Neurotoxicology 72 (2019) 61–73

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Full Length Article

d-Amphetamine and methylmercury exposure during adolescence alters sensitivity to monoamine uptake inhibitors in adult mice

Steven R. Boomhower[a,*], M. Christopher Newland[b]

[a] Department of Environmental Health, Harvard T.H. Chan School of Public Health, 665 Huntington Ave, Bldg 1, Boston, MA, United States
[b] Department of Psychology, Auburn University, Auburn, AL, United States

ARTICLE INFO

Keywords:
Adolescence
Clomipramine
d-amphetamine
Desipramine
Mathematical principles of reinforcement
Methylmercury

ABSTRACT

Gestational exposure to methylmercury (MeHg), an environmental neurotoxicant, and adolescent administration of *d*-amphetamine (*d*-AMP) disrupt dopamine neurotransmission and alter voluntary behavior in adult rodents. We determined the impact of adolescent exposure to MeHg and *d*-AMP on monoamine neurotransmission in mice by assessing sensitivity to acute *d*-AMP, desipramine, and clomipramine, drugs that target dopamine, norepinephrine, and serotonin reuptake, respectively. Male C57Bl/6n mice were given 0 (control) or 3 ppm MeHg via drinking water from postnatal day 21 to 60 (murine adolescence). Within each group, mice were given once-daily injections of *d*-AMP or saline (i.p.) from postnatal day 28 to 42. This exposure regimen produced four treatment groups (n = 10–12/group): control, *d*-AMP, MeHg, and *d*-AMP + MeHg. As adults, the mice lever pressed under fixed-ratio schedules of reinforcement (FR 1, 5, 15, 30, 60, and 120). Acute i.p. injections of *d*-AMP (.3–1.7 mg/kg), desipramine (5.6–30 mg/kg), and clomipramine (5.6–30 mg/kg) were administered in adulthood after a stable behavioral baseline was established. Adolescent MeHg exposure increased saturation rate and minimum response time, an effect that was mitigated by chronic administration of *d*-AMP in adolescence. In unexposed mice, the three monoamine reuptake inhibitors had separable behavioral effects. Adolescent *d*-AMP increased sensitivity to acute *d*-AMP, desipramine, and clomipramine. Adolescent MeHg exposure alone did not alter drug sensitivity. Combined adolescent *d*-AMP + MeHg exposure enhanced sensitivity to acute *d*-AMP's and desipramine's effects on minimum response time. Adolescence is a vulnerable developmental period during which exposure to chemicals can have lasting effects on monoamine function and behavior.

1. Introduction

Adolescence marks the final developmental stage of monoamine neurotransmitter systems and is implicated in the etiology of a variety of psychological disorders (Chambers et al., 2003; Paus et al., 2008). Rodent adolescence spans postnatal days (PND) 21–60, a period during which expression of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) transporters undergoes dramatic alterations in brain regions implicated in reward and choice, such as the striatum (Galineau et al., 2004; Tarazi et al., 1998) and prefrontal cortex (Moll et al., 2000; Sanders et al., 2005). These changes coincide with behavior reflecting differences in executive functioning, such as increased impulsive choice (Doremus-Fitzwater et al., 2012; Pinkston and Lamb, 2011) and behavioral inflexibility in rats and mice (Newman and McGaughy, 2011), as compared with adults. Developmental changes in monoamine signaling and their interactions (Tassin, 2008) may enhance adolescent vulnerability to behavior-altering drugs and neurotoxicants, both alone and in combination.

Human adolescents may be at risk of exposure to methylmercury (MeHg), an environmental neurotoxicant, and the stimulant *d*-amphetamine (*d*-AMP). Human adolescents consume fish known to have high mercury concentrations (Butler et al., 2017; Nielsen et al., 2015), such as tuna (Tran et al., 2004; Wang et al., 2013), and are encouraged to consume more for health reasons (Gidding et al., 2005). Both the therapeutic and recreational use of stimulant drugs, such as *d*-AMP, also increases during the adolescent period (Johnston et al., 2014). Therefore, it is important to have experimental models of the long-term impact of these combined exposures to better inform public-health policy, particularly because both MeHg and *d*-AMP alter brain monoamines.

MeHg and *d*-AMP both alter DA and affect behavior in adulthood following developmental exposure. MeHg increases DA efflux and inhibits DA reuptake both *in vitro* and in the striatum of behaving rats (Dreiem et al., 2009; Faro et al., 2002). Adolescent MeHg exposure dose-dependently reduces sensitivity to reinforcement magnitude (Boomhower and Newland, 2016) and slows spatial-discrimination

* Corresponding author.
E-mail address: sboomhower@hsph.harvard.edu (S.R. Boomhower).

https://doi.org/10.1016/j.neuro.2019.02.001
Received 11 November 2018; Received in revised form 4 January 2019; Accepted 3 February 2019
Available online 12 February 2019
0161-813X/

reversals and extradimensional shift in adult mice (Boomhower and Newland, 2017). *d*-AMP also increases striatal and prefrontal cortical DA (Andersen et al., 2001) but the effects of chronic adolescent *d*-AMP on choice in adulthood are mixed. Adolescent *d*-AMP administration impairs reversal learning in rodents (Boomhower and Newland, 2017; Hankosky et al., 2013), but no impairment was reported in monkeys (Soto et al., 2012). In combination with adolescent MeHg exposure, chronic *d*-AMP enhanced the effects of MeHg on reversal learning but prevented MeHg-induced impairment on an extradimensional shift (Boomhower and Newland, 2017). These findings suggest the adolescent brain may be vulnerable to *d*-AMP and MeHg exposure, both alone and in combination.

Targeted drug challenges can reveal the impact of developmental exposures on monoamine signaling. In adult rats, gestational MeHg exposure increases sensitivity to the DA-reuptake inhibitors *d*-AMP and cocaine but not to the NE-reuptake inhibitor desipramine (Rasmussen and Newland, 2001; Reed and Newland, 2009). Chronic *d*-AMP administration in adolescent rats dose-dependently enhances firing rates of 5-HT neurons in adulthood, whereas the effects on DA and NE neuron firing rates depend on the dose examined (Labonte et al., 2012). Altered sensitivity to the DA, NE, and 5-HT re-uptake inhibitors *d*-AMP, desipramine, and clomipramine (respectively) would reveal a long-lasting neurochemical impact of adolescent MeHg and *d*-AMP exposure. These three drugs are well suited to assess changes in monoamine function due to their receptor specificity (Artaiz et al., 2005; Han and Gu, 2006; Millan et al., 2001) and their behavioral effects in past work following gestational MeHg exposure in rats (Rasmussen and Newland, 2001; Reed and Newland, 2009).

A theoretically-driven model called Mathematical Principles of Reinforcement (MPR) (Killeen, 1994) can be especially helpful in parsing the behavioral effects of exposure (Boomhower and Newland, 2019). MPR posits that three processes govern voluntary behavior: (a) the value or *specific activation* of a reinforcer, (b) the maximal rate of a target response (e.g., lever pressing), which is the inverse of the *minimum response time* required to complete that behavior, and (c) the rate at which coupling of reinforcers to preceding responses increases, called the *saturation rate* (Table 1). For fixed-ratio schedules of reinforcement, a fixed number of target responses (e.g., lever presses) must occur before a reinforcer is delivered so the saturation rate simply describes how quickly response-reinforcer coupling is maximized as a function of the number of target responses made before reinforcer delivery (Fig. 1). In this way, the saturation rate carries information about the number of preceding responses a reinforcer strengthens. MPR predicts response rate (*b*, responses/sec) as a function of fixed ratio (*n*) using Eq. (1) (Killeen and Sitomer, 2003):

$$b = \frac{1 - e^{-\lambda\delta n}}{\delta} - \frac{n}{\delta a} \quad (1)$$

where a represents specific activation, δ is the minimum response time, and λ is the saturation rate. Boomhower and Newland (2018) found that adolescent MeHg exposure increased saturation rates across two experiments and decreased minimum response times in one experiment. Here, we assess the behavioral effects of adolescent *d*-AMP and MeHg exposure both alone and in combination, and whether adolescent exposure to *d*-AMP and MeHg alter sensitivity to acute *d*-AMP, desipramine, and clomipramine as revealed by the parameters of MPR.

2. Method

2.1. Subjects and exposures

Male C57Bl/6n mice ($n = 48$) were obtained from Envigo Laboratories (Indianapolis, IN) and arrived on postnatal day (PND) 21. Mice were derived from 12 litters with each litter comprising 4 littermates. The mice were pair housed in plexiglas Optimice® cages, given free access to food, and were maintained in a humidity- and

Table 1
A summary of the parameters of Mathematical Principles of Reinforcement (MPR), including their units, meaningful graphical relations, and explanations of the behavioral components they measure.

