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DOPAMINE RELEASE DYNAMICS AND RELATED BEHAVIOR IN C57BL/6J & DBA/2J MICE FOLLOWING SOCIAL ISOLATION AND ENVIRONMENTAL ENRICHMENT

by

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Abstract

Environmental enrichment for rodents consists of cage mates and physical stimuli (such as tunnels and running wheels) and has been shown to provide a protective effect against anxiety-like and addiction-related behaviors. These enrichment effects are dependent on the sex and strain of mice, potentially related to differences in mesolimbic dopamine activity. This study employed *in vivo* fixed potential amperometry to quantify dopamine release before and after cocaine administration, and open-field behavioral testing to measure behaviors associated with anxiety and substance use. These tests included males and females of 2 mouse strains (C57BL/6J and DBA/2J) separated into 3 housing groups (environmentally enriched, group housed, and isolated). The use of 3 housing groups allows distinction between the protective environmental influence of social and physical stimuli combined and social without physical stimuli. The current study found that C57BL/6J mice had more sensitive dopamine autoreceptor functioning, while DBA/2J mice had a higher overall dopamine supply available in the nucleus accumbens. Enriched housing groups were shown to have a decreased incidence of anxiety-related behaviors in the open field test versus standard housing or isolated housing groups. B6 mice had higher overall activity, specifically B6 males, generally showing more locomotor behavior than D2 mice. These measures may be used to more fully characterize the behavioral and neurological traits of the two mouse strains. Identifying interactive genetic and environmental risk factors for drug abuse and understanding the associated neurochemical mechanisms is critical for improving success of drug prevention and treatment programs.

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Dopamine Release Dynamics and Related Behavior in C57BL/6J & DBA/2J Mice Following Social Isolation and Environmental Enrichment

Exposure to adverse events during early developmental periods has long lasting and deep-seated effects on brain development and behavior, continuing into adulthood. In humans, childhood neglect is linked to higher risks of developing major depression, schizophrenia, anxiety, and other psychological disorders, along with heightened risk for drug abuse during adolescence and adulthood (Dube et al., 2003; Heim et al., 2004; Rapoport et al., 2005).

Social isolation in rodent models of anxiety and drug abuse

In rodent models, mice reared in isolation show long-term behavioral aberrations including heightened aggression, anxiety-like behavior, cognitive rigidity, and increased neurochemical response to psychostimulants (Fone and Porkess, 2008; Yorgason et al., 2015). In mouse models, the stress of social isolation leads to endocrine dysfunction, alteration of the hypothalamic-pituitary-adrenal axis functioning, and glucocorticoid dysregulation including changes in the expression of the stress hormone, corticosterone (Ieraci, Mallei, & Popoli, 2016).

Regarding measures of behavior related to anxiety, social isolation during adolescence reduces habituation in the open field, impairs fear-conditioning, and reduces time spent in the open arms of an elevated plus maze (Voiker et al., 2005; Walker et al., 2005). Increased anxiety-related behaviors following adolescent social isolation is a relatively consistent behavioral finding in rodents across context and measuring paradigms (see Walker et al., 2019).

Anxiety disorders and substance use disorders seem to have implied bidirectional mechanisms that commonly result in comorbid diagnoses (see Stoychev, 2019). Similarly, social isolation during adolescence has also been shown to alter the way rodents respond to rewarding

stimuli. Studies have shown that adolescent social isolation leads to increased drug seeking and liking in rodents (see Walker et al., 2019). For example, social isolation increases self-administration of ethanol, morphine, cocaine, and other psychostimulants (Ding et al., 2015; Fone & Porkess, 2008; McCool and Chappell, 2009; Schenk et al., 1987). Adolescent social isolation in rodents also increases motivation for natural rewards such as sucrose (Fone & Porkess, 2008) and enhances the development of conditioned place preference to ethanol, morphine, cocaine, and other psychostimulants (Couderreau, et al., 1997; Whitaker et al., 2013; Zakharova et al., 2009). Thus, social isolation during adolescence is often used to create an animal model of a population vulnerable for developing substance use disorder.

