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EXAMINING THE EFFECTS OF SOCIAL ISOLATION ON NICOTINE PREFERENCE AND MESOLIMBIC DOPAMINE

by

Nicholas Bross Paige

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Abstract

Factors relating to social interaction have been shown to alter patterns of psychostimulant use in preclinical and clinical models. The present study aimed to determine the effects of social isolation on nicotine preference using conditioned place preference (CPP) and the effects of social isolation and nicotine exposure on reward-related dopamine release in the nucleus accumbens (NAc). Regarding CPP results, there was a significant housing (group or isolated) x drug (nicotine or saline) x trial interaction on time spent in the drug-paired chamber. Regarding dopamine recordings, there was a significant housing x drug exposure (nicotine or saline) x time (60 min recording period) interaction on percent change in dopamine half-life following cocaine. At cocaine's peak effect, isolation and nicotine exposure both independently increased this dopaminergic response, but an interactive effect between these variables was not significant. Identifying risk factors for drug abuse is critical for prevention and treatment programs.

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Abbreviations

ANOVA = Analysis of Variance

CPP = Conditioned Place Preference

DAT = Dopamine Transporter

FSCV = Fast Scan Cyclic Voltammetry

FPA = Fixed Potential Amperometry

NAc = Nucleus Accumbens

nAChR = Nicotinic Acetylcholine Receptor

VTA = Ventral Tegmental Area

Examining the Effects of Social Isolation on Nicotine Preference and Mesolimbic Dopamine Functioning

Approximately 90% of today's smokers initiated smoking before the age of 18, and roughly 8% of high school students smoke cigarettes (Center for Disease Control, 2017). Difranza and colleagues (2002) found that adolescents can undergo tobacco dependency within a day of the first inhale and experience a number of withdrawal symptoms such as cravings, restlessness, and sadness. In the past few decades cigarette prevalence has steadily declined among adolescents (Miech et al., 2018); however, recent years have brought an alarming increase in adolescent nicotine exposure due to the popularity of vaping devices. From 2017 to 2018, nicotine vaping increased by 10.9 percent in 12th graders (Johnston et al., 2018). Additionally, there is evidence that suggests nicotine vaping predicts future cigarette experimentation (Miech et al., 2017). For these reasons, it is imperative that we work to gain a better understanding of the environmental risk factors that contribute to adolescent nicotine use and seek to determine how nicotine exposure affects reward circuitry in adulthood.

Adolescence is a sensitive period, characterized by rapid brain maturation and restructuring. During this time, environmental factors may drastically impact this process and the disruption of maturation can lead to abnormalities in brain function that persist into adulthood (Fuhrmann et al., 2015). Social interaction is believed to play a critical role in adolescent development of mammals. In human adolescents, peer and family connectedness is one of the strongest indicators of psychological health (Hall-Lande et al., 2007). Adolescents who do not report having close friendships consistently have lower levels of self-esteem and more psychological symptoms of maladjustment (Berndt, Hawkins, & Jiao, 1999; Stocker, 1994). For these reasons, it is believed that social interaction is a critical promoter of

psychological health during adolescence. Conversely, social isolation, or a lack of social interaction, is especially problematic for adolescents. It has been shown that isolated human adolescents are more likely to smoke cigarettes and engage in risky behavior (Aloise-Young & Kaeppner, 2005; Pearson et al., 2006; Seo & Huang, 2012). While human studies are often confounded with a variety of factors that play a role in social interaction or lack thereof, there is a robust amount of animal studies supporting the hypothesis that social isolation alters responses to rewarding stimuli.

Social Isolation and Drug Seeking

Numerous behavioral studies have supported the hypothesis that social isolation increases sensitivity to drugs of abuse. Isolated rodents have displayed increased self-administration of psychostimulants such as cocaine (Boyle et al., 1991; Schenk et al., 1987; Gipson et al., 2011; Howes et al., 2000; Ding et al., 2005; Baarendse et al., 2014; Fosnocht et al., 2019) and amphetamine (Bardo et al., 2001; Green, Gehrkie, & Bardo, 2002). Herrmann et al, (2014) found that isolated mice had an increased locomotor response to amphetamine. The findings of these behavioral tests support the general idea that social isolation increases reward salience of abused substances; however, fewer studies have assessed the impact of isolation on rodent nicotine seeking or liking. Given that nicotine has been shown to have a greater anxiolytic effect in isolated mice compared to group housed mice (Cheeta et al., 2001), we expected to find that nicotine has greater reinforcing properties in isolated mice as well.

Social Isolation and Dopamine Functioning

The mesolimbic pathway, consisting of dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), acts as a driving force for reward seeking. Nicotine acts on nAChRs, which in turn activate dopaminergic projections of the VTA.

Previous research shows that there are age-dependent differences in how the mesolimbic pathway responds to nicotine administration. Placzek and colleagues (2009) showed that a single dose of nicotine significantly increased long term potentiation in dopaminergic neurons of adolescent, but not adult rodents. Additionally, McQuown and colleagues (2007) found that adolescent, but not adult nicotine exposure increased lever pressing for cocaine. These results support the idea that the mesolimbic dopamine pathway is particularly responsive to nicotine and other drugs of abuse during adolescence versus adulthood, and that administration of nicotine during adolescence has a lasting impact on this pathway.

For decades scientists have suspected that social isolation acts on the dopamine system, producing an increased reactivity to drugs of abuse. Jones et al. (1990) found that social isolation increased rat's sensitivity to an intra-NAc infusion of amphetamine and hypothesized that it was caused by disruption in dopamine signaling of the NAc. Lewis et al. (1990) wanted to see if early social isolation resulted in long term alterations of DA receptor function in adult rhesus monkeys. They found that isolated monkeys responded significantly more to an apomorphine challenge compared to monkeys who never experienced social isolation and concluded it must have been due to changes in DA receptor transmission. Just as these earlier groups hypothesized, it has now been shown that isolation does in fact induce morphological changes in dopamine neurons. Karkhanis et al. (2018) used FSCV on slices of NAc core and found that isolated mice had a larger stimulation-evoked dopamine response than group housed mice. Yorgason et al. (2016) did voltammetry in slices of striatum from isolated vs group housed mice and found that social isolation increases both dopamine uptake and psychostimulant potency in the striatum, again suggesting that isolation leaves an individual more vulnerable to the addictive properties of drugs acting on the mesolimbic pathway. It has also been

hypothesized that mesolimbic DA neurons in the VTA and NAc mediate the experience of social reward. Gunaydin et al. (2014) found that the VTA-NAc pathway encodes and predicts social interaction and Dölen et al. (2013) found that NAc activity is essential for experiencing social reward. DA neurons in the dorsal raphe nucleus showed increased activity when experiencing social contact after isolation (Matthews et al., 2016), again suggesting DA systems may become hyper responsive after social isolation.