Parameter	Functional description	Units	Graphical relations	Construct/explanation
δ	Minimum response time	$\frac{seconds}{response}$	*Y intercept* $= \frac{1}{\delta}$	1 The duration of the fastest response cycle under the experimental conditions, including the response device and the subject's health. 2 Its inverse is the maximum response rate.
a	Specific activation	$\frac{responses}{reinforcer}$	X intercept	1 The value of the reinforcer under the experimental conditions; conceptually, it is similar to break point under a progressive ratio. 2 The behavior-activating effects of a single reinforcer.
λ	Saturation rate	$\frac{1}{seconds}$	Slope of the curve's ascending limb	1 The slope of the delay-of-reinforcement gradient, or the rate at which a reinforcer loses value the longer it is temporally removed from a target response. 2 Its inverse is the window of eligibility, which represents the average number of preceding target responses that are strengthened by a reinforcer.
b	Response rate	$\frac{responses}{second}$	The dependent variable along the Y axis	Average response rate, which varies with the fixed ratio schedule and from which parameter estimates are derived.
n	FR value	$\frac{responses}{reinforcer}$	The independent variable along the X axis	The number of responses required for reinforcement.

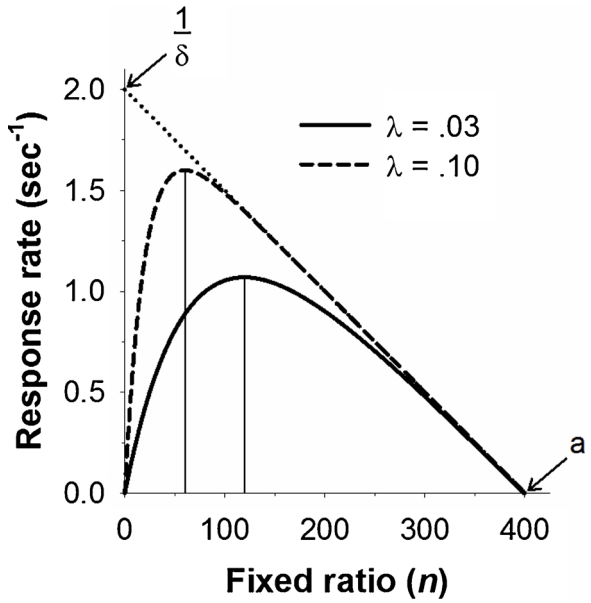

Fig. 1. Mathematical Principles of Reinforcement (MPR) uses specific activation (a), minimum response time (δ), and saturation rate (λ) to predict response rate as a function of fixed ratio (FR). The dotted line is an asymptote, which is defined by the maximum response rate (Y-intercept: $1/\delta$) and the specific activation or value of a reinforcer (X-intercept: a). The solid and dashed lines are drawn using Eq. (1) with $\delta = 0.50$, $a = 400$, and two different saturation rates (see legend above the figure). The dashed line has a relatively high saturation rate ($\lambda = 0.10\,s^{-1}$), which indicates that a lower FR (i.e., FR 60) maximizes response rates. The solid line has a relatively low saturation rate ($\lambda = 0.03\,s^{-1}$), which indicates that a higher FR (i.e., FR 120) maximizes response rates. Both curves indicate an equivalent reinforcer value ($a = 400$ responses) and minimum response time ($\delta = 0.50$ s).

temperature-controlled, AAALAC-accredited animal facility under a 12-hr light/dark cycle (lights on at 6:00 AM). Upon arrival, littermates were randomly distributed across four experimental groups ($n = 12$ mice in each) in a 2 × 2 full-factorial design: control, *d*-AMP, MeHg, and *d*-AMP + MeHg. Littermates were equally distributed among groups, so no litter was represented more than once in a group. Mice were pair housed, and cagemates were always in the same experimental group.

The control and *d*-AMP groups were given tap water, and the MeHg and *d*-AMP + MeHg groups were given 3 ppm MeHg (calculated as Hg) as methylmercuric chloride (Sigma) dissolved in their drinking water. MeHg exposure spanned PND 21 through 59 (Fig. 2) and resulted in a daily dose of about 400 *μg/kg* MeHg (see Boomhower and Newland, 2017). This dose and exposure duration produces neurobehavioral impairment in the absence of overt sensorimotor disturbances in rodents exposed during gestation (Newland et al., 2004; Newland and Rasmussen, 2000) or adolescence (Boomhower and Newland, 2017, 2016). Further, the exposure regimen was identical to the exposure regimen in Boomhower and Newland (2016), who showed that

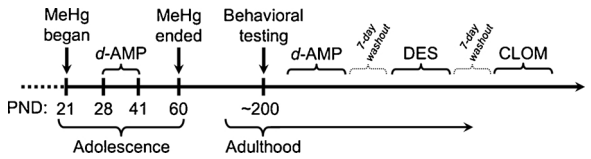

Fig. 2. A timeline of experimental events demonstrating that adolescent mice were exposed to tap water (control) or 3 ppm MeHg. Within these groups, mice received acute injections of saline or *d*-AMP once daily for two weeks. As adults, acute dose-response curves for *d*-AMP, desipramine, and clomipramine were generated, each separated by a week. PND = postnatal day, *d*-AMP = *d*-amphetamine, MeHg = methylmercury, DES = desipramine, CLOM = clomipramine.

adolescent exposure to 3 ppm MeHg via drinking water produced total brain mercury levels of about 2 ppm by the end of the exposure period. At the time of behavioral testing, brain mercury levels were nearly undetectable.

The control and MeHg groups were given once daily i.p. injections of 0.9% saline (injection volume: 0.01 mL/g) for fourteen consecutive days during adolescence (PND 28–41). During the same timespan (PND 28–41), the *d*-AMP and *d*-AMP + MeHg groups received once daily i.p. injections of 1.0 mg/kg/day *d*-AMP. *d*-AMP was delivered as *d*-amphetamine sulfate (Sigma) dissolved in 0.9% saline. This age range was selected because DA-receptor expression peaks in the striatum (Andersen et al., 1997; Gelbard et al., 2000; Teicher et al., 1995), and NE- and 5-HT-transporter expression peaks in the frontal cortex (Moll et al., 2000; Sanders et al., 2005) during this two-week period. To establish milk as a reinforcer and prevent obesity, all mice were maintained at a body mass of 25 (± 1) g by restricting the amount of rodent chow given after experimental sessions to about 2.5 g daily. For reasons unrelated to the present study, three mice ($n = 1$, control; $n = 2$, *d*-AMP alone) died before behavioral testing, and one additional mouse ($n = 1$, *d*-AMP alone) died before acute-drug challenges. All procedures were conducted in accordance with NIH guidelines (National Research Council, 2011) and approved by the Auburn University Institutional Animal Care and Use Committee.

2.2. Apparatuses

Twelve operant chambers (Med Associates®, St. Albans, VT) modified for mice were used for data collection. Two retractable levers were located on the right wall with an alcove centered between them for liquid-reinforcement delivery. Reinforcement consisted of a 3-sec dipper presentation of 0.01-cc of a sweetened-condensed milk solution (3 parts water, 1 part sweetened-condensed milk). Two Sonalert® tone generators (high tone: 4500 Hz, low tone: 2700 Hz) were mounted at the top of the right wall above each lever. Each chamber was enclosed in a sound-attenuating cubicle, and a Windows®-based computer located in a separate, adjacent room controlled all reinforcement contingencies with a 0.01-s resolution. Each mouse was assigned a chamber for the duration of the experiment.

2.3. Procedure

All mice had previous experience pressing levers (see Boomhower and Newland, 2017 for details) so lever-press training was not necessary for this experiment. At approximately PND 200, mice were trained on a multiple fixed-ratio (FR) schedule of reinforcement based on Reilly (2003) with some modifications. A session was divided into six components, each associated with a different FR schedule and signaled by a unique, low/high-alternating tone. The FR schedule increased across components in the following order: FR 1, 5, 15, 30, 60, and 120. The low/high-alternating tone durations (in seconds) associated with each respective FR were as follows: 0.15/1.19, 0.74/.60, 0.92/.42, 1.04/.30, 1.13/.21, 1.19/.15. At the beginning of a session, one lever was inserted into the chamber and the tones sounded. Whether the lever was the left or right lever was counterbalanced across subjects. Upon completion of the FR requirement, the lever was retracted, the tones were extinguished, and 3-sec access to milk reinforcement was made available. The lever was then re-inserted into the chamber. A component ended after the delivery of 12 reinforcers or 10 min, whichever came first. Components were separated by 30 s during which the lever was retracted and tones were extinguished. The multiple FR schedule was in effect for 40 sessions to allow responding to stabilize. An equal number of mice from each exposure group were run in four separate sessions between 1:00 PM and 5:00 PM Monday through Friday.