Social Isolation and Dopamine Transmission

Mesolimbic dopamine transmission has been classically identified as a major contributor to behaviors related to reward and motivation (see Ikemoto & Panksepp, 1999). The mesolimbic dopamine pathway consists of dopamine neurons in the ventral tegmental area (VTA) that project to areas of the limbic system, including the nucleus accumbens (NAc). Most drugs of abuse, including psychostimulants, share the neural mechanism of increasing dopamine efflux in the NAc (Ikemoto & Panksepp, 1999). Thus, some altered behavioral responses to drugs of abuse likely stem from altered functioning of the mesolimbic dopamine pathway.

Social isolation alters dopaminergic functioning. Utilizing fast scan cyclic voltammetry in rats, Yorgason et al. (2016) showed that social isolation for 6 weeks post weaning increased efflux and reuptake of NAc dopamine . Using western blot analysis, researchers showed that this increased reuptake functioning was likely due to a higher NAc dopamine transporter (DAT) population in isolated versus group housed animals (Yorgason et al., 2016). Karkhanis et al. (2019) also found increased dopamine release following adolescent social isolation and

attributed this hyperdopaminergic profile to an increase in dopamine synthesis and the size of readily releasable pools. Isolation-induced changes in dopamine release are more robust in the NAc as compared to the prefrontal cortex (Fone & Porkess, 2008). Other studies, however, report no effect of social isolation on baseline dopamine levels (Karkhanis et al., 2014; Miura et al., 2002; Yorgason et al., 2016). With regard to the effects of rewarding drugs on NAc dopamine release, rodents raised in social isolation exhibit a greater response to psychostimulants. This increased response has been found in response to amphetamine, methylphenidate, ethanol, and cocaine (Howes et al., 2000; Karkhanis et al., 2014; Yorgason et al., 2016).

Factors Affecting Responses to Housing Conditions

Sex

Much of the existing literature on isolation housing effects on dopamine transmission and dopamine-related behaviors exclusively employed male animals (Howes et al., 2000; Karkhanis et al., 2016; Karkhanis et al., 2019; Miura et al., 2002; Yorgason et al., 2016), preventing insight into potential sex differences. However, studies do suggest that sex plays a role in the way dopaminergic transmission is altered in response to isolation and early life stress. Following adolescent social isolation, male rodents exhibit increased anxiety scores on acoustic startle response prepulse inhibition testing (Weiss and Feldon, 2001), increased reactivity in the hypothalamic-pituitary axis leading to increased corticosterone levels, and increased isolation-induced sucrose preference (Pisu et al., 2016). Results of these studies suggest that females may be less vulnerable to anxiety-related symptomology in response to isolation housing.

Strain

Interestingly, the response to early life stress in mice is strain dependent, indicating a genetic basis for variation in stress responsiveness that may reflect individual differences within humans. C57BL/6J (B6) and DBA/2J (D2) are two inbred mouse strains that are commonly used in behavioral neuroscience research and show opposite responses to several behavioral tests.

Generally speaking, B6 mice are characterized as more active and show higher levels of self-administration of cocaine versus D2 mice, while D2 mice are more prone to anxiety-like behavior (Crawley et al., 1997; Morse et al., 1993). Early life social disturbances affect D2 mice to a greater extent than B6 mice, leading to increased isolation-induced self-administration of cocaine in D2 mice (van der Veen et al., 2007), increased responses to stressful stimuli in D2 mice (Millstein et al., 2006), and increased isolation-induced locomotor activity in D2 mice (Morse et al., 1993).

Physical and Social Stimuli

In most colonies, group housing is standard for rodents (typically paired housing for rats). Animals are housed with multiple individuals in an enclosure, divided by sex and strain. In this condition the animals are exposed to social stimulation, which compared to social isolation, can be classified as social enrichment (SE) (Holgate et al., 2017; Toyoshima et al., 2018).

Environmental enrichment (EE) on the other hand, is defined as a combination of complex inanimate and social stimulation (Sztainberg & Chen, 2010). While the specifics of EE protocols lack standardization between laboratories and studies, typically they consist of using larger than standard cages to house the animals, along with the placement of props, objects, and structures in these cages. Examples are running wheels, tubes or shelters, and thicker nesting material to allow for burrowing behaviors. These props allow for enhanced sensory, cognitive,

motor, and social stimulation versus standard laboratory housing conditions. Importantly, this allows for the performance of more natural and species-typical behaviors.