Current Study

Drug seeking is driven by increased mesolimbic dopamine activity, and social interaction has been shown to increase drug salience in several studies, suggesting that isolation produces its effects by altering reward circuitry of the midbrain. The proposed study aimed to determine how SI impacts nicotine preference and NAc dopamine functioning. Adolescent male and female mice were housed in isolation or groups for 3 weeks and were tested for nicotine conditioned place preference, during which half of the mice in each housing group were exposed to nicotine and the other half saline (control). Following behavioral testing, all mice underwent dopamine recordings; thus, we were able to assess the influence of social isolation and nicotine exposure on aspects of dopamine transmission. During dopamine recordings, a drug was administered to test the response of the reward system when challenged with a psychostimulant (cocaine) that directly acts on the dopamine system. Cocaine blocks the reuptake of cocaine by inhibiting the dopamine transporter (DAT) and is known to greatly increase extracellular dopamine concentrations (Kuhar et al., 1991). We hypothesized that isolation would result in a significant increase in nicotine CPP compared to group housing. In terms of dopamine transmission, we expected that isolation and nicotine exposure would both lead to a hyperdopaminergic profile (increased baseline release and increased dopaminergic response to cocaine relative to control

mice). Additionally, we hypothesized that social isolation combined with nicotine exposure would exhibit an interactive effect, resulting in an even greater hyperdopaminergic profile (increased baseline release and increased dopaminergic response to cocaine relative to mice that did not experience both isolation and nicotine exposure). This study benefits the scientific community, as well as the clinical population, by strengthening our understanding of housing effects and risk factors for drug use.

Methods

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Memphis and were also aligned with those outlined in The Public Health and Service Policy on Humane Care and Use of Laboratory Animals (National Institutes of Health 2012) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2013).

Mice and Housing Conditions

C57BL/6J mice were purchased from Jackson Laboratory and arrived to the University of Memphis at 3 weeks of age. 24 male and 24 female mice were single-housed while the remainder of the experimental mice (24 males and 24 females) remained with their original cage mates (4 per cage). Mice remained in these housing conditions for at least 2 weeks (from PND 21-end of experiments) prior to beginning behavioral testing. The experimental timeline is outlined in Figure 1.

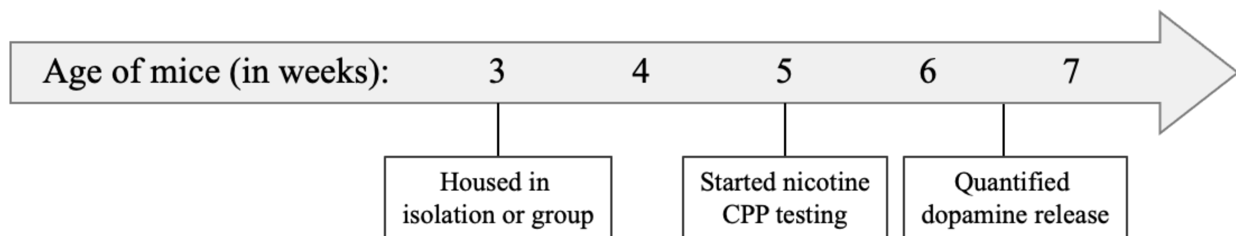

Figure 1. Depiction of experimental timeline. Mice will be in the housing conditions for 2 weeks prior to behavioral testing and 3.5 weeks prior to neurochemical testing.

Nicotine-Preference Testing

Four identical, 2- chambered conditioned place preference (CPP) Plexiglass boxes were used for testing. A removable door separated the two chambers, which are symmetrical in size (20 cm x 18.5 cm x 29.5 cm each) The exterior walls of one chamber were covered in white and black vertical stripes about an inch thick. The exterior walls of the other chamber were covered with an alternating black and white diamond pattern of the same thickness. Both sides of the interior wall and the door separating the chambers were solid black. Additionally, the bedding in one chamber was made of Spruce, Fir, and Pine shavings, while bedding in the opposite chamber was made of Aspen shavings. The apparatus contained infrared sensors placed along both the X and Y dimensions to detect the exact movement and location of the mouse during testing.

Nicotine CPP followed the procedure performed by Kutlu et al. (2015) on C57BL/6J mice. An overview of the procedure is depicted in Figure 2. The entire experiment took 10 consecutive days. On day 1 of experiments, mice were habituated to the CPP box during their first testing session (T1). Each mouse was taken to the testing room, isolated from its cage-mates, and habituated to the testing room in a holding cage for 20 minutes. Following habituation, each mouse was gently handled for ~1 min and held as if a subcutaneous injection was being administered. Each mouse was then randomly placed into a chamber and was given 15

minutes to roam the box with unrestricted access to either chamber. Time spent in each chamber was recorded, and on conditioning days nicotine was paired with the least preferred chamber. Nicotine CPP has been shown to be more robust with the biased CPP procedure (Acquas et al., 1989; Calcagnetti & Schechter, 1994; Le Foll & Goldberg, 2005). On conditioning days, mice went through 2 conditioning sessions separated by 5 hours. On conditioning days 2, 3, and 4 (phase 1 conditioning) mice received a subcutaneous injection of nicotine (0.35 mg/kg) or saline (0.9% in equal volume as nicotine injection) and were placed in the confined nicotine or saline-paired chamber, respectively. After 15 min, mice were returned to their home cages. Five hours later, the mice were habituated to the room for 20 min, given the alternate drug, and were placed in the respective chamber for 15 min.