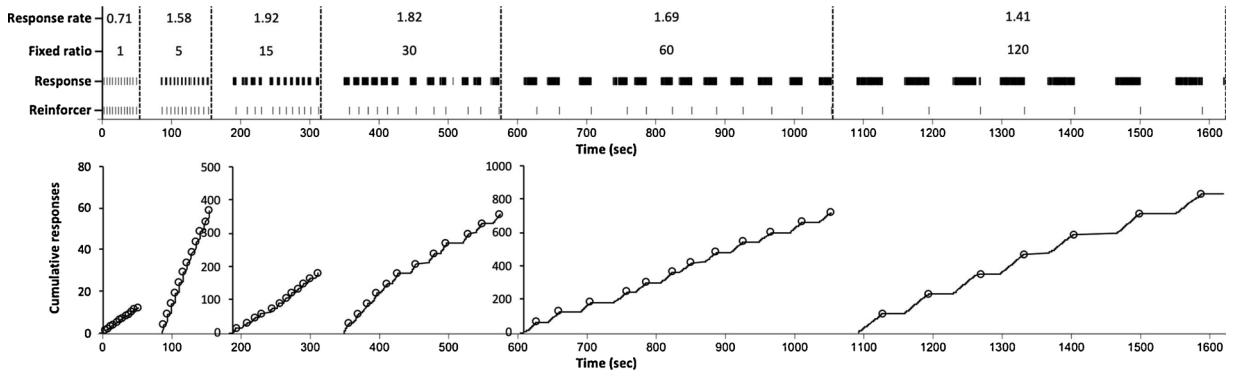

Fig. 3. *Top*: Individual data from a single control mouse under all FR schedules. Vertical dashed lines indicate the termination of a component, either after 12 reinforcers or 10 min (whichever came first), and its corresponding fixed ratio. Response rates (responses/sec) are shown in the top row and are the primary dependent variable for fitting the MPR model. FR value is in Row 2. Responses (Row 3) and reinforcers (Row 4) are shown as tick marks.
Bottom: Data from the same mouse in the top panel are shown as cumulative responses as a function of session duration. Each line shows responding under the different FR values, and open circles denote the delivery of a reinforcer. Note the increase in Y-axis scaling as the session progresses. Note, also, the steady response rate toward the end of each ratio.

2.4. Drugs and drug administration

Following baseline, acute dose-response curves were generated for *d*-AMP (0.3–1.7 mg/kg), then desipramine (5.6–30 mg/kg), and finally clomipramine (5.6–30 mg/kg). *d*-Amphetamine sulfate, desipramine hydrochloride, and clomipramine hydrochloride were dissolved in 0.9% saline as the vehicle. These drugs were chosen for their selectivity to their respective receptor subtypes (Artaiz et al., 2005; Han and Gu, 2006; Millan et al., 2001) and their behavioral effects in past work (Rasmussen and Newland, 2001; Reed and Newland, 2009), which demonstrated that the dose ranges used here captured changes in drug sensitivity following gestational MeHg exposure in rats. Previous work reported that *d*-amphetamine, desipramine, and clomipramine have half-lives of approximately 60 min (Fuller et al., 1972; Miller et al., 1971), 3–4 h (Kozisek et al., 2007), and 54 min (Marty et al., 1992), respectively, in rats and mice. Drug injections occurred on Tuesdays and Fridays, saline vehicle was given on Thursdays, and Mondays and Wednesdays served as non-injection controls. All injections were given i.p. at a volume of 0.01 ml/g and occurred 5 min prior to sessions. The order of dosing was counterbalanced across squads using a Latin-Square design. Each mouse received an acute dose once. Only in three instances, each in a different mouse, was it necessary to repeat a dose because no responding was recorded for an entire session. In these instances, data from both the first and second dose were averaged together. Following the completion of a dose-response curve, a seven-day washout period in which no injections were given before sessions occurred before the next dose-response curve was generated (Fig. 2).

2.5. Data analysis

The primary dependent variables were response rates under each component of the multiple FR procedure and parameter estimates derived from Eq. (1). Response rates were calculated as the number of responses divided by the time (in seconds) available to respond (i.e., the lever was extended) during a component. Response rates were averaged across the last 10 sessions for baseline and were compared among groups using a linear mixed effects (LME) model (Systat Software, v13, Richmond, CA) with exposure (control, *d*-AMP, MeHg, and *d*-AMP + MeHg) and FR (1, 5, 15, 30, 60, and 120) as a fixed effect and litter as a random effect and repeated measure. LME was chosen for all analyses because it is able to model incomplete repeated-measures data more effectively than traditional repeated-measures ANOVA. Eq. (1) was fit to individual-subject response-rate functions using nonlinear least-squares regression. Because of the presence of outliers, estimates of a, δ, and λ were compared among groups using 20% Winsorized means and variance (Boomhower and Newland, 2017; Pope et al., 2016), which minimizes the influence of outliers and stabilizes variability without compromising sample size (Wilcox, 2012, 1998; Wilcox and Keselman, 2003). In some cases, the distribution of a remained highly skewed so the inverse of a ($1/a$) was used for all analyses and is shown as that variable in figures (Reilly, 2003). Where appropriate, Tukey post-hoc comparisons were used to determine differences between groups. For dose-response determinations, response rates were compared among groups using a LME model with exposure, FR, and dose as fixed effects and litter as a random effect. Behavior was also examined during the washout period between each acute dose-effect determination. For this analysis, response rates were averaged across the last five non-injection days. Parameter estimates were compared among groups using a LME model with exposure and dose as fixed effects and litter as a random effect.

3. Results

3.1. Baseline

Fig. 3 shows an event and cumulative record from a single control mouse. Response rate, FR, responses, and reinforcers (top panel) and cumulative responses (bottom panel) are shown as a function of time. Overall, the response rate toward the end of each FR was stable. Response rate is the primary dependent variable of the MPR analysis.

The top of Fig. 4 shows mean (left panels) and individual-subject (right panels) response rates as a function of FR for mice exposed to *d*-AMP, MeHg, and *d*-AMP + MeHg during adolescence. Fits from Eq. (1) are shown as lines. A main effect of FR [$F(5, 245) = 29.32$, $p < 0.001$] indicated that response rates were a bitonic function of FR, dramatically increasing and peaking around FR 15 and then gradually decreasing. Even the poorest fits of Eq. (1) fit the data well. Differences in the shapes of the curve were captured in the parameter estimates derived from Eq. (1) shown at the bottom of Fig. 3. There were main effects of exposure on both λ [$F(3, 40) = 6.41$, $p < 0.01$] and δ [$F(3, 40) = 2.88$, $p < 0.05$]. Post-hoc comparisons revealed the MeHg group had significantly higher estimates of λ than the other groups of mice, indicating that a lower FR maximized response rates for MeHg-exposed mice, and these differences were mitigated by *d*-AMP + MeHg exposure. Post-hoc comparisons revealed also that MeHg-exposed mice had longer estimates of minimum response time, evident in a lower maximum response rate (Y-intercept). There were no effects on estimates of $1/a$ [$F(3, 40) = 2.02$, $p = 0.13$].