While most studies compare isolation to either SE or EE, the use of all 3 housing conditions may provide insight into the relative protective effects of social vs. physical stimuli. Aarde, Miller, Creehan, Vanderwater, and Taffe (2015) and Cosgrove, Hunter, and Carroll, (2002) found that exposure to a running wheel lead to an immediate decrease in self-administration of psychostimulants. Other studies indicate social interaction may be the driving force behind altered behaviors related to drug reinforcement (Gipson, Beckman, El-Maraghi, Marusich, & Bardo, 2011; Morse, Erwin, & Jones, 1993). Comparing groups using open field testing, isolated mice display increased anxiety compared to mice in the other conditions, while EE mice exhibited significantly less anxiety than SE (Sáenz, Villagra, & Trías, 2006). Regarding comparison of SE and EE conditions related to dopaminergic differences, results have not been consistent. For example, El Rawas et al. (2008) found similar drug-induced dopamine levels in the NAc of EE and SE, but Bardo et al. (1999) found increased drug-induced dopamine release in EE mice relative to SE mice.

Current Study

The goal of the proposed study was to examine the effects of various housing conditions on neurochemical and behavioral characteristics related to addiction, with specific interest the added influence of sex and strain. Males and females from 2 mouse strains (B6 and D2) at 3 weeks old were placed in 1 of 3 housing conditions: environmental enrichment (EE: larger cage with structures, wheels, and cage mates), socially enriched (SE: standard size cage with cage mates but no physically interactive objects), and isolated (standard size cage with no cage mates and no physically interactive objects). Mice remained in their respective housing conditions

throughout adolescence. In vivo fixed potential amperometry was used to quantify NAc dopamine release before and after a drug challenge (cocaine injection). Larger dopaminergic responses in the NAc after cocaine administration typically denotes an increased likelihood of drug seeking (Baik, 2013). The current study hypothesized that isolated mice would show a heightened NAc dopamine response to cocaine, while EE groups should have an equivalent or smaller response compared to socially enriched groups. It was expected that B6 mice, being more prone to drug self-admin, would show a higher NAc response overall compared to D2 groups. Open field testing was used to measure exploratory behaviors (locomotor activity and rearing) and provide a quick measurement of anxiety-related behaviors (time in center of chamber). Based on previous studies, I hypothesized that the mice in the EE groups of both B6 and D2 strains will show reduced locomotor activity and reduced anxiety relative to the isolation housed mice, and that D2 mice in all housing conditions would show lower locomotor activity levels overall compared to B6 groups. Socially enriched groups should score in between isolation and EE housed groups for both strains. The findings of the present study work towards increasing our understanding of the effects of factors such as strain, sex, physical enrichment, social enrichment, and isolation on behavioral and neurochemical characteristics related to drug use.

Method

All procedures were approved by our university Institutional Animal Care and Use Committee (IACUC) and are aligned with those outlined in The Public Health 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 2003).

Subjects

Ninety eight C57BL/6J mice (51 male, 47 female) and 101 DBA/2J mice (46 male, 55 female) were obtained from Jackson Laboratory (Bar Harbor, ME, USA) at 3 weeks old. Mice were housed in a temperature-controlled room at 21 ± 1ºC. The room was set on a 12 h light / 12 h dark cycle. Food and water were available ad libitum.

Procedure

Housing Conditions: Mice arrived at our facility at 3 weeks old and were immediately (and randomly) placed into one of 3 housing groups: EE, SE, or isolated (see Table 1). Mice in the EE group were housed in large cages (height: 8.00 in, length: 18.25 in, width: 9.75 in) with running wheels, nesting material, tunnels, and same sex cage mates (3-5 per group). Cage bedding was changed twice per week, and EE items were washed weekly in a solution of distilled water and bleach followed by double rinsing in distilled water and air-drying. SE mice were housed in standard mouse cages with no EE items, in same sex groups (3-5 mice per cage). Isolated mice were housed alone in standard cages with no EE items. SE and isolated mouse cages were also changed twice per week. Mice remained in their respective housing conditions for 10-12 weeks.