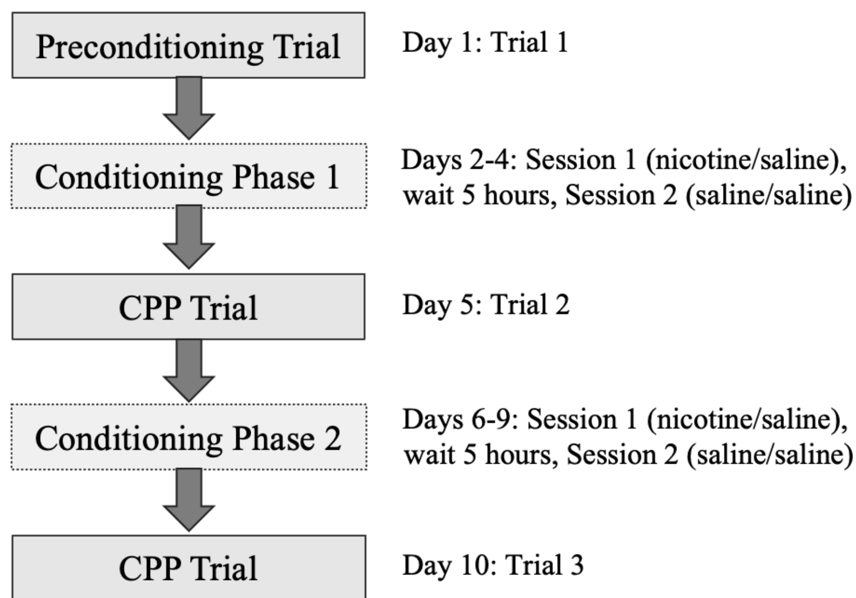

Figure 2. Overview of conditioned place preference procedure (CPP). This procedure has been shown to induce nicotine CPP in mice (Kutlu et al., 2015).

The schedule of injections was counterbalanced within conditioning sessions so that half of the mice received saline and the other half nicotine during their first conditioning trial. Mice

that were assigned to saline CPP groups went through the same conditioning schedule but received subcutaneous injections of saline before being placed in either one of the chambers for 15 minutes. On day 5, test 2 (T2) was performed. Mice were placed in the saline chamber and were given open access to either chamber for 15 min. Time spent in either chamber was recorded. Days 6, 7, 8, and 9 (phase 2 conditioning) followed the procedure from phase 1. On day 10, test 3 (T3) was performed using the same procedure as test 1 and 2. Time spent in either chamber was measured, and a repeated measures ANOVA was run to examine differences in time spent in the nicotine chamber for isolated versus group housed mice.

Behavioral Data Analysis

Time spent in the nicotine-or saline-paired chamber was calculated for each mouse on test days (experimental days 1,5, and 10). A mixed factorial ANOVA was used to determine the effect of housing (group/isolated), drug (nicotine/saline), and sex on time spent in the drug-paired chamber across the testing days (within-subjects factor, 3 testing days). Two-way between-subjects ANOVAs were used to determine the effect of sex and housing during specific test days when appropriate.

Dopamine Recordings

The day after behavioral testing, all mice underwent dopamine recordings via in vivo fixed potential amperometry. For dopamine recordings, each individual mouse was anesthetized permanently with urethane (1.5 g/kg i.p.). The mice was assessed 15 min after urethane by eye-blink, mild tail and foot pinch-induced reflexes to ensure initial and complete induction of anesthesia. If needed, a supplemental dose of urethane (0.5 g/kg i.p.) was administered. Anesthesia records were kept in a designated notebook in the lab.

Once mice were fully anesthetized, they were placed in a standard stereotaxic frame within a mouse head-holder adaptor, to enable accurate placement of electrodes. Core body temperature of each animal was monitored and maintained at 38°C. A single longitudinal incision of the scalp (1cm in length) was made to expose the surface of the skull. Three trephine holes (~1-1.5 mm o.d.) were drilled through the skull to permit the insertion of a stimulating electrode (SNE-100 outer diam. 100 um; Rhodes Medical Co., CA, USA) into the left VTA (coordinates: AP -3.3 mm from bregma, ML +0.3 mm from midline, and DV -4.0 mm from dura), a carbon fiber dopamine recording electrode into the NAc (10 µm o.d. and 250 µm long, Thornel Type P, Union Carbide, PA, USA) (coordinates: AP +1.5 mm from bregma, ML +1.0 mm from midline, and DV -4.0 mm from dura), and a silver-chloride reference and auxiliary electrode combination placed in contact with the surface of the parietal cortex (outer diameter of tip in contact with tissue 300 um) (see Figure 3). All electrodes were accurately guided into brain tissue via standard stereotaxic carriers mounted in place on the stereotaxic frame (David Kopf Instruments) (Paxinos & Franklin, 2001).

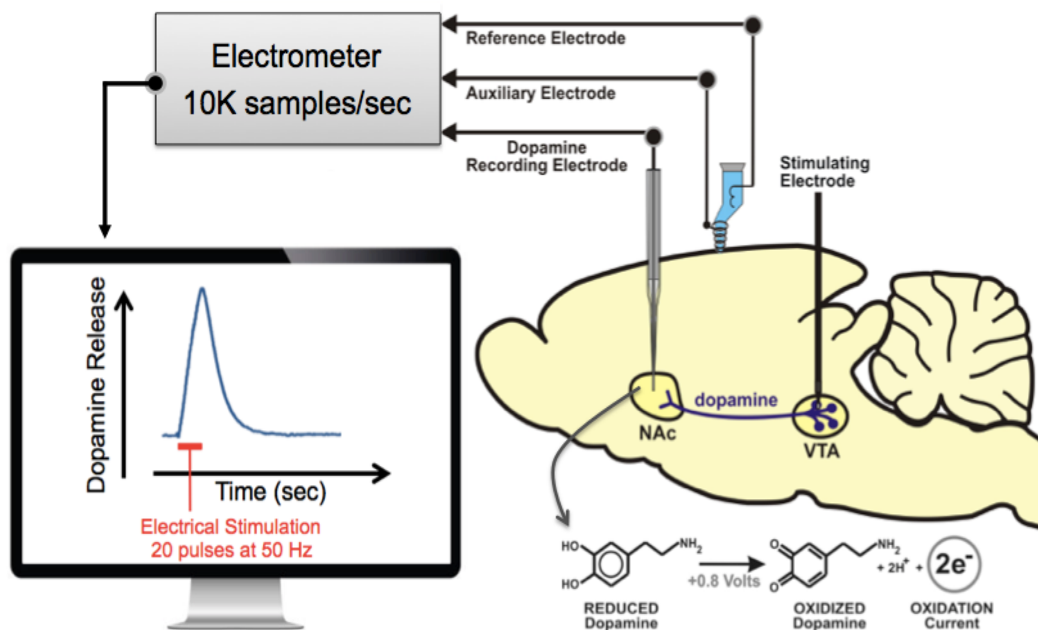

Figure 3. Depiction of surgical setup for dopamine recordings. In vivo fixed potential amperometry was used to measure dopamine release in the nucleus accumbens (NAc) elicited by electrical stimulation of the ventral tegmental area (VTA).

