIA upon ICSS reward thresholds during AMPH withdrawal, repeated daily AMPH (4mg/kg) injections were administered for 4 days to MIA and control offspring. ICSS measurements were determined 8 and 12 hours following each injection. As previously identified (Lin et al 2000), control offspring exhibited a progressive increase in magnitude of reward current threshold across repeated bouts of intoxication/withdrawal (Fig. 5). ANOVA identified a blunting of this progression in MIA offspring, with significant effects of MIA treatment ($F=3.478$, $p=0.040$), day ($F=25.87$, $p<0.0001$), and time of day ($F=9.048$, $p=0.004$). ANOVA did not identify significant treatment x day ($F=121$, $p=0.305$) or treatment x time of day ($F=0.312$, $p=0.734$) interactions. Post hoc comparisons identified significantly attenuated reward threshold elevations in both poly I:C and LPS compared to control offspring at the 12 hour

withdrawal time points on day 3 ($p<0.05$, poly I:C and LPS compared to control), and day 4 [poly I:C ($p<0.05$) and LPS ($p<0.01$) compared to control]. Both MIA and control groups returned to baseline reward threshold within 48 hours following the final AMPH administration, with no significant differences between groups (data not shown).

Effects of AMPH withdrawal on ICSS response latencies in control and MIA offspring are shown in Fig. 6. ANOVA identified a significant effect of time of day ($F=6.296$, $p=0.016$), but did not identify a significant effect of day ($F=1.739$, $p=0.163$). There was no statistically significant effect of MIA treatment on response latency ($F=1.789$, $p=0.180$).

3.4 Correlations between outcome measures

In order to investigate relationships between different outcome measures within individual animals, we examined correlations between outcome measures in control and MIA offspring.

Correlations between the acute and withdrawal effects of AMPH upon ICSS reward threshold are shown in Table 1. Significant correlations were identified between measures of facilitation of reward threshold by the 0.125mg/kg dose and withdrawal on day 1 - 8 hour time point, along with facilitation of reward threshold by the 0.5mg/kg dose and withdrawal on day 3 - 8 hour time point. Correlations between ICSS reward threshold measurements at different time points of AMPH withdrawal are also shown on

Table 1. As expected, demonstrating internal consistency, there are numerous significant correlations between time points.

Correlations between AMPH facilitated decreases in reward threshold and response latency at different doses are shown on Table 2. Only one significant correlation was identified between reward threshold facilitation at the 0.25mg/kg dose and response latency facilitation at the 0.5mg/kg dose. The lack of other significant correlations within individual animals suggests an overall weak relationship between the reward threshold facilitation and response latency facilitation measures.

Correlations between 24-hour change in weight in the pregnant dams in response to MIA or vehicle injection on gestation day 14, and adult offspring measures of AMPH facilitated decreases in ICSS reward threshold at different doses, and ICSS reward threshold increases at different withdrawal time points are shown on Table 3. A significant correlation was identified between acute ICSS reward threshold facilitation at the 0.125mg/kg dose and dam weight change. A highly significant correlation was identified between the final (day 4, 12 hours post injection) withdrawal time point and change in dam weight. The final withdrawal time point demonstrates the most significant group difference between MIA and control offspring in post-hoc analysis. This time point's highly significant correlation to change in dam weight suggests a close relationship between MIA treatment - induced weight

loss in pregnant dams and attenuated ICSS reward threshold increases caused by AMPH withdrawal in the individual adult offspring.

We also examined correlations between increases in ICSS reward threshold and response latency caused by AMPH withdrawal at all time points. Only 2 out of 30 measures tested for correlation showed significant correlations (data not shown).

Discussion

The MIA model developed by Patterson and colleagues causes developmental abnormalities of relevance to schizophrenia in the offspring of affected dams (Meyer et al 2008, Ozawa et al 2006, Shi et al 2003) (Boksa 2010, Meyer & Feldon 2010, Patterson 2009). Based upon the 50% co-morbidity for drug and alcohol dependence in schizophrenia patients (Buckley 2006), we (Richtand et al 2012) and others (Borcoi et al 2015, Labouesse et al 2015) identified behavioral abnormalities of relevance to drug dependence in MIA offspring. Drug-stimulated reinstatement of conditioned place preference to AMPH is significantly enhanced in MIA offspring in a rat MIA model (Richtand et al 2012). In a mouse model of maternal immune activation, acquisition of conditioned place preference to AMPH (Borcoi et al 2015) and cocaine (Labouesse et al 2015) are enhanced in MIA offspring. While these studies differ significantly in methodological details, MIA enhancement of the acquisition and reinstatement of a drug-conditioned response is suggestive of an underlying biological process which may elevate addiction risk. Here we extend those earlier findings, directly demonstrating effects of maternal immune activation upon reward function in MIA offspring.