Stereotaxic Surgery and Dopamine Recordings: Each mouse was permanently anaesthetized with urethane (1.5g/kg, i.p.). Successful anesthesia was assessed using reflex checks including eye-blink and mild tail and foot-pinch. If necessary, a supplemental dose of urethane (0.5 g/kg, i.p.) was given. Mice were then mounted in a stereotaxic frame with a warming pad to maintain body temperature at 36*C (± 0.5 °C). Three trephine holes (~ 1-1.5 mm o.d.) were drilled through the skull. According to the coordinates from the mouse atlas of Paxinos and Franklin (2001), a concentric bipolar stimulating electrode (SNE-100; Rhodes

Medical Co., CA, USA) was inserted into the left VTA (AP +3.3mm, ML +0.3mm, and DV -4.0mm). A stainless-steel auxiliary and silver/silver chloride reference combination electrode was then placed in contact with cortical tissue contralateral to the stimulating electrode. Next, the carbon fiber recording electrode (500 um length by 7 um o.d.; Thornel Type P, Union carbide, PA) was implanted into the left NAc (AP +1.5, ML +0.8, and DV -3.8). A fixed +0.8 V current was continuously applied to the recording electrode, and dopamine oxidation currents were monitored via an electrometer (filtered at 50 Hz) at 10k samples per second (Figure 2).

Dopamine autoreceptor sensitivity was assessed by applying paired test pulses (T1 and T2, 10 pulses each at 50 Hz, 10 seconds between pulses) to the VTA at 30 second intervals. Five rounds of conditioning pulses (1, 5, 10, 20, and 40; 0.5 msec pulse duration at 15 Hz) were delivered prior to T2 such that there is 0.3 s between the end of the conditioning pulse train and initiation of T2. Autoreceptor-mediated inhibition of evoked dopamine efflux was calculated as the change in the amplitude of T2 with respect to T1 for each set of conditioning pulses; low-to-high dopamine autoreceptor sensitivity is represented as low-to-high percent inhibition of evoked dopamine efflux (i.e. high sensitivity results in a lower amplitude of T2 relative to T1).

After the autoreceptor test is completed, stimulation parameters were then reset to 20 pulses at 50 Hz every 30 seconds. Baseline levels of VTA stimulation-evoked dopamine were monitored for 5 min in each mouse prior to cocaine administration (10 mg/kg, i.p.), and dopamine recordings were continued for 1 hour post-injection. From this baseline recording, dopamine release was quantified as the magnitude of the response, and dopamine uptake, an indication of DAT functioning measured by dopamine half-life decay (i.e. the time for 50% decrease from the maximum evoked increase to the prestimulus baseline level). Finally, a 3 min

continuous stimulation was applied to assess overall dopamine supply levels (Fielding et al., 2013).

When recordings were complete, direct anodic current of 100 mAmps was applied for 10 seconds to mark the electrode positions. Mice were subsequently euthanized (intracardial urethane injection at 0.345 g/ml) and brains removed and stored in 30% sucrose/10% formalin solution with 0.1% potassium ferricyanide. Using a cryostat at -20°C, coronal sectioning of each brain was performed, and electrode placements were identified using a light microscope and recorded on coronal diagrams (Paxinos & Franklin, 2001). Also, following each FPA experiment, *in vitro* electrode calibrations were accomplished by exposing each carbon fiber recording electrode to a series of known solutions of dopamine concentrations (0.2, 0.4, 0.8, and 1.2 uM) via a flow injection system (Dugast, Suaud-Chagny, & Gonon, 1994; Prater et al., 2018). This process allows us to convert our raw current data (nAmp) to dopamine concentration (uM).

Behavioral Testing: One week prior to dopamine recordings, mice were tested in the open field chamber. The apparatus used for the open field test was a HamiltonKinder SmartFrame™ (HamiltonKinder, Poway, CA), with a 4 x 8 photo beam strip and a 4 x 8 photo beam rearing attachment. The chamber is made of clear Plexiglas and lighted with a 15-W bulb. Locomotor activity was recorded with MotorMonitor version 4.14 (HamiltonKinder, Poway, CA). Behavioral testing occured at roughly mid-day/noon for all groups. On behavioral testing days, each mouse was placed in an individual holding cage and allowed to habituate in a soundproof chamber in the testing room for at least 30 min. Following habituation, each mouse was placed in the center of the open field chamber. Behaviors including total distance travelled, total number of rears, and percent time spent in the center of the chamber were evaluated based

on infrared beam breaks that are automatically recorded by a computer. Percent time spent in the center of the chamber is an index of anxiety-related behavior, with increased percent time in center indicating decreased anxiety (Seibenhener & Wooten, 2015). Number of rears serves as an index of risk assessment and exploration, while the total distance traveled reflects overall locomotor activity. At the end of each session, the test mouse was returned to its home cage, and the open field chamber was cleaned with 10% isopropyl alcohol and allowed to dry.