Following the implantation of all electrodes, an electrometer (ED401 e-corder 401 and EA162 Picostat, eDAQ Inc., Colorado Springs, CO) was used to apply a fixed positive potential (+0.8 V) to the dopamine-recording electrode of each mouse. Changes in dopamine release in the NAc were monitored continuously in response to brief trains of electrical stimulation (20 pulses at 100 Hz; 0.5 msec pulse duration; 800 microamps; every 30 sec) applied to the VTA. After a 10 min baseline recording (gathering pre-drug responses), the mouse received an i.p. injection of cocaine (10 mg/kg) to measure dopamine transporter function and dopaminergic response to a dopamine agonist. Administration of cocaine during amperometic recordings allowed us to examine the impact of sex, housing, and nicotine exposure on dopaminergic responses to a psychostimulant. This dose of cocaine was selected based on the effective dose used to inhibit dopamine reuptake in previous experiments in our lab (Lester et al., 2010).

After recordings were complete, each mouse was euthanized by intra-cardiac injection of an overdose of urethane. The mean change in dopamine oxidation current (nAmp), corresponding to stimulation-evoked dopamine efflux, was converted to a mean dopamine concentration (μM) by post-experiment in vitro calibration of the carbon fiber electrode in solutions of dopamine (0.5-2.0 μM) using a flow injection system (Michael and Wightman, 1999).

Neurochemical Data Analysis

In order to quantify the recorded dopamine efflux, data points occurring at 0.25 sec pre- and 10 sec post-stimulation were extracted at 10 minute intervals. From baseline (pre-cocaine) recordings we quantified dopamine release (the magnitude of the response peak) and dopamine half-life (i.e. the time for 50% decrease from the maximum evoked increase to the prestimulus baseline level). A three-way between subjects ANOVA was used to determine the effect of housing (group/isolated), drug exposure (nicotine/saline), and sex on baseline dopamine release and half-life. In order to determine the effect of the i.p. drug challenge (cocaine) during amperometric recordings, changes in stimulation-evoked dopamine release and half-life were converted to mean percent change with respect to baseline (Lester et al., 2008; Estes et al., 2019). A mixed factorial ANOVA was used to determine the effect of housing (group/isolated), drug exposure (nicotine/saline), and sex on percent change in dopamine release and half-life over the 60 minute recording period (within-subjects factor) following the drug challenge. A three-way between-subjects ANOVA was used to determine effects of sex, isolation, and nicotine exposure specifically at the peak effect time of cocaine.

Results

Nicotine Conditioned Place Preference (CPP)

A mixed factorial ANOVA was used to determine the effects of sex, housing (isolation/group), and drug (nicotine/saline) on time spent in the drug-paired chamber across the 3 CPP trials. All CPP ANOVA results are listed in Table 1. There was a main effect of trial on time spent in the drug paired chamber [$F(2,142)= 25.78$, $p < .001$, $\eta_p^2 = .27$], but no trial x sex or trial x housing interactions were observed, indicating that neither sex nor housing significantly affected the time spent in the drug-paired chamber across the 3 trials. However, as expected, there was a significant interaction between trial and drug [$F(2,142) = 4.49$, $p = .013$, $\eta_p^2 = .06$], indicating that the drug administered (nicotine or saline) significantly altered the time spent in the drug-paired chamber across the 3 trials (see Figure 4A).

Regarding the three-way interactions, no significant interactions were observed between trial x sex x housing or trial x sex x drug. However, a trial x housing x drug interaction was observed right at the significance level [$F(2,142) =3.06$, $p = .050$, $\eta_p^2 = .04$], indicating that the isolated mice responded differently to the drug across the trials relative to the group-housed mice. Sex did not alter this effect as there was no significant trial x sex x housing x drug interaction.

For each CPP trial, two-way between-subjects ANOVAs were used to determine the effects of sex, housing, and drug on time spent in the drug-paired chamber at each trial. Neither sex nor housing had a main effect on time spent in the drug-paired chamber during any of the trials. Drug had no main effect during the first trial but did significantly alter the time spent in the drug-paired chamber during the second and third trial [Trial 2: $F(1,71) = 15.12$, $p < .001$, $\eta_p^2 = .18$; Trial 3: $F(1,71) = 5.21$, $p = .026$, $\eta_p^2 = .07$]. Regarding the interactive effects, no

significant interactions were observed between sex x housing or sex x drug during any of the CPP trials. During the first and second trials, there were no significant housing x drug interactions; however, a significant housing x drug interaction was observed during the third trial [$F(1,71) = 5.25$, $p = .025$, $\eta_p^2 = .07$], indicating that isolated mice responded differently to the drug relative to the group-housed mice during the third CPP trial (see Figure 4B). Specifically, isolated mice spent more time in the nicotine-paired chamber compared to the other groups. Sex did not alter this effect as there was no significant sex x housing x drug interaction during any of the CPP trials.

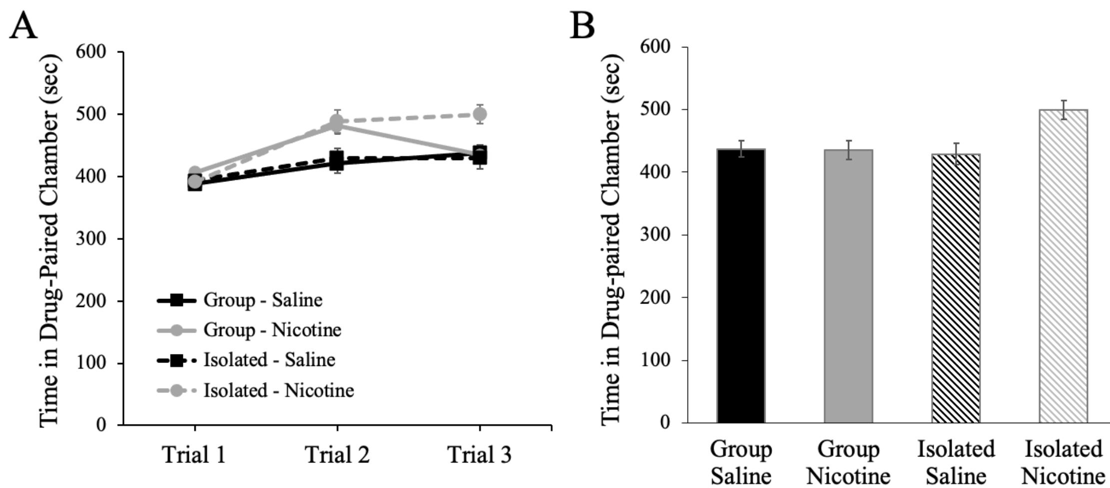

Figure 4. CPP Results. (A) A significant trial x housing x drug interaction indicates that isolated mice responded differently to the drug across the trials relative to group-housed mice. (B) During Trial 3, a significant housing x drug interaction was observed, revealing that isolated mice spent more time in the nicotine-paired chamber compared to the other groups. * indicates $p < .05$.