Following acute AMPH injection, the effect of AMPH upon brain stimulation reward threshold is significantly attenuated in MIA offspring generated with either poly I:C or LPS *in utero* exposure, compared to control offspring (Figure 3). Thus, MIA offspring require more drug to achieve similar

drug reward effect. During the withdrawal phase following chronic AMPH injections (Figure 5), the progressive increase in magnitude of reward threshold across repeated bouts of intoxication/withdrawal was blunted in MIA offspring. Thus, MIA attenuates the effects of acute AMPH intoxication on reward thresholds, and reduces the consequences of withdrawal from repeated bouts of intoxication on reward threshold.

Genetics

The observed attenuation of drug reward function responses are notable in their similarity to prior observations in the addictions literature of attenuated physiological response in both human and animal high genetic addiction risk models. This phenomenon was first identified as the low level of response (LR) to alcohol in individuals at increased genetic risk for alcoholism (Schuckit 1984a, Schuckit 1984b, Schuckit 1985, Schuckit 1994). The attenuated physiological response to alcohol thereby requires higher alcohol levels to experience the same drug effect. The need for higher alcohol consumption to achieve intoxication could thereby increase addiction risk (Schuckit 1994, Schuckit et al 2004). Other human studies have identified genetic influences on drug reward in schizophrenia patients, demonstrating a genome-wide association between increased risk for schizophrenia and attenuated amphetamine response (Hart et al 2014).

This phenomenon has also been observed in rodent genetic studies. Mice bred for low sensitivity to the locomotor activating effects of methamphetamine (METH) also consumed significantly more METH and cocaine than mice bred for high sensitivity (de Wit & Phillips 2012, Kamens et al 2005, Shabani et al 2012, Wheeler et al 2009). Additionally, mice bred for high levels of oral METH consumption exhibited lower levels of conditioned aversion to METH (Shabani et al 2012, Shabani et al 2011), suggesting a lessened sensitivity to aversive effects of the drug. Rats bred for low cocaine response exhibited higher levels of self-administration than high responders (Mandt et al 2008). Thus, genetics studies suggest mechanisms impacting addiction risk may have close parallels to MIA's environmental influence on brain reward. In other genetic studies, however, the opposite finding has been observed (de Wit & Phillips 2012).

Lowered sensitivity of reward circuits to drugs of abuse is consistent with a previously described stress/withdrawal model of drug dependence (Koob & Le Moal 2001). In this model, during the transition into addiction, reward systems involved in the acute effects of drugs of abuse undergo adaptations that decrease reward response (Koob & Volkow 2010). Evidence in support of this model includes human brain imaging studies which have identified decreased striatal dopamine release and D2-family receptor binding in drug-dependent individuals (Volkow et al 2004). These adaptations are hypothesized to reduce acute sensitivity to rewarding reinforcers. Thus, this

model characterizes a central role for reward system hypoactivity in the escalation of drug consumption and transition into drug addiction (Koob & Kreek 2007).

Dopamine-mediated reward function has been evaluated in animal genetic models as an individual risk factor for drug abuse. Dopamine D2 receptor knockout mice, and rats treated with D2 receptor antagonist showed significant increases in cocaine self-administration (Caine et al 2002). Conversely, reduction in ethanol self-administration in the rat was achieved through over-expression of the D2 receptor in the nucleus accumbens, suggesting a protective effect of robust dopaminergic signaling in reward targets of drugs of abuse (Thanos et al 2001). Thus, the effects of MIA on offsprings' reward response to drugs of abuse may mirror the transition into addiction, and could confer similar risk for continued drug use.

Dopamine Mechanisms

Identification of altered ICSS reward threshold provides an opportunity to identify mechanisms underlying this action. We propose that the changes in drug reward in MIA offspring presented here are consequence of alterations in Limbic systems critically involved in brain reward. The most prominent and widely replicated effect has been within the nucleus accumbens, where a developmental progression of dopamine system

functional abnormalities has been widely replicated in MIA offspring. Adolescent MIA animals exhibit overall diminished dopamine function as a result of decreased dopamine production, along with decreased dopamine transporter and receptor expression (Romero et al 2010, Vuillermot et al 2010). In adult MIA offspring, evidence of dopamine alterations in nucleus accumbens has shown both increased (Romero et al 2010, Vuillermot et al 2010) or decreased (Bakos et al 2004) baseline dopamine levels, and increased dopamine turnover (Ozawa et al 2006). The dopamine abnormalities observed in MIA offspring may impact the sensitivity of reward circuits to drugs of abuse.