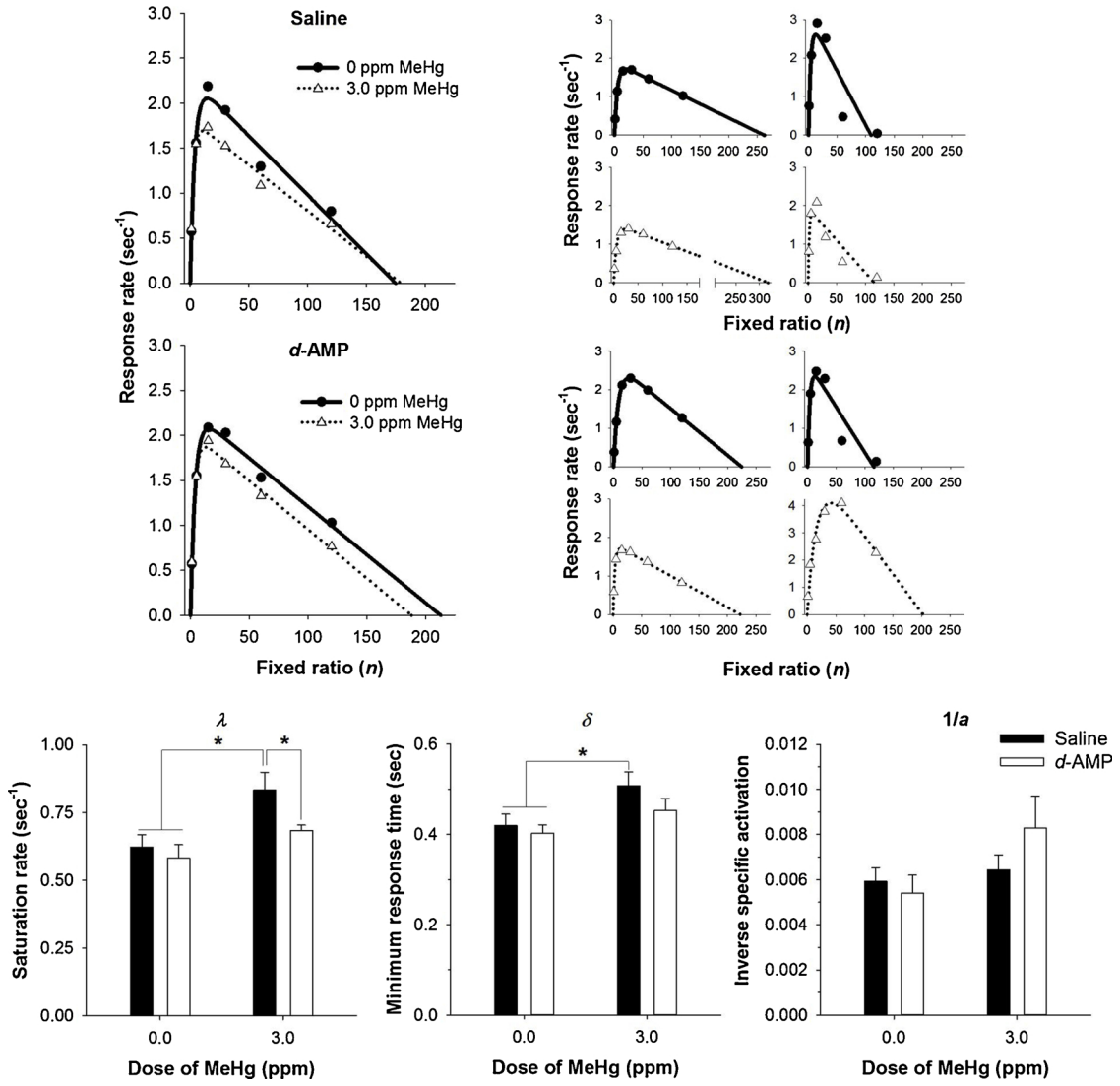

Fig. 4. *Top left*: Mean response rate as a function of FR requirement for mice exposed to MeHg during adolescence. Data from mice who received injections of saline or *d*-AMP are shown in the top and bottom panel, respectively. Lines represent the mean fits of Eq. (1).
Top right: Individual-subject data from mice in each experimental group with the best (left column) and worst (right column) fits of Eq. (1). The top four panels show data from the saline group, and the bottom four panels show data from the *d*-AMP group.
Bottom: Mean (+SEM) parameter estimates derived from Eq. (1) for mice exposed to MeHg, *d*-AMP, or *d*-AMP + MeHg during adolescence. Note specific-activation estimates are inverse transformed ($1/a$).
d-AMP = *d*-amphetamine, MeHg = methylmercury; *$p < 0.05$.

3.2. Acute d-AMP administration

Fig. 5 shows mean response rates as a function of FR after vehicle and different doses of *d*-AMP for mice exposed to *d*-AMP, MeHg, and *d*-AMP + MeHg during adolescence. Lines represent mean fits of Eq. (1) for each dose of *d*-AMP. A significant FR X dose interaction [$F(25, 1424) = 4.27$, $p < 0.001$] indicated that *d*-AMP dose-dependently reduced response rates to the greatest extent under midrange FRs. Main effects of dose [$F(5, 1424) = 52.24$, $p < 0.001$] and FR [$F(5, 1424) = 110.91$, $p < 0.001$] were reflected in a dose-dependent decrease in response rate as well as a generally bitonic effect of FR on response rate, respectively. A main effect of exposure [$F(3, 1424) = 11.46$, $p < 0.001$] indicated that control mice on average responded more than the other groups across all FR values and *d*-AMP doses.

Dose-effect curves showing mean parameter estimates as a function of acute drug dose are shown in Fig. 8. The top row of Fig. 8 shows the effect of *d*-AMP on saturation rate, minimum response time, and specific activation. For λ, main effects of dose [$F(5, 235) = 28.48$, $p < 0.001$] and exposure [$F(3, 235) = 14.86$, $p < 0.001$] indicated that acute *d*-AMP dose-dependently decreased λ from vehicle, and *d*-AMP mice generally had the lowest λ estimates, respectively. There was no dose X exposure interaction, indicating that *d*-AMP's decrease in λ depended only on the baseline value, which was determined by adolescent exposures. For δ, a significant exposure X dose interaction [$F(15, 235) = 2.33$, $p < 0.01$] was reflected in an enhanced sensitivity to acute *d*-AMP in the *d*-AMP + MeHg group, particularly at the 1.0 mg/kg dose. Main effects of dose [$F(5, 235) = 6.84$, $p < 0.001$] and exposure [$F(3, 235) = 5.22$, $p < 0.01$] indicated, respectively, that minimum response times lengthened as a function of *d*-amphetamine dose and were longest among MeHg and *d*-AMP + MeHg mice. For $1/a$, main effects of exposure [$F(3, 235) = 5.39$, $p = 0.001$] and dose [$F(5, 235) = 13.50$, $p < 0.001$] were reflected in generally lower $1/a$ estimates for control and *d*-AMP-exposed mice and a dose-dependent decrease in $1/a$ estimates for all groups, respectively. Thus, the reinforcer value (a) increased as a function of *d*-AMP dose.

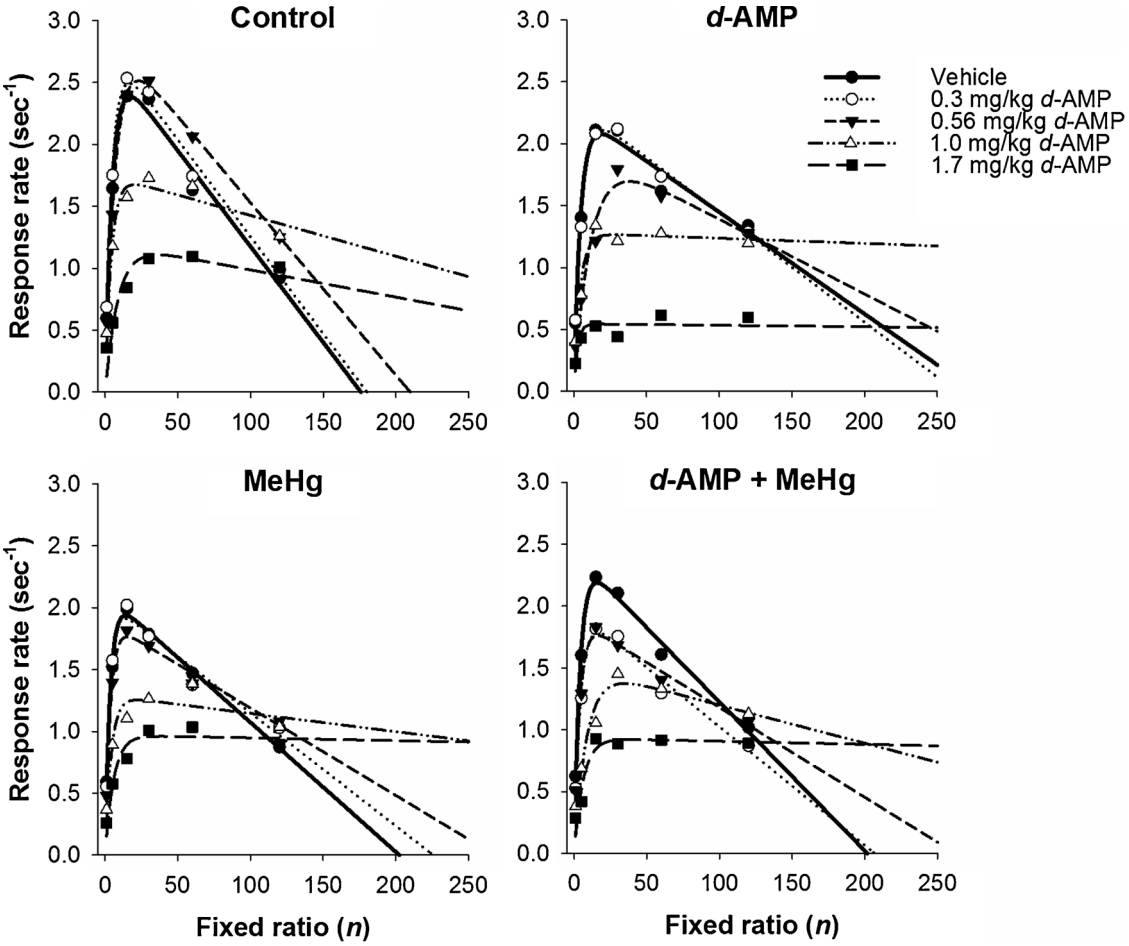

Fig. 5. Mean response rate as a function of FR requirement under vehicle and doses of *d*-AMP for mice exposed to MeHg, *d*-AMP, and *d*-AMP + MeHg during adolescence. Lines represent the mean fits of Eq. (1) for each dose. *d*-AMP = *d*-amphetamine, MeHg = methylmercury.

3.3. Acute desipramine administration

Fig. 6 shows mean response rates as a function of FR for each dose of desipramine in control, *d*-AMP, MeHg, and *d*-AMP + MeHg mice. Lines represent the mean fits of Eq. (1) for each dose. A significant FR X dose interaction [$F(25, 1433) = 1.51$, $p = 0.05$] revealed that desipramine, like *d*-AMP, typically reduced response rates to a greater extent under midrange FRs than low (FR 1) or high (FR 120) ones. Main effects of dose [$F(5, 1433) = 16.21$, $p < 0.001$] and FR [$F(5, 1433) = 154.96$, $p < 0.001$] indicated a dose-dependent decrease in response rates as well as a bitonic effect of FR on response rate, respectively. A main effect of exposure [$F(3, 1433) = 3.69$, $p = 0.01$] was evidenced in generally higher response rates for control and *d*-AMP + MeHg mice.