Table 1. *CPP Time Spent in the Drug-Paired Chamber – ANOVA Results*

	Trial 1					Trial 2					Trial 3				
Source	*df*	MS	*F*	*p*	Effect Size	*df*	MS	*F*	*p*	Effect Size	*df*	MS	*F*	*p*	Effect Size
Sex (S)	1	1117.07	.459	.433	.009	1	2150.74	.439	.51	.006	1	86.549	.019	.892	<.001
Housing (H)	1	714.227	.397	.531	.006	1	2197.41	.449	.505	.006	1	16877.224	3.648	.06	.049
CPP Drug (D)	1	728.848	.405	.526	.006	1	74077.398	15.124	<.001*	.176	1	24076.913	5.205	.026*	.068
S x H	1	4035.656	2.244	.139	.031	1	14262.532	2.912	.092	.039	1	962.017	.208	.65	.103
S x D	1	35.859	.02	.888	<.001	1	578.817	.118	.732	.002	1	6617.661	1.431	.236	.02
H x D	1	1514.521	.842	.362	.012	1	117.525	.024	.877	<.001	1	24300.967	5.253	.025*	.069
S x H x D	1	1635.007	.909	.344	.013	1	6745.182	1.377	.245	.019	1	6953.263	1.503	.224	.021
Error	71	1798.394				71	4898.103				71	4626.108			

Note. MS = Mean squares, effect size = partial η^2.

Baseline Dopamine Release and Half-Life

Stimulation-evoked dopamine efflux was recorded before and after a dopaminergic drug challenge (cocaine). Baseline dopamine release and synaptic half-life were assessed in each mouse prior to the challenge injection. Three-way between-subjects ANOVAs were used to determine the effect of sex, housing (isolation/group), and previous drug exposure (nicotine/saline from CPP trials) on dopamine release and half-life. None of the independent variables or interactions between variables significantly altered baseline dopamine release or half-life (see Figure 5A-C). All ANOVA results for dopamine release and half-life are listed in Table 2.

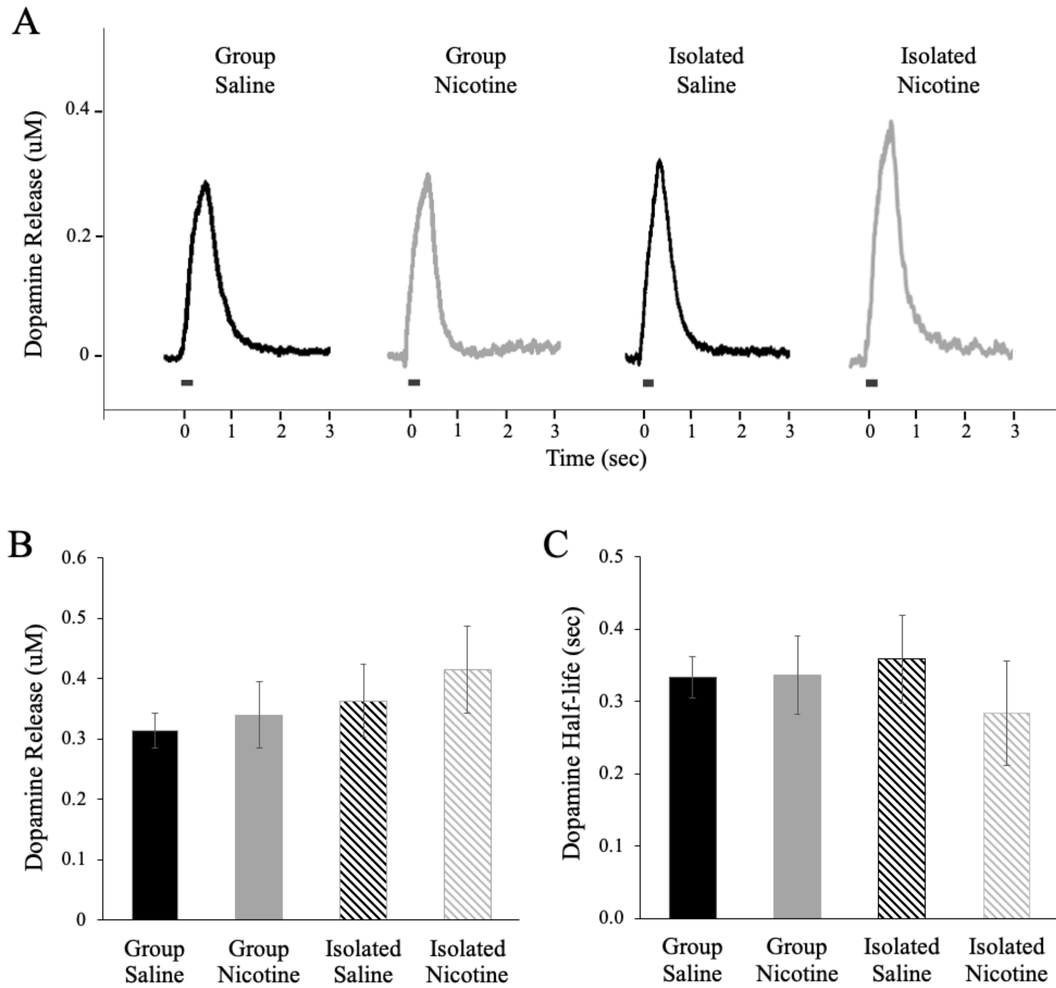

Figure 5. Baseline dopamine release and half-life. (A) Representative responses from each housing and drug group. No significant differences in (B) dopamine release or (C) half-life were observed between housing or drug groups. Data is displayed as mean ± SEM.

Table 2. *Dopamine Release and Half-life – ANOVA Results*

	Dopamine Release					Dopamine Half-life				
Source	*df*	MS	*F*	*p*	Effect Size	*df*	MS	*F*	*p*	Effect Size
Sex (S)	1	0.028	0.565	.455	.010	1	4.930	0.006	.936	<.001
Housing (H)	1	0.059	1.199	.278	.021	1	0.003	0.413	.523	.007
CPP Drug (D)	1	0.021	0.426	.516	.007	1	0.021	2.732	.104	.046
S x H	1	0.167	3.422	.070	.057	1	2.554	0.003	.954	<.001
S x D	1	0.032	0.659	.420	.011	1	0.03	3.927	.052	.064
H x D	1	0.005	0.101	.752	.002	1	0.028	3.717	.059	.061
S x H x D	1	0.004	0.085	.771	.001	1	0.002	0.234	.63	.004
Error	57	0.049				57	0.008			

Note. MS = Mean squares, effect size = partial η^2.