Other neurochemical abnormalities observed in MIA offspring include elevated basal extracellular glutamate (Roenker et al 2011), and altered synaptophysin expression in the prefrontal cortex (Romero et al 2010). Within hippocampus, MIA offspring exhibit glutamate system abnormalities including decreased NMDA receptor-dependent synaptic current and plasticity (Escobar et al 2011, Lante et al 2007) and elevated basal extracellular glutamate (Ibi et al 2009).

Cytokines/Inflammation

MIA offspring also exhibit altered peripheral cytokine expression, including cytokines IL-6, TNF- α, and IL-1β in response to LPS-stimulation (Beloosesky et al 2010, Hodyl et al 2008, Hodyl et al 2007, Lasala & Zhou 2007). These data support a suggested model that maternal immune exposure primes subsequent aberrant cytokine responses later in development (Bilbo & Schwarz 2009). This is relevant to nervous system function because astrocytes and microglia synthesize and express cytokines within the central nervous system. These glial cells are targets of abused drugs, as they express dopamine, opioid, cannabinoid, $GABA_B$, and nicotinic cholinergic receptors. A rapidly growing literature demonstrates cytokine modulation of drug reward and dependence induced by stimulants, opiates, and alcohol (Coller & Hutchinson 2012, Frank et al 2011, Miguel-Hidalgo 2009). Cytokine actions on limbic system function may be highly conserved, as this may underlie inflammatory system actions on reward behaviors with beneficial effects upon infections or wound healing (Felger & Miller 2012). Individual cytokines differ in pro- vs. anti-inflammatory effects, and similar contrasts have been observed regarding effects upon reward function. For example, IP TNF-α injection elevates ICSS reward thresholds, suggesting inhibitory effects of this cytokine on reward system function (van Heesch et al 2013). Consistent with cytokine TNF-α inhibition of reward system function, TNF-α knockout mice exhibit increased fixed-ratio METH self-administration and higher progressive-ratio breakpoints (Yan et al 2012). In contrast, other

cytokines appear to facilitate reward function and drug self-administration. For example, inhibition of pro- inflammatory cytokine expression using microglial inhibitors reduces METH administration (Snider et al 2013), and attenuates drug-induced reinstatement of METH self-administration (Beardsley et al 2010).

Weight Change

Our observations in the present study are consistent with previous findings of significant weight loss in pregnant dams following MIA treatment (Fortier et al 2004a, Fortier et al 2004b, Zuckerman et al 2003). We have previously observed that maternal weight loss was predictive of the severity of alterations in behavioral outcome measures in response to amphetamine and MK-801 (Bronson et al 2011). In the present study, dam weight loss in response to MIA was significantly correlated with attenuated withdrawal response in MIA offspring at the day 4, 12-hour time point, which also exhibited the most significant MIA effect. Physiological changes including reduced food intake, body temperature fluctuation, and elevation of pro-inflammatory cytokines have been previously associated with weight loss following MIA treatment (Fortier et al 2004b). Such effects along with previous data linking MIA maternal weight loss and offspring behavioral outcomes suggest further investigation into maternal MIA response on offspring development.

Future Directions

Drug self-administration will further expand translation of MIA animal studies human addictions. Animal models using extended drug access and self-administration have identified important relationships linking temporal changes in the homeostatic response of drug reward to the transition to compulsive drug use (Koob et al 2014). Self-administration allows measurement of motivation for drug consumption, quantity of consumption, and escalation in dose over time. Such a model with ad-libitum and long-term drug access would be closely analogous to the process of human transition into addiction and drug abuse. Combining self-administration with ICSS would allow for measurement of reward threshold adaptations in response to self-administration.

MIA reduces the effect of AMPH upon drug reward during acute intoxication, and attenuates the reward deficit seen during withdrawal, and this mirrors attenuated responses to acute intoxication consistently observed in human and animal models with high addiction risk. The requirement for greater drug consumption to achieve similar drug reward may increase drug consumption and thereby enhance addiction risk. Schizophrenia has a right co-morbidity with smoking, and considering the strong evidence linking MIA and schizophrenia, nicotine would be an important drug to investigate in MIA models.

Figures

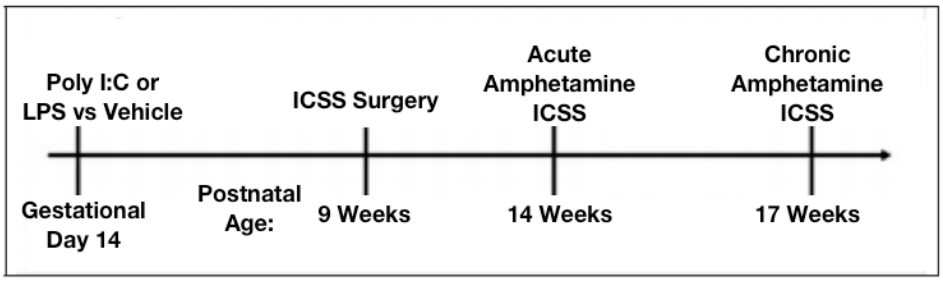

Figure 1. Experimental design summary

Mothers received MIA treatment on gestational day 14. ICSS surgery was performed on postnatal week 9. Acute AMPH experiments were performed at 14 weeks of age. Chronic AMPH experiments were performed at 17 weeks of age.