The second row of Fig. 8 shows parameter estimates as a function of desipramine dose for the four experimental groups. For λ, a significant exposure X dose interaction [$F(15, 236) = 2.58$, $p < 0.01$] was evident in that differences in λ estimates among groups were greatest for control conditions and after the low doses of desipramine and were eliminated under the high doses. A main effect of dose [$F(5, 236) = 36.80$, $p < 0.001$] reflected a dose-dependent decrease in saturation rate by desipramine. There was, however, an increase in λ over vehicle at the 17 mg/kg dose for the *d*-AMP group. A main effect of exposure [$F(3, 236) = 11.36$, $p < 0.001$] indicated that *d*-AMP mice typically had lower λ estimates, whereas the *d*-AMP + MeHg group had the highest. For δ, a significant dose X exposure interaction [$F(15, 236) = 2.39$, $p < 0.01$] showed that δ estimates in both *d*-AMP mice and *d*-AMP + MeHg mice were more sensitive to desipramine than controls. A main effect of dose [$F(5, 236) = 9.26$, $p < 0.001$] indicated a dose-dependent increase in minimum response time. For $1/a$ estimates, there was a main effect of exposure [$F(3, 236) = 15.47$, $p < 0.001$] in that $1/a$ estimates for *d*-AMP mice were lowest relative to other groups. Further, a main effect of dose [$F(5, 236) = 3.45$, $p < 0.01$] indicated that desipramine increased $1/a$ estimates under low doses though this was primarily for the *d*-AMP + MeHg group.

3.4. Acute clomipramine administration

Fig. 7 shows mean response rates as a function of FR for each experimental group under acute doses of clomipramine. Again, lines represent mean fits of Eq. (1). A significant dose X FR interaction [$F(25, 1433 = 4.55$, $p < 0.001$] showed that response rates under midrange FRs were typically suppressed more than response rates under the lowest and highest FRs. Also, a significant exposure X dose interaction [$F(15, 1433) = 2.71$, $p < 0.001$] indicated that clomipramine dose-dependently decreased response rates for *d*-AMP mice to the greatest extent relative to other groups. Main effects of FR [$F(5, 1433) = 118.61$, $p < 0.001$] and exposure [$F(3, 1433) = 5.96$, $p < 0.001$] indicated response rates were a bitonic function of FR and control animals typically responded more, respectively.

The bottom row of Fig. 8 shows mean parameter estimates from Eq. (1) as a function of clomipramine dose. For λ, a main effect of exposure [$F(3, 224) = 15.75$, $p < 0.001$] indicated that λ estimates were lowest for control mice and all adolescent exposures blunted the impact of clomipramine on λ. A main effect of dose [$F(5, 224) = 49.87$, $p < 0.001$] indicated that clomipramine dose-dependently decreased λ estimates for all groups, respectively. For δ, a significant exposure X

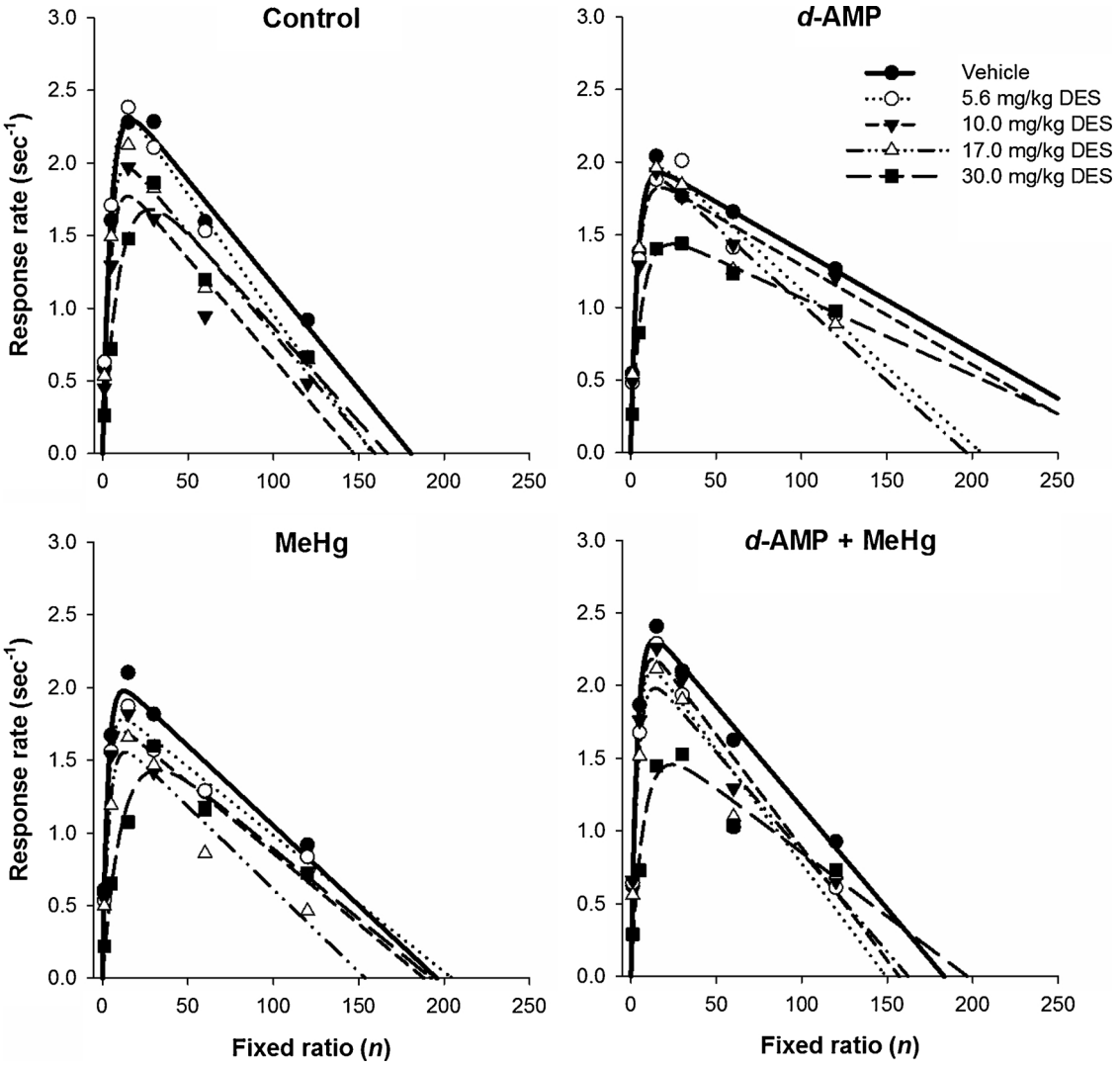

Fig. 6. Mean response rate as a function of FR requirement under vehicle and doses of desipramine for mice exposed to MeHg, *d*-AMP, and *d*-AMP + MeHg during adolescence. Lines represent the mean fits of Eq. (1) for each dose. *d*-AMP = *d*-amphetamine, MeHg = methylmercury, DES = desipramine.

dose interaction [$F(15, 224) = 4.99$, $p < 0.001$] was evident in that δ estimates for *d*-AMP group were increased to a greater extent by clomipramine, corresponding with the decrease in maximum response rate, whereas minimum response times did not systematically change as a function of clomipramine for control, MeHg, and *d*-AMP + MeHg mice. There were main effects of exposure [$F(3, 224) = 7.90$, $p < 0.001$] and dose [$F(5, 224) = 4.91$, $p < 0.001$], but these were subsumed by the exposure X dose interaction. For $1/a$, a main effect of dose [$F(5, 224) = 12.53$, $p < 0.001$] indicated a bitonic effect of clomipramine on $1/a$ estimates with the highest dose reducing $1/a$ estimates, indicative of an increase in reinforcer value. There was no effect of exposure or significant exposure X dose interactions on $1/a$ estimates.

3.5. Washout data

Fig. 9 shows saturation rate (left), minimum response time (middle), and inverse specific activation (right) in the sessions preceding each dose-effect determination for each group of mice. For λ, there were main effects of exposure [$F(3, 120) = 7.16$, $p < 0.001$] and timepoint [$F(2, 120) = 8.28$, $p < 0.001$], indicating that MeHg-exposed mice had higher saturation rates than controls and these were highest before the *d*-AMP dose-response determination (respectively). There was no significant interaction. For δ, there were main effects of exposure [$F(3, 120) = 5.14$, $p < 0.01$] and timepoint [$F(2, 120) = 14.90$, $p < 0.001$], indicating that MeHg-exposed mice had higher minimum response times on average than the other groups and minimum response time decreased after the *d*-AMP dose-response determination (respectively). There was no significant interaction. For $1/a$, main effects of exposure [$F(3, 119) = 3.42$, $p = 0.02$] and timepoint [$F(2, 119) = 5.05$, $p < 0.01$] indicated that the *d*-AMP + MeHg group typically had higher $1/a$ estimates and $1/a$ estimates decreased following the *d*-AMP dose-response determination.