Dopamine Autoreceptor Functioning

During dopamine autoreceptor testing, autoreceptor functioning was quantified by determining the degree to which the conditioning pulses altered the dopamine release elicited by the test stimulations (T2 relative to T1). A mixed factorial ANOVA was used to determine the effect of sex, housing (isolation/group), and previous drug exposure (nicotine/saline from CPP trials) on autoreceptor functioning across the different conditioning pulse settings (within-subjects factor). With 0 conditioning pulses, dopamine release following T1 and T2 are similar (T2/T1 x 100 = 100% dopamine release). Greater decreases in dopamine release (% of T2/T1) indicates increased autoreceptor functioning (see Figure 6A). As expected, there was a significant main effect of number of conditioning pulses on percent change in dopamine release [$F(6,378) = 51.67$, $p < .001$, $\eta_p^2 = .45$], indicating that the number of conditioning pulses alters autoreceptor-mediated dopamine inhibition. There were no significant main effects of sex, housing, or drug exposure and no significant interactive effects between these variables on dopamine autoreceptor functioning (see Figure 6B).

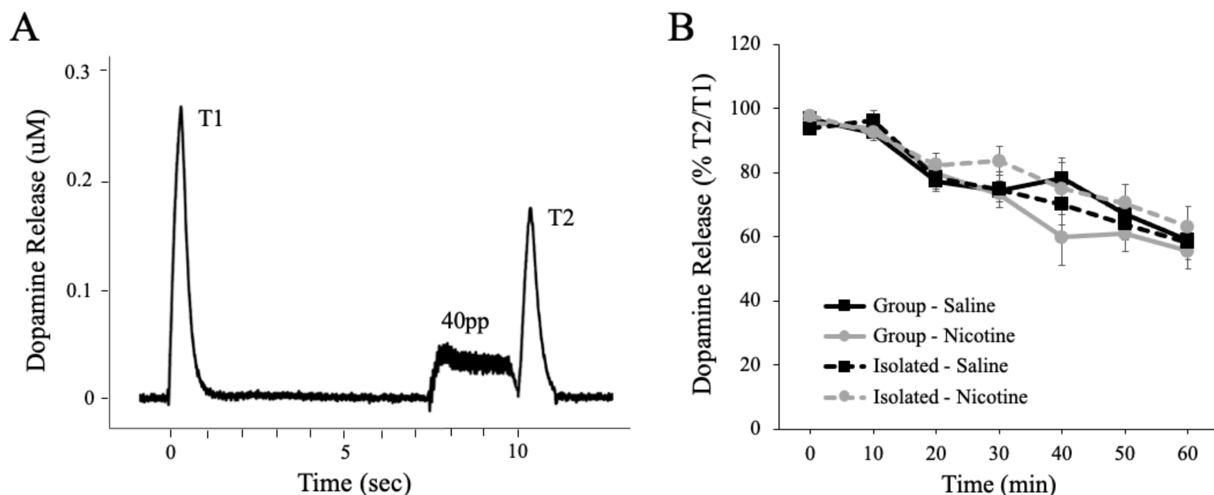

Figure 6. Autoreceptor-mediated inhibition of dopamine release. (A) Example amperometric recording of autoreceptor test stimulations (T1,T2) separated by 40 pre-pulses (pp). Greater decreases in dopamine release (% of T2/T1) indicates increased autoreceptor functioning. (B) As the number of pp increases, autoreceptor-mediated dopamine release decreases. No significant effects of housing or nicotine exposure were observed in autoreceptor functioning.

Dopaminergic Response to DAT inhibition

During dopamine recordings, cocaine (10 mg/kg, ip) was administered as a challenge to the dopamine system. Cocaine acts directly on the dopamine system by blocking synaptic reuptake through DAT (Kuhar et al., 1991). Dopamine synaptic half-life is commonly used to indicate the influence of DAT inhibition (Holloway et al., 2018; Mittleman et al., 2011; Siciliano et al., 2014). Dopamine half-life following cocaine was converted into percent change of baseline (with pre-cocaine responses being 100%).

Mixed factorial ANOVAs were used to determine the effect of sex, housing (isolation/group), and previous drug exposure (nicotine/saline from CPP trials) on percent change of dopamine half-life over the 1-hour recording period following the cocaine injection. As expected, there was a significant main effect of time post injection on percent change in dopamine half-life [$F(6,342) = 112.51$, $p < .001$, $\eta_p^2 = .66$]. No time x sex interaction was observed; however, both housing and drug altered this dopaminergic response over time. The significant interaction between time and housing [$F(6,342) = 4.10$, $p = .001$, $\eta_p^2 = .07$] indicated that isolated mice responded differently to cocaine over time compared to group-housed mice, and the significant interaction between time and drug [$F(6,342) = 3.13$, $p = .005$, $\eta_p^2 = .05$] indicated that the mice that received nicotine during CPP responded differently to cocaine over time compared to the mice given saline during CPP (see Figure 7B). There were no significant time x sex x housing or time x sex x drug interactive effects on percent change in dopamine half-life following cocaine; however, a significant interaction between time x housing x drug was observed [$F(6,342) =3.28$, $p = .004$, $\eta_p^2 = .05$], indicating a combined effect of isolation and nicotine-exposure on this response to the dopaminergic drug challenge (cocaine).

Further analyses were conducted at the peak effect time of cocaine. A three-way between-subjects ANOVA was used to determine the effect of sex, housing, and previous drug exposure (nicotine/saline from CPP trials) on percent change in dopamine half-life 20 min post cocaine injection. No main effect of sex was observed, but there was a main effect of housing [$F(1,57) = 7.48$, $p = .008$, $\eta_p^2 = .12$] and CPP drug [$F(1,57) = 6.79$, $p = .012$, $\eta_p^2 = .11$] on percent change in dopamine half-life at this time point, with isolated mice displaying an increased response to cocaine compared to group-housed mice and nicotine-exposed mice displaying an increased response compared to saline-exposed mice (see Figure 7A and C). There were no significant interactive effects between these variables on percent change in dopamine half-life 20 min post cocaine (see Table 3).