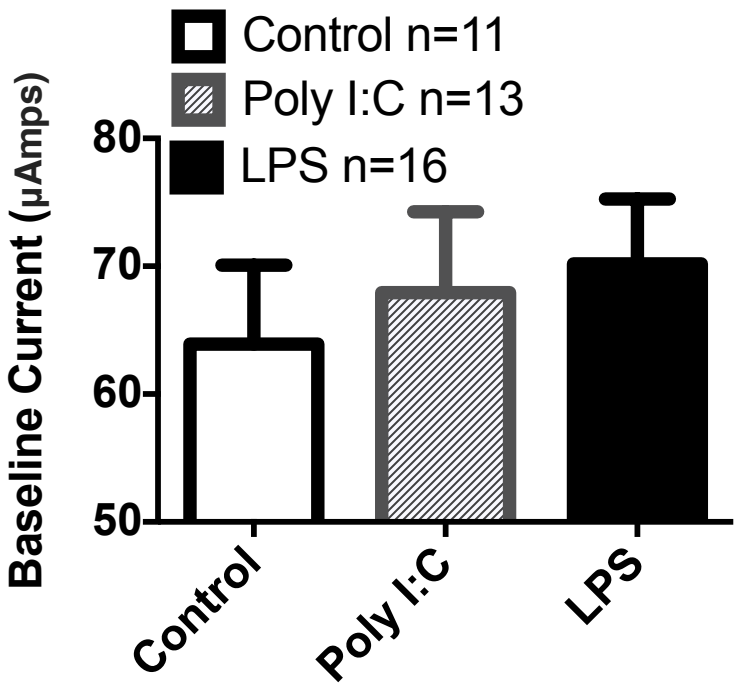

Figure 2. Analysis of ICSS baseline currents

Rats were trained in ICSS and baseline reward thresholds were established prior to drug testing. Analysis did not identify significant effect of MIA treatment on baseline reward thresholds with this sample size. ($F=0.2848$, $p=0.7539$)