4. Discussion

4.1. Behavioral effects of adolescent MeHg and d-AMP exposure

Adolescent MeHg exposure increased saturation rate indicating that coupling between the target response immediately preceding reinforcement and the reinforcer increased more rapidly as a function of FR for that group. Stated differently, fewer responses on average were coupled to reinforcers. This suggests that the delay-of-reinforcer gradient was steeper in adolescent MeHg-exposed mice than controls. Boomhower and Newland (2018) also found in two independent experiments that adolescent MeHg exposure increased saturation rate in mice, so this effect is reproducible. MeHg exposure in adolescence impairs performance on a second reversal and an extradimensional shift as well as reinforcer sensitivity in mice (Boomhower and Newland,

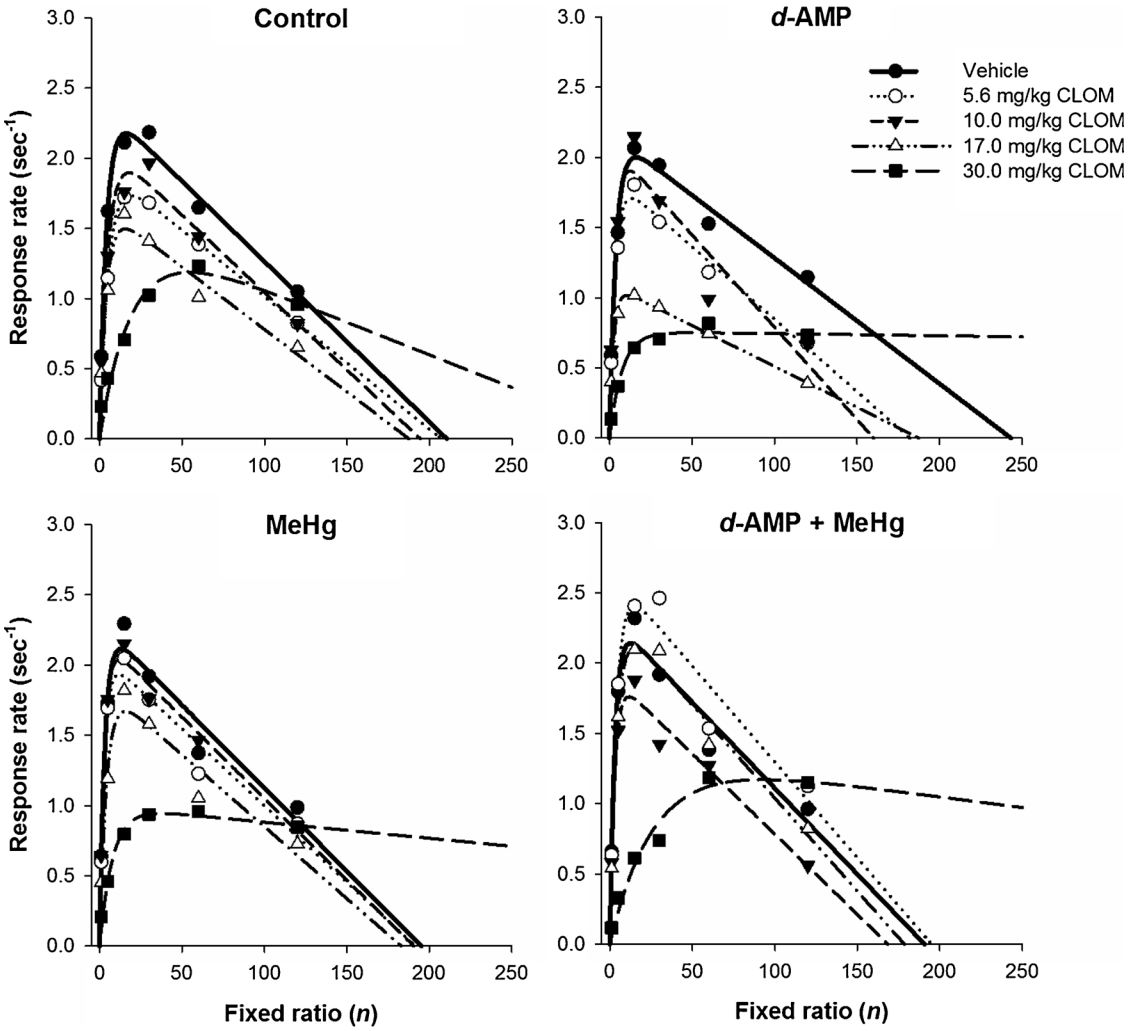

Fig. 7. Mean response rate as a function of FR requirement under vehicle and doses of clomipramine for mice exposed to MeHg, *d*-AMP, and *d*-AMP + MeHg during adolescence. Lines represent the mean fits of Eq. (1) for each dose. *d*-AMP = *d*-amphetamine, MeHg = methylmercury, CLOM = clomipramine.

2017, 2016). The present study adds to a growing body of work suggesting that deficits in the impact of reinforcement on responding may be related to impaired reversal learning and reinforcer processing. Specifically, deficits in reversal learning following developmental exposures could result from a degradation in the coupling of responses to reinforcers in that reinforcers only strengthen the last one or two responses before reinforcement.

MeHg exposure alone in adolescence increased estimates of minimum response time in the present study, an outcome that would reduce response rate. Conversely, Boomhower and Newland (2018) found that 3 ppm MeHg in adolescence reduced estimates of minimum response time after controlling for the influence of an outlier in one experiment, but minimum response time was unchanged following exposure in a second experiment. In that regard, whether the effects of MeHg on minimum response time represent an important behavioral effect of adolescent exposure is debatable. If the effect is real, then it is subtle and difficult to reproduce. It is well-established also that chronic adult-onset MeHg exposure impairs motor behavior and function in rodents after prolonged exposure (Heath et al., 2010; Shen et al., 2016). If minimum response time changes following a much shorter MeHg exposure duration then that would suggest that the adolescent period may be particularly susceptible to the motoric effects of MeHg exposure, δ is an especially sensitive measure, or both. For example, MeHg exposure during gestation, another sensitive developmental window, increased response durations in aged rats (Newland and Rasmussen, 2000). It can be noted though that minimum response time is subtler than the grosser measures of motor behavior, such as wheel running, grip strength, and limb flexion, measured in Heath et al. (2010) and nose poking in Shen et al. (2016).

Chronic administration of *d*-AMP in adolescence mitigated MeHg-induced changes in minimum response time and saturation rate. We also observed that baseline differences in MPR's parameters among the treatment groups were mitigated following the dose-response determination of *d*-AMP. That is, the MeHg group, which had higher saturation rates and minimum response times than the other groups under baseline, tended to resemble controls after the acute *d*-AMP dose-response determination. Previous work has shown that concurrent administration of chronic *d*-AMP during adolescence prevented MeHg-induced impairment of an extradimensional shift but enhanced the effects of MeHg on reversal learning in these mice (Boomhower and Newland, 2017). Our results provide further support that chronic administration of *d*-AMP in adolescence can modulate the behavioral effects of adolescent MeHg exposure in mice in addition to its direct effects when administered alone.

It should be noted that we did not include adult comparison groups who received identical exposures as the adolescent mice in our study, which would be necessary to determine whether adolescents were more vulnerable than adults to the effects of MeHg or *d*-AMP on behavior. However, past work in adult mice chronically exposed to MeHg showed that sensorimotor and learning effects only appeared following longer