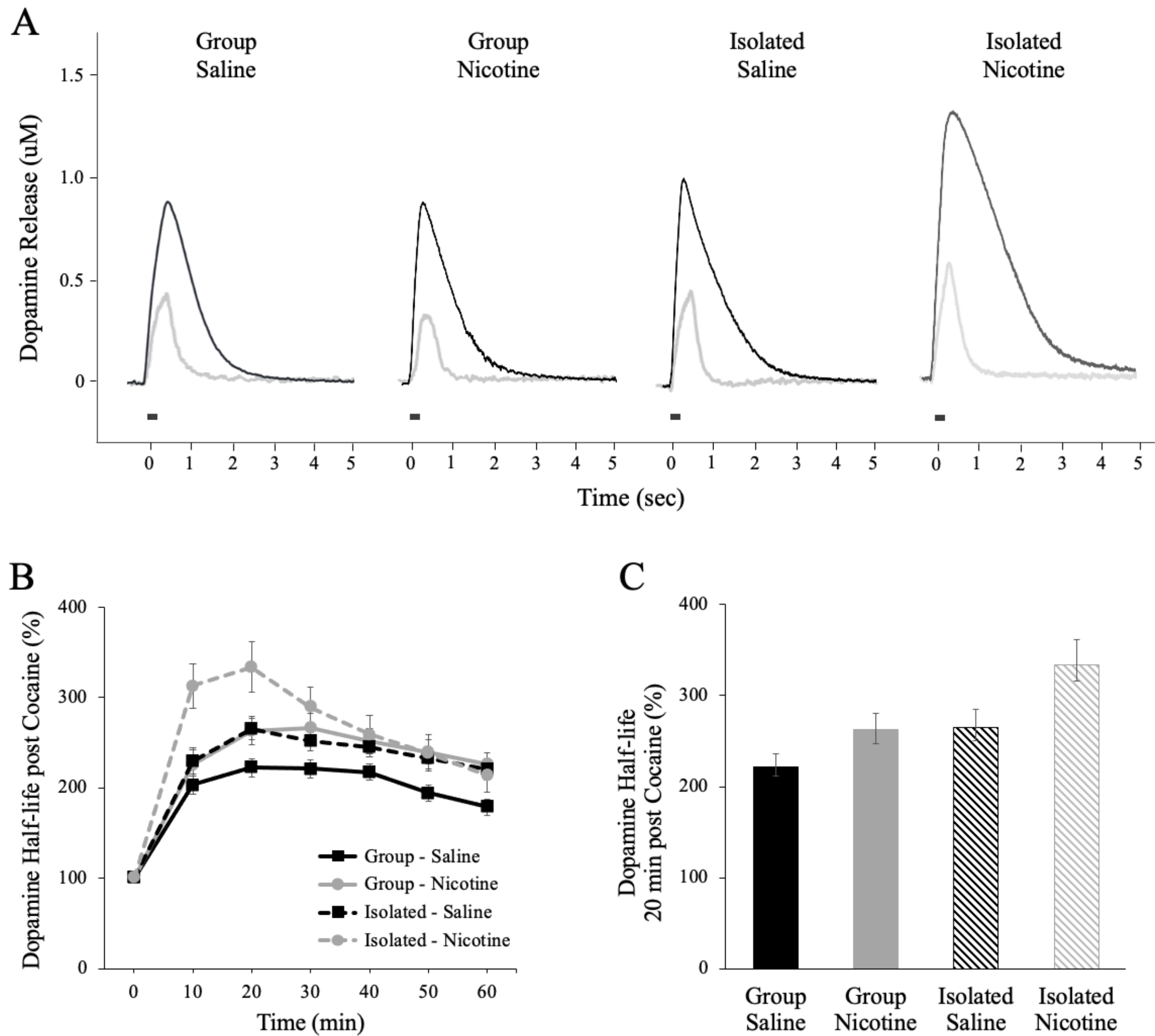

Figure 7. Dopaminergic response to cocaine. (A) Profiles indicate example responses from each drug and housing group at 20 min post injection. Light lines represent pre-cocaine response. (B) Mean (± SEM) dopamine half-life over the 1 hour recording period following cocaine administration was significantly altered by isolation, nicotine-exposure, and the combined effect of both variables. (C) At the peak effect of cocaine (20 min post injection), isolation and nicotine-exposure increased percent change in dopamine half-life, but an interactive effect between these variables was not observed.

Table 3. *Percent Change in Half-life at 20 Min post Cocaine – ANOVA Results*

Source	*df*	MS	*F*	*p*	Effect Size
Sex (S)	1	981.441	.143	.706	.003
Housing (H)	1	51264.074	7.483	.008*	.116
CPP Drug (D)	1	46499.797	6.787	.012*	.106
S x H	1	508.902	.074	.786	.001
S x D	1	655.648	.096	.758	.002
H x D	1	3286.761	.48	.491	.008
S x H x D	1	1309.095	.191	.664	.003
Error	57	6851.099			

Note. MS = Mean squares, effect size = partial η^2.

Discussion

Social interaction is believed to play a critical role in the maturation of adolescent mammals. Reduced/impaired social interaction has been shown to alter behaviors related to drug use and seeking in both humans and rodents (Seo & Huang, 2012; Howes et al., 2000; Schenk et al., 1987). Here, we hypothesized that a lack of social interaction (social isolation) in mice during adolescence would increase preference for nicotine and that social isolation combined with nicotine exposure would lead to a hyperdopaminergic reward profile. Adolescent mice were separated into group or isolated housing for 2 weeks prior to being tested for nicotine preference in a CPP paradigm. Then mice were assessed for baseline dopamine efflux as well as percent change in dopamine efflux following a challenge to the dopamine system (administration of the DAT inhibitor cocaine) using *in vivo* fixed potential amperometry.

Nicotine Preference

No sex effects were found regarding nicotine preference, and isolation altered nicotine preference similarly in males and females. During CPP testing, chamber preference was examined on 3 testing trial days, with no injections administered during trial 1. During trial 2, both group-housed and isolated mice showed an increased preference for nicotine over saline;

however, during trial 3, nicotine preference was only displayed by isolated mice. These results indicate that social isolation influences adolescent rodents in a way that increases their preference for nicotine administration, which mirrors the findings from studies using other psychostimulants (Boyle et al., 1991; Bardo et al., 2001; Herrmann et al., 2014). It's unclear whether this increase in nicotine preference is driven by an increased reward response, relief of negative affect from social stress, or both. Given that nicotine has been shown to have a greater anxiolytic effect in isolated mice compared to group housed mice (Cheeta et al., 2001), future studies expanding on this line of research could benefit by including measures of anxiety (such as light/dark box testing, elevated plus maze testing, or measures of corticosterone).