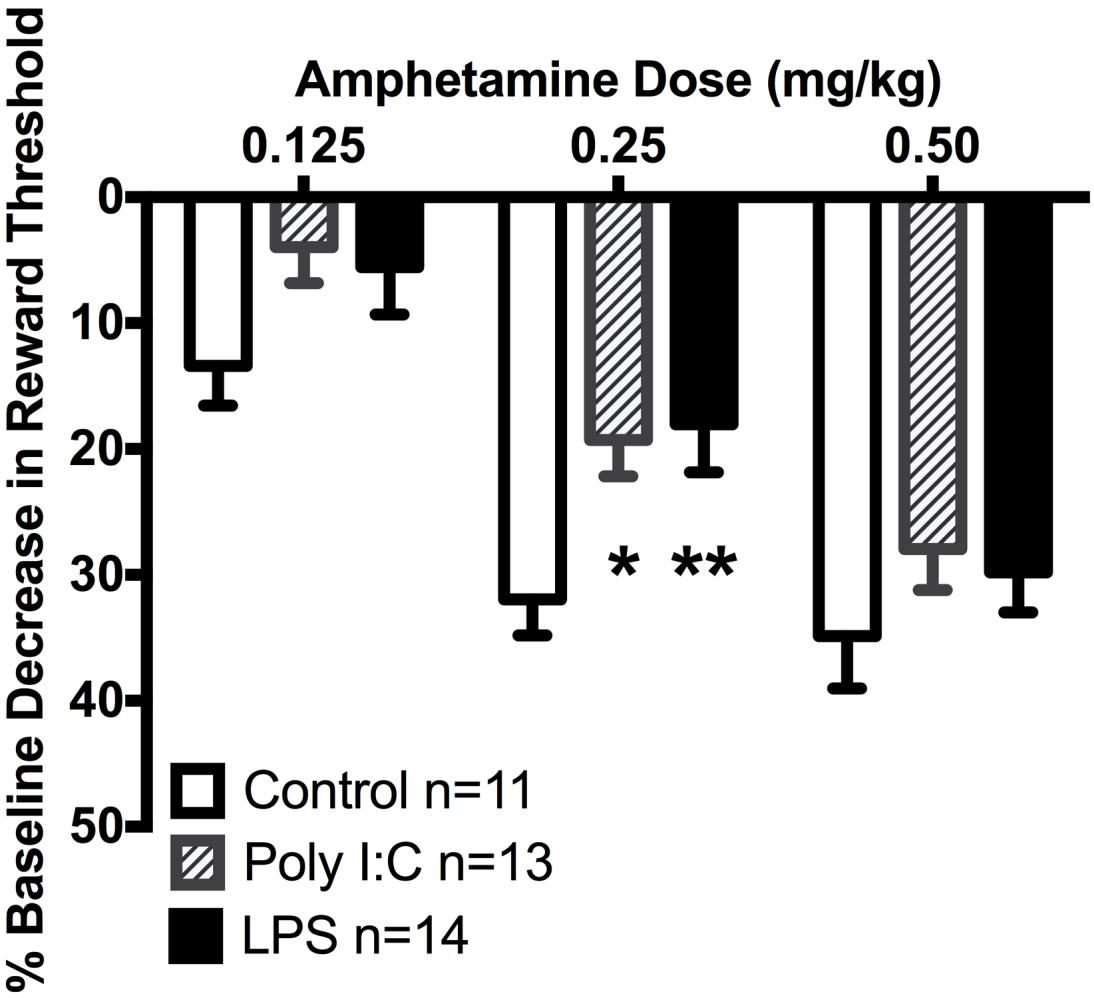

Figure 3. AMPH facilitation of ICSS reward thresholds are significantly attenuated in MIA compared to control offspring.

Reward thresholds were measured 10 minutes following AMPH administration. ANOVA identified significant effects of treatment ($F=5.383$, $p=0.0092$) and AMPH dose ($F=33.69$, $p<0.0001$). ANOVA did not identify a significant treatment x dose interaction ($F=0.7445$, $p=0.5648$). Post hoc comparisons identified a significant reduction in reward threshold at the 0.25mg/kg dose for poly I:C and LPS offspring compared to control (* $p<0.05$)

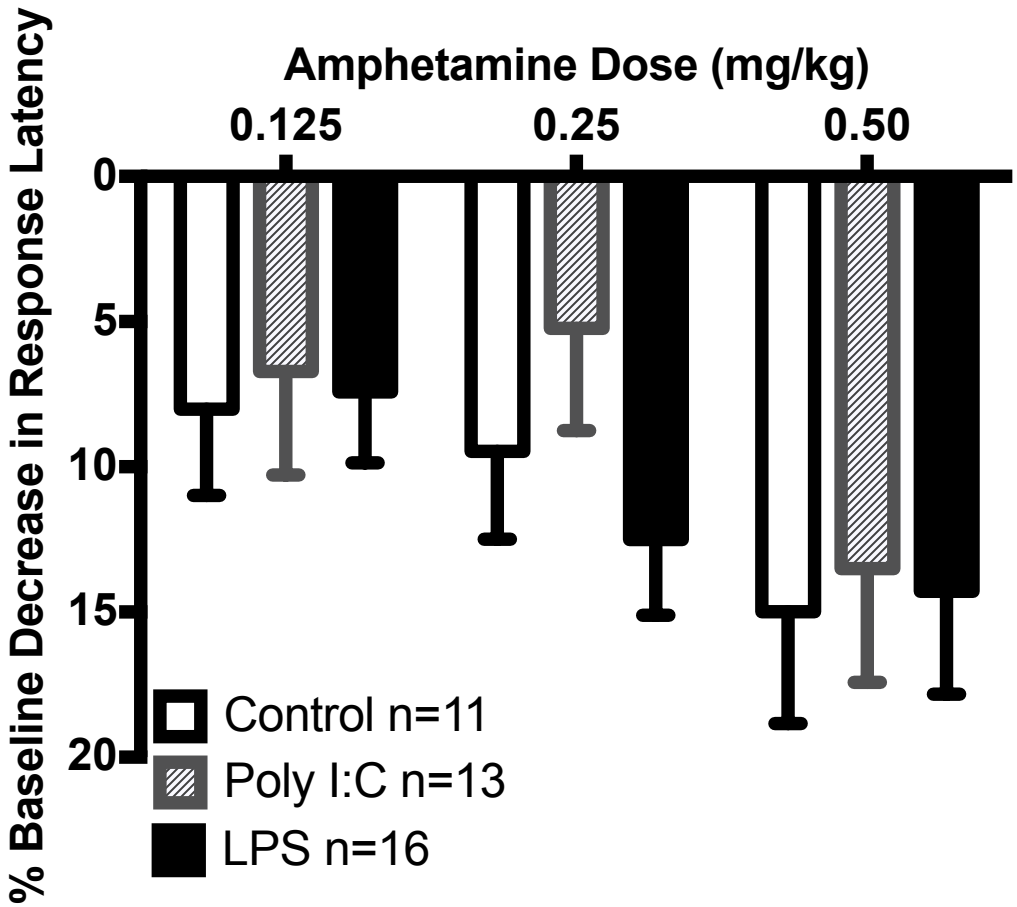

Figure 4. ICSS response latencies are not significantly altered in MIA compared to control offspring. Response latencies were measured 10 minutes following AMPH administration. ANOVA identified a significant effect of AMPH dose ($F=5.280$, $p=0.0072$). ANOVA did not identify significant effects of treatment ($F=0.4083$, $p=0.6678$) or treatment x dose interaction ($F=0.5378$, $p=0.7084$).