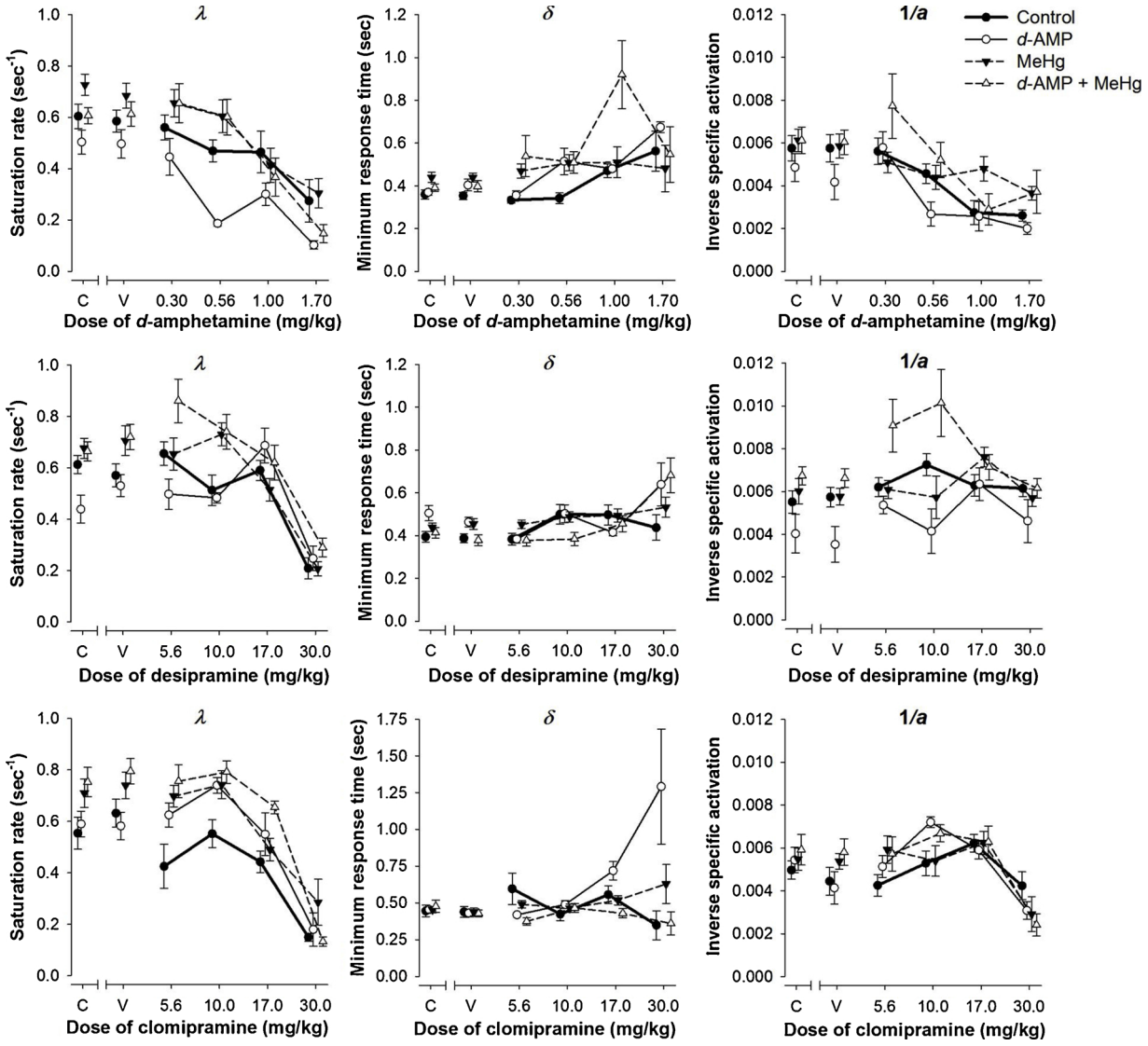

Fig. 8. Mean (± SEM) parameter estimates derived from Eq. (1) for mice exposed to MeHg, *d*-AMP, or *d*-AMP + MeHg during adolescence. Data for *d*-amphetamine (top row), desipramine (middle row), and clomipramine (bottom row) are shown. Note the change in Y-axis scaling for the panel showing the effects of clomipramine on minimum response time. Also note specific-activation estimates are inverse transformed (1/*a*). *d*-AMP = *d*-amphetamine, MeHg = methylmercury, C = non-injection control, V = vehicle.

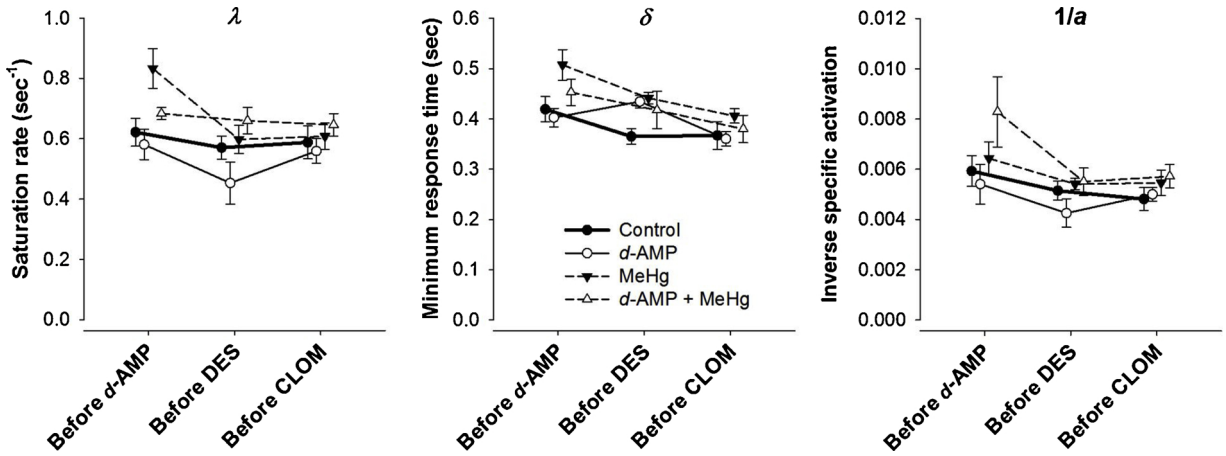

Fig. 9. Mean (± SEM) parameter estimates from the five sessions prior to each acute dose-response determination. *d*-AMP = *d*-amphetamine, MeHg = methylmercury, DES = desipramine, CLOM = clomipramine.

exposures to higher concentrations of MeHg (i.e., 10 ppm MeHg for greater than 60 days) (Bailey et al., 2013; Shen et al., 2016). This suggests that adult MeHg-exposed animals are probably less sensitive to MeHg's behavioral effects than adolescent mice but a direct comparison is impossible because of the different behavioral procedures used.

4.2. Behavioral effects of acute d-AMP, desipramine, and clomipramine in unexposed mice

This is the first study to report effects of three monoamine reuptake inhibitors on the parameters deriving from MPR, and the results support the idea that drugs can target separate and independent aspects of behavior. Acute administration of *d*-AMP changed all estimates of MPR's parameters in control mice in a dose-related fashion. *d*-AMP reduced saturation rate monotonically, indicating that reinforcers strengthened more responses than under vehicle. *d*-AMP also dose-dependently increased minimum response time, which is reflected in a decrease in maximum response rates. Finally, *d*-AMP dose-dependently increased specific activation (i.e., reduced $1/a$ estimates), reflecting enhanced reinforcer efficacy. The sensitivity of MPR parameters to acute *d*-AMP reported here is consistent with previous work using an MPR approach (Mobini et al., 2000; Reilly, 2003) as well as those from a different model (Heyman and Seiden, 1985) in rats. It should be noted, however, that Reilly (2003) found that acute *d*-AMP in rats increased λ and $1/a$ estimates, which is opposite to our findings in the C57Bl/6n mouse. The effects of acute *d*-AMP on choice for delayed reinforcers depends on procedural variables (Krebs et al., 2016; Maguire et al., 2014), particularly related to stimuli signaling reinforcement (Slezak and Anderson, 2009). Reilly (2003) used visual stimuli to signal the fixed ratio in effect, whereas we used auditory stimuli. These procedural differences or genetic differences may explain the opposing effects of *d*-AMP on saturation rate and the impact of reinforcement on past responding.

The greatest effects of desipramine in control mice were confined to saturation rate. The highest dose of desipramine reduced saturation rate relative to vehicle, indicating the impact of reinforcement on previous responses was enhanced. Midrange doses of desipramine (10 and 17 mg/kg) increased minimum response times in control mice. Desipramine enhances choice for larger-delayed reinforcers (Bizot et al., 2011) and reduces premature responses under a differential reinforcement of low-rate schedule in rats (Dekeyne et al., 2002). These effects are consistent with the results we report here in that desipramine both strengthened the impact of reinforcers on previous responses and slowed response rates.

Similar to desipramine, the effects of clomipramine were mostly confined to saturation rate in control mice. Clomipramine reduced saturation rates at all doses but more so at the highest doses (17 and 30 mg/kg), indicating reinforcers captured a greater number of previous responses. Clomipramine did not systematically alter minimum response time. Reinforcer value decreased ($1/a$ estimates increased) at the middle doses and increased at the highest dose. Past work suggests clomipramine does not alter responding for delayed reinforcers (Bayley et al., 1998), though the doses used in Bayley et al. (1–10 mg/kg) were lower than the doses that reduced saturation rate in our study. Other 5-HT reuptake inhibitors, such as fluoxetine, have also been shown to reduce reinforcer value using an MPR analysis in rats, an effect similar to our results (Sanabria et al., 2008). We report that high doses of clomipramine enhance the number of responses coupled to reinforcers.

A primary goal of the present study was to evaluate how adolescent exposure to *d*-AMP and MeHg altered sensitivity to monoaminergic drugs. Thus, the effects of adolescent exposure to *d*-AMP and MeHg both alone and in combination on adult sensitivity to *d*-AMP, desipramine, and clomipramine were interpreted relative to the drug effects in control animals.