Dopamine Functioning

Effects of sex on dopamine functioning: No main effects of sex were observed on any of the measured dopamine release variables. Previous studies on sex-related differences in dopamine release have shown conflicting results (Becker & Chartoff, 2018). Female rodents have been shown to have greater striatal dopamine release than males (Arvidsson et al., 2014; Walker et al., 2000), while other studies have shown male rodents to have greater dopamine release than females (Cummings et al., 2014; Xiao & Becker, 1998). Yet similar to our findings, no sex differences have also been found in measurements of striatal dopamine release and neural activity in the ventral tegmental area (Calipari et al., 2017; Griffin & Middaugh, 2006). Furthermore, sex differences did not alter the dopaminergic effects of isolation or nicotine exposure.

Effects of isolation on dopamine functioning: In terms of baseline dopamine release, we saw no significant effect of isolation. These results were a bit unexpected seeing as other researchers have found that adolescent isolation increases dopamine release using a similar

technique *ex vivo* (in brain slices) (Karkhanis et al., 2019; Yorgason et al., 2016) and that social isolation increases basal dopamine content levels using *in vivo* microdialysis (Karkhanis et al., 2014). Conflicting results are likely due to differences in techniques used to assess dopamine release and/or variations in isolation protocols. There was also no significant effect of isolation on dopamine autoreceptor functioning, which is similar to findings from a study by Yorgason et al. (2013) that used voltammetry in brain slices to assess D2-type autoreceptor activity following social isolation. Following DAT inhibition by cocaine, there was a significant effect of isolation on percent change in dopamine half-life over the 1-hour recording period post injection. At the drug's peak effect time, isolated mice displayed a greater dopaminergic response to cocaine compared to group housed mice. These results fall in line with previous findings that social isolation increases psychostimulant potency (Ding et al., 2005; Howes et al., 2005; Jones et al., 1990; Yorgason et al., 2016).

Protocols surrounding rodent isolation are often variable and difficult to standardize. Our isolation period of 3 weeks was relatively short compared to other protocols, which can require subjects to be isolated for up to several months. Additionally, although the isolated mice were housed alone, the cages were transparent and positioned side-by-side on racks in one room. Therefore, the mice could still receive a limited amount of social interaction via smell, sight, and sound. This is a common issue in research on this subject, as housing subjects in completely separate rooms would require an extensive amount of space. It is possible that a more severe isolation protocol could have produced more robust behavioral and neurochemical phenotypes.

Effects of nicotine exposure on dopamine functioning: Nicotine exposure did not alter baseline dopamine release or half-life. These results were surprising because previous research has shown that even a single dose of nicotine can produce long term potentiation of excitatory

synapses of dopamine neurons in the NAc (Mansvelder & McGehee, 2000; Saal et al., 2003). Our results suggest that these altered synaptic strengths and NMDA/AMPA ratios do not necessarily result in increased dopamine release. However, nicotine exposure did alter the way the mesolimbic dopamine system responded to the drug challenge. Following DAT inhibition by cocaine, there was a significant effect of nicotine exposure on percent change in dopamine half-life over the recording period. At the cocaine's peak effect time, nicotine-exposed mice exhibited a greater dopaminergic response to cocaine than the group-housed mice. These results provide neurochemical support for behavioral studies that have shown that adolescent nicotine exposure increases sensitivity to psychostimulants and rewarding properties of cocaine into adulthood (Alajaji et al., 2016; Kenny & Markou, 2005; Kota et al., 2009; McQuown et al., 2007).

Interactive effects of isolation and nicotine-exposure on dopamine functioning: Combining the experiences of isolation and nicotine exposure did not significantly affect baseline dopamine release, half-life, or autoreceptor functioning. However, isolation and nicotine exposure did seem to have an interactive effect on the dopaminergic response pattern following cocaine administration, as a significant housing x drug x time interaction was observed on percent change in dopamine half-life post injection. These response patterns indicate a greater dopaminergic response at the early time points following cocaine in mice that were both isolated and nicotine-exposed; however, the interactive effect of isolation and nicotine exposure did not hold up with further analyses of percent change in dopamine half-life at cocaine's peak effect time (20 min post injection). Overall these findings suggest that isolation and nicotine exposure alter dopamine functioning independently in ways that do not have an additive effect when these experiences combine.

Conclusions

The results of this study reiterate the importance of understanding how experiences during adolescent development plays into later risk for drug abuse. We found that social isolation during adolescence increases nicotine preference in male and female mice. Additionally, our results suggest that social isolation and nicotine exposure may both alter dopamine functioning in the direction of enhanced reinforcing effects of rewards. Our results did not indicate, however, that these experiences (isolation and nicotine exposure) affect reward sensitivity in an additive manner. These findings hold importance for both researchers and clinicians. Our results highlight the importance of controlling for factors related to social interaction in rodent research on the rewarding effects of drugs. Experimental designs often require periods of isolation, which should be reported and controlled for appropriately. Understanding how factors such as social interaction and drug exposure play into an individual's risk for substance abuse could help clinicians develop more effective intervention strategies for at-risk patients.

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IACUC PROTOCOL ACTION FORM

To:	Deranda Lester
From	Institutional Animal Care and Use Committee
Subject	Animal Research Protocol
Date	October 23, 2018

The institutional Animal Care and Use Committee (IACUC) has taken the following action concerning your Animal Research Protocol No. 829

0829 Examining the Effects of Social Interaction and Nicotine Exposure on Mesolimbic Dopamine Functioning

☒ Your protocol is approved for the following period:

From: October 19, 2018 To: October 18, 2021

☐ Your protocol is not approved for the following reasons (see attached memo).

☐ Your protocol is renewed without changes for the following period:

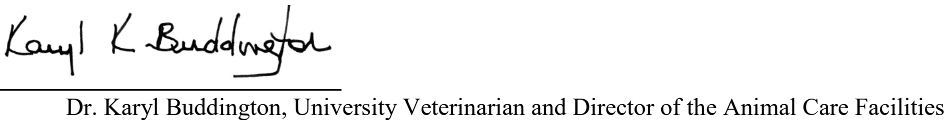
From: To:

☐ Your protocol is renewed with the changes described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated for the following period:

From: To:

☐ Your protocol is not renewed and the animals have been properly disposed of as described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated

Amy L. de Jongh Curry, PhD, Chair of the IACUC



Dr. Karyl Buddington, University Veterinarian and Director of the Animal Care Facilities