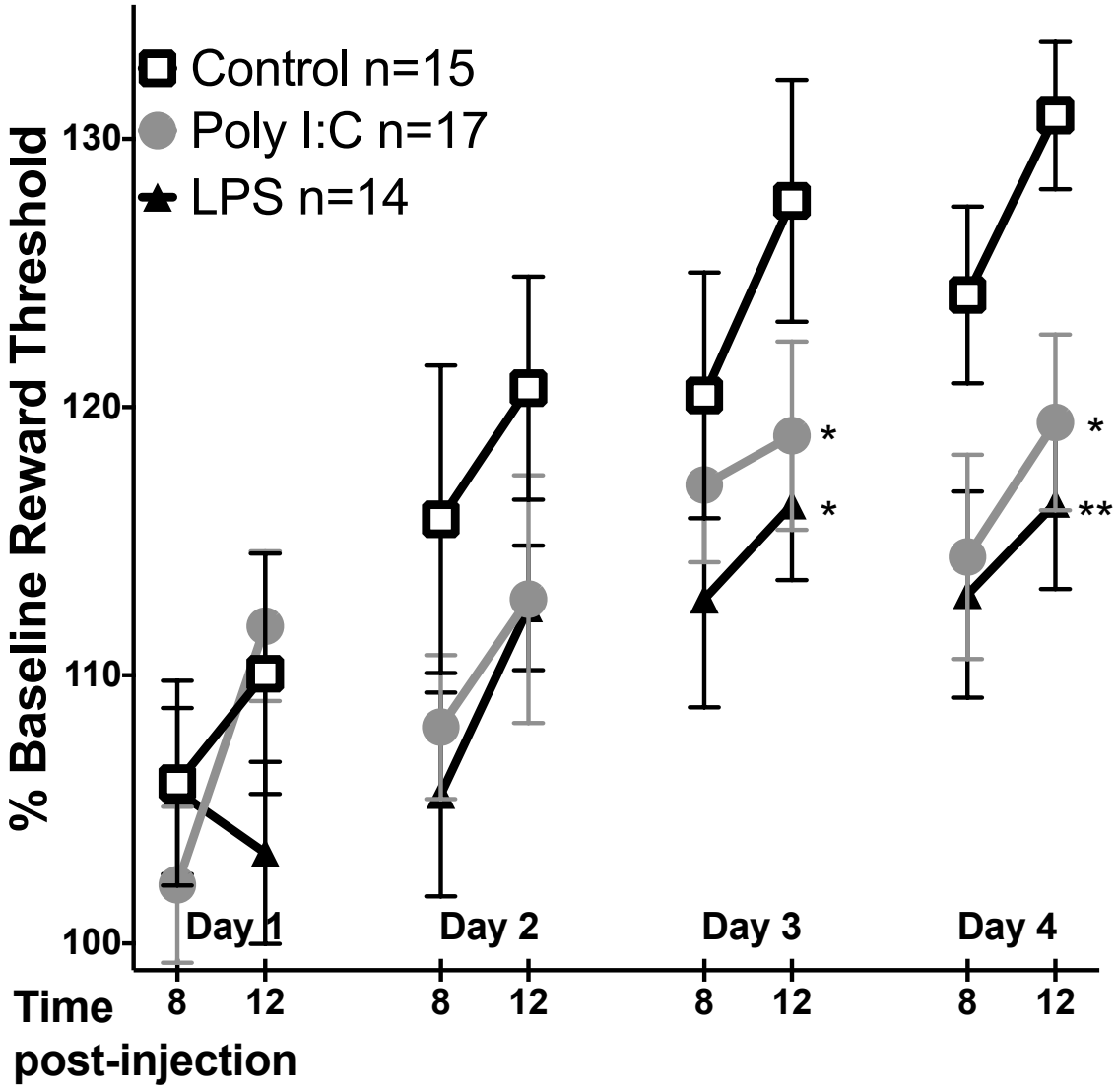

Figure 5. Effects of AMPH withdrawal on ICSS reward thresholds are significantly attenuated in MIA compared to control offspring following repetitive AMPH injections. Reward thresholds were measured 8 and 12 hours following daily injection of AMPH for four days. ANOVA identified a significant effect of treatment ($F=3.478$, $p=0.040$), day ($F=25.87$, $p<0.0001$), and time of day ($F=9.048$, $p=0.004$). Post hoc comparisons identified

significantly raised reward thresholds at 12 hours post-injection on day 3 in both poly I:C and LPS offspring compared to control (* $p<0.05$), and significantly raised reward thresholds at 12 hours post-injection on day 4 in poly I:C (* $p<0.05$) and LPS (** $p<0.01$) offspring compared to control.