4.3. Effects of adolescent d-AMP on sensitivity to d-AMP, desipramine, and clomipramine

Mice exposed to *d*-AMP during adolescence had the lowest saturation rates under acute *d*-AMP of all groups. Chronic adolescent *d*-AMP enhances DA neuron firing rates in the rat midbrain (Labonte et al., 2012) and impairs reversal learning in mice and rats (Boomhower and Newland, 2017; Hankosky et al., 2013). Our results here indicate that chronic adolescent exposure to *d*-AMP enhances in an additive fashion *d*-AMP's effects on the coupling of responses to reinforcers in adulthood.

Adolescent *d*-AMP administration increased sensitivity to desipramine, and this was mostly confined to minimum response time. The highest dose of desipramine (30 mg/kg) increased minimum response times in *d*-AMP mice, an effect not present in control mice. Dopamine depletions (Guiard et al., 2008) as well as chronic administration of 5 mg/kg *d*-AMP in adolescent rats enhance firing rates of NE neurons in the locus coeruleus, which projects to the prefrontal cortex (Labonte et al., 2012). That alterations in desipramine sensitivity were only noted with minimum response time is consistent with past work on desipramine's motoric effects (Weber et al., 2009) as well as the use of desipramine to treat motor impairment following DA depletions (Kamińska et al., 2017). Our findings show chronic administration of 1 mg/kg *d*-AMP in adolescence enhances sensitivity to the response-slowing effects of a high dose of desipramine, suggesting NE signaling is altered by low-dose adolescent *d*-AMP administration.

d-AMP mice also displayed increased sensitivity to clomipramine's effects on minimum response time. Whereas clomipramine did not systematically alter minimum response time in control mice, clomipramine dose-dependently increased minimum response times in *d*-AMP mice. Adolescent *d*-AMP administration enhances 5-HT neuron firing rates in the adult rat dorsal raphe (Labonte et al., 2012), most likely due to the reciprocal interactions of midbrain DA and dorsal-raphe 5-HT neurons (Guiard et al., 2008). Here, we demonstrate that adolescent *d*-AMP administration increases sensitivity to the motoric effects of clomipramine, similar to desipramine. That *d*-AMP-induced sensitivity to clomipramine was confined to minimum response time is consistent with studies using clomipramine (and other 5-HT reuptake inhibitors) to treat stimulant-induced stereotypies in rodents (Eilam and Szechtman, 2005). The lasting effects of chronic *d*-AMP administration in adolescence on adult sensitivity to *d*-AMP, desipramine, and clomipramine underscore the vulnerability of the adolescent brain and behavior to monoamine-altering drugs.

4.4. Effects of adolescent MeHg on sensitivity to d-AMP, desipramine, and clomipramine

Adolescent MeHg exposure overall did not significantly alter sensitivity to *d*-AMP, desipramine, or clomipramine. That is, the effects of *d*-AMP, desipramine, and clomipramine on MPR's parameters in MeHg-exposed mice largely resembled the effects in control mice. Gestational MeHg exposure increases sensitivity to *d*-AMP and cocaine but not desipramine in adult rats (Rasmussen and Newland, 2001; Reed and Newland, 2009). In regard to 5-HT, past work suggests MeHg exposure reduces extracellular 5-HT in the zebrafish brain (Maximino et al., 2011; Puty et al., 2014), and chronic treatment with fluoxetine, a 5-HT reuptake inhibitor, rescues gestational MeHg-induced behavioral deficits in rats (Onishchenko et al., 2008). Our data show that adolescent exposure to 3 ppm MeHg alone does not significantly alter sensitivity to drugs that enhance DA, NE, and 5-HT. Thus, the adolescent brain may display less vulnerability to MeHg's monoamine-altering effects than the developing brain *in utero*, particularly related to MeHg's effects on DA and 5-HT in past work.

4.5. Effects of adolescent d-AMP + MeHg on sensitivity to d-AMP, desipramine, and clomipramine

Though adolescent MeHg exposure alone did not alter sensitivity to *d*-AMP, desipramine, or clomipramine, combined exposure to MeHg + *d*-AMP in adolescence did alter drug sensitivity over and above the effects of adolescent MeHg or *d*-AMP alone. Adolescent *d*-AMP + MeHg exposure increased sensitivity to acute *d*-AMP in adulthood, an effect that was confined to minimum response time. The 1 mg/kg dose in particular increased minimum response times in *d*-AMP + MeHg mice by approximately two-fold relative to the control, *d*-AMP, and MeHg mice. In previous work, combined exposure to *d*-AMP + MeHg in adolescence enhances perseverative errors and slows the transition through a spatial-discrimination reversal in mice (Boomhower and Newland, 2017). DA signaling in the striatum is both important for reversal learning in monkeys and rodents (Clarke et al., 2011; O'Neill and Brown, 2007) as well as motor learning (Hikosaka et al., 2002). Further, DA depletions in the substantia nigra selectively increase minimum response time using an MPR analysis in rats (Avila et al., 2009). Thus, enhanced sensitivity to the response-slowing effects of acute *d*-AMP following *d*-AMP + MeHg exposure in adolescence is consistent with the notion that striatal DA may be a target of these combined exposures.

Combined exposure to *d*-AMP + MeHg in adolescence increased sensitivity to desipramine's effects on minimum response time and specific activation. Specifically, desipramine dose-dependently increased minimum response times in *d*-AMP + MeHg mice, an effect that was nearly identical in *d*-AMP mice but not present in controls. Both 5.6 and 10 mg/kg of desipramine also reduced reinforcer value (increased $1/a$ estimates) in *d*-AMP + MeHg mice, whereas only the 10 mg/kg dose reduced reinforcer value in control mice. Mice exposed to adolescent MeHg alone are slower to acquire an extradimensional shift, and these deficits are reversed by concurrent administration of chronic *d*-AMP in adolescence (Boomhower and Newland, 2017). Impaired extradimensional shifting is associated with reduced NE signaling in the rodent medial prefrontal cortex (McGaughy et al., 2008), whereas increasing NE activity facilitates an extradimensional shift (Lapiz and Morilak, 2006). As noted above, we show that adolescent *d*-AMP administration functionally enhanced the behavioral effects of a NE agonist in adulthood. This interpretation could explain why chronic *d*-AMP in adolescence mitigated the effects of adolescent MeHg exposure on an extradimensional shift in past work (Boomhower and Newland, 2017).

Adolescent *d*-AMP + MeHg exposure did not alter sensitivity to clomipramine relative to control mice. Whereas chronic *d*-AMP administration alone in adolescence greatly enhanced clomipramine's effects on minimum response time, combined exposure to *d*-AMP + MeHg in adolescence mitigated this sensitization. MeHg exposure reduces whole-brain extracellular 5-HT in zebrafish (Maximino et al., 2011). It is unlikely, though, that adolescent MeHg exposure reduced 5-HT levels sufficiently to counteract the effects of adolescent *d*-AMP on clomipramine sensitivity—particularly because adolescent MeHg alone did not alter clomipramine sensitivity. Regardless, our data suggest 5-HT signaling is particularly vulnerable to chronic *d*-AMP administration in adolescence, and MeHg exposure during adolescence can alter this relation.

5. Conclusions

Through the use of a theoretically-driven mathematical model of behavior, we dissected the acute effects of monoamine drugs on three behavioral mechanisms as well as determined how adolescent exposure to *d*-AMP + MeHg altered drug effects. Importantly, all three monoamine uptake inhibitors had a unique pattern of effects on MPR's parameters. This underscores the utility of the MPR model in characterizing drug effects on separable aspects of behavior. Adolescent exposure to chronic *d*-AMP, both alone or in combination with adolescent MeHg exposure, can modify sensitivity to the monoamine uptake inhibitors *d*-AMP, desipramine, and clomipramine. Adolescent *d*-AMP administration enhanced sensitivity to all three drugs, particularly clomipramine, but in different ways. Motor effects dominated the desipramine and clomipramine dose-effect profiles for mice exposed to *d*-AMP during adolescence while response-reinforcer coupling and reinforcer efficacy dominated acute *d*-AMP's dose-effect profile for these mice.

Adolescent MeHg exposure alone impaired response-reinforcer coupling and minimum response times but did not alter sensitivity to acute *d*-AMP, desipramine, or clomipramine. However, the combination of adolescent MeHg and *d*-AMP exposure altered sensitivity to acute *d*-AMP and desipramine. Specifically, minimum response time dominated *d*-AMP's dose-effect profile while both minimum response time and reinforcer value dominated desipramine's dose-effect profile following combined MeHg + *d*-AMP exposure. This study implicates monoamine uptake in mediating the behavioral effects of adolescent *d*-AMP and MeHg exposure. Determining the extent to which developmental exposures to drugs and neurotoxicants alter sensitivity to psychopharmaceuticals later in life is crucial to public health.

Funding and disclosure

This research was supported by the National Science Foundation Graduate Research Fellowship Program (DGE-1414475) to SRB and NIHES024850 to MCN. The authors declare no conflict of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

Special thanks to Katelyn Johnson, Joseph McIlwain, Madison Morlan, Alex Sauer, and Savannah Simpson for help with data collection.

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