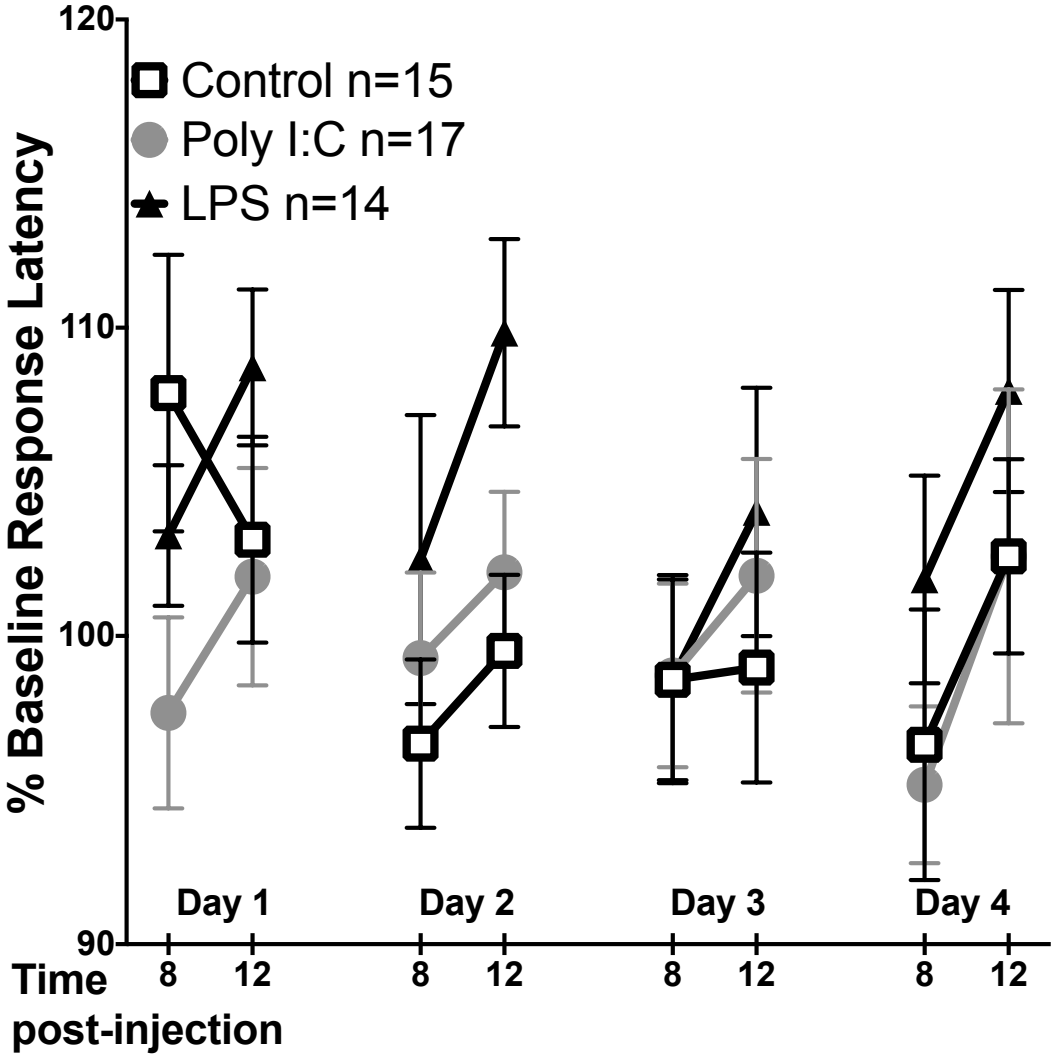

Figure 6. Effects of AMPH withdrawal on ICSS response latencies are not significant in MIA compared to control offspring. Response latencies were measured 8 and 12 hours following daily injection of AMPH for four days ANOVA identified a significant effect of time of day ($F=6.296$, $p=0.016$), and did not identify a significant effect of treatment ($F=1.789$, $p=0.180$) or day ($F=1.739$, $p=0.163$).

Tables

Table 1. Correlations between acute AMPH facilitation of ICSS reward threshold and effects of AMPH withdrawal on ICSS reward threshold.

n = 38		Chronic Day 1 8 hours	Chronic Day 1 12 hours	Chronic Day 2 8 hours	Chronic Day 2 12 hours	Chronic Day 3 8 hours	Chronic Day 3 12 hours	Chronic Day 4 8 hours	Chronic Day 4 12 hours
Acute 0.125 mg/kg	Pearson Correlation	.340*	.035	.283	.212	.025	.020	.139	.106
	Sig. (2-tailed)	.037	.836	.085	.201	.882	.903	.406	.525
Acute 0.25 mg/kg	Pearson Correlation	.190	.300	.003	.233	-.093	-.143	.107	.150
	Sig. (2-tailed)	.254	.067	.984	.160	.577	.393	.523	.370
Acute 0.5 mg/kg	Pearson Correlation	.302	.228	.043	.349*	.225	.187	.150	.255
	Sig. (2-tailed)	.066	.168	.797	.032	.174	.262	.370	.122
Chronic Day 1 8 hours	Pearson Correlation	1	.291	.438**	.477**	.451**	.273	.297	.193
	Sig. (2-tailed)		.076	.006	.002	.004	.097	.070	.246
Chronic Day 1 12 hours	Pearson Correlation	.291	1	.227	.612**	.383*	.500**	.410*	.218
	Sig. (2-tailed)	.076		.171	.000	.018	.001	.011	.188
Chronic Day 2 8 hours	Pearson Correlation	.438**	.227	1	.420**	.654**	.618**	.336*	.244
	Sig. (2-tailed)	.006	.171		.009	.000	.000	.039	.139
Chronic Day 2 12 hours	Pearson Correlation	.477**	.612**	.420**	1	.367*	.536**	.358*	.168
	Sig. (2-tailed)	.002	.000	.009		.023	.001	.028	.312
Chronic Day 3 8 hours	Pearson Correlation	.451**	.383*	.654**	.367*	1	.750**	.491**	.265
	Sig. (2-tailed)	.004	.018	.000	.023		.000	.002	.107
Chronic Day 3 12 hours	Pearson Correlation	.273	.500**	.618**	.536**	.750**	1	.458**	.440**
	Sig. (2-tailed)	.097	.001	.000	.001	.000		.004	.006
Chronic Day 4 8 hours	Pearson Correlation	.297	.410*	.336*	.358*	.491**	.458**	1	.267
	Sig. (2-tailed)	.070	.011	.039	.028	.002	.004		.106
Chronic Day 4 12 hours	Pearson Correlation	.193	.218	.244	.168	.265	.440**	.267	1
	Sig. (2-tailed)	.246	.188	.139	.312	.107	.006	.106	

Table 2. Correlations between AMPH facilitated decreases in reward threshold and response latency.

n = 38		Response Latency 0.125 mg/kg	Response Latency 0.25 mg/kg	Response Latency 0.5 mg/kg
Reward Threshold 0.125 mg/kg	Pearson Correlation	.210	.087	.023
	Sig. (2-tailed)	.207	.603	.891
Reward Threshold 0.25 mg/kg	Pearson Correlation	.238	.045	.329*
	Sig. (2-tailed)	.151	.790	.043
Reward Threshold 0.5 mg/kg	Pearson Correlation	.287	-.051	.210
	Sig. (2-tailed)	.081	.761	.207

Table 3. Correlations between a 24-hour change in pregnant dam weight in response to GD14 MIA treatment or vehicle and adult offspring measures of AMPH facilitated decreases and AMPH withdrawal-related increases in ICSS reward threshold

n = 38		Dam Weight Change
Acute 0.125 mg/kg	Pearson Correlation	.352*
	Sig. (2-tailed)	.030
Acute 0.25 mg/kg	Pearson Correlation	.247
	Sig. (2-tailed)	.135
Acute 0.5 mg/kg	Pearson Correlation	.068
	Sig. (2-tailed)	.683
Chronic Day 1 8 hours	Pearson Correlation	-.073
	Sig. (2-tailed)	.664
Chronic Day 1 12 hours	Pearson Correlation	-.045
	Sig. (2-tailed)	.790
Chronic Day 2 8 hours	Pearson Correlation	.128
	Sig. (2-tailed)	.442
Chronic Day 2 12 hours	Pearson Correlation	-.016
	Sig. (2-tailed)	.923
Chronic Day 3 8 hours	Pearson Correlation	-.060
	Sig. (2-tailed)	.722
Chronic Day 3 12 hours	Pearson Correlation	.061
	Sig. (2-tailed)	.717
Chronic Day 4 8 hours	Pearson Correlation	.147
	Sig. (2-tailed)	.379
Chronic Day 4 12 hours	Pearson Correlation	.465**
	Sig. (2-tailed)	.003

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