Results

Baseline Dopamine Release and Half-Life

Dopamine release (μM) and half-life (sec) were assessed in each mouse prior to cocaine administration. Three-way between subjects ANOVAs were used to determine the effect of sex, strain (B6 or D2), and housing (EE, SE, isolated) on dopamine release and half-life. Regarding baseline dopamine release, no main effects of sex, strain, or housing were observed [$F(1,48) = .28$, $p = .600$; $F(2,48) = 1.87$, $p = .178$; $F(2,48) = 1.90$, $p = .161$; respectively]. There were no significant interactions between sex and strain [$F(1,48) = .58$, $p = .451$], sex and housing [$F(2,48) = .80$, $p = .454$], housing and strain [$F(2,48) = 1.44$, $p = .248$], or all 3 variables [sex, strain, and housing: $F(2,48) = .16$, $p = .853$] on baseline dopamine release (Figure 3A and B).

Similarly, regarding baseline dopamine half-life, no main effects of sex, strain, or housing were observed [$F(1,48) = .05$, $p = .822$; $F(1,48) = 2.69$, $p = .107$; $F(2,48) = .72$, $p = .491$; respectively]. There were no significant interactions between sex and strain [$F(1,48) = .31$, $p = .582$], sex and housing [$F(2,48) = .44$, $p = .649$], housing and strain [$F(2,48) = .65$, $p = .527$], or all 3 variables [sex, strain, and housing: $F(2,48) = .28$, $p = .754$] on baseline dopamine half-

life (Figure 3A and C). Altogether, none of the independent variables or combination of variables altered baseline (pre-drug) dopamine release or half-life.

Dopamine Autoreceptor Functioning

For autoreceptor functioning a mixed factorial ANOVA was used to determine the effect of sex, strain, and housing (between-subjects factors) on autoreceptor functioning across the different prepulse settings (within-subjects factor). Autoreceptor-mediated inhibition of evoked dopamine release was expressed in terms of the percentage change between test stimulations (T1 and T2) for each set of conditioning pulses (or pre-pulses). With 0 pre-pulse stimulation pairs near 100% dopamine release (no change between T1 and T2), percent release of T2/T1 that is decreased from 100% indicates autoreceptor-mediated inhibition of dopamine release. Thus, greater decreases in dopamine release (% of T2/T1) indicates increased autoreceptor functioning (Figure 4A).

As expected, there was a significant main effect of number of prepulses on percent change in dopamine release [$F(6,276) = 69.95$, $p < .001$, $\eta_p^2 = 0.60$], indicating that the number of prepulses impacts autoreceptor-mediated dopamine inhibition. There was a significant interaction between prepulses and strain [$F(6,276) = 14.98$, $p < .001$, $\eta_p^2 = .25$], with B6 mice displaying increased autoreceptor functioning versus D2 mice (Figure 4B and C). There were no significant interactions between prepulses x sex, or prepulses x housing [$F(6,276) = 1.60$, $p = .147$; $F(12,276) = .72$, $p = .728$ respectively]. There were no significant interactions between prepulses x strain x sex, or prepulses x strain x housing, or prepulses x sex x housing [$F(6,276) = .47$, $p = .831$; $F(12,276) = 1.13$, $p = .336$; $F(12,276) = .46$, $p = .936$ respectively]. No significant interactions were seen between all four variables [prepulses x sex x strain x housing: $F(12,276) = .66$, $p = .791$].

Dopamine Supply

Dopamine supply was determined by continuously stimulating the VTA to evoke the release of all available dopamine in the NAc (Figure 5A). A three-way between-subjects ANOVA was used to determine the effect of sex, strain, and housing on dopamine supply. Sex did not affect dopamine supply [$F(1,48) = 3.17$, $p = .083$]; however, a significant main effect of strain on dopamine supply levels was observed [$F(1,48) = 6.02$, $p = .019$, $\eta_p^2 = .14$], with B6 mice displaying reduced dopamine supply levels compared to D2 mice. Housing also significantly altered dopamine supply [$F(2,48) = 7.94$, $p = .001$, $\eta_p^2 = .31$], with Bonferroni post hoc test showing that EE mice had reduced dopamine supply compared to both SE and isolated mice (p =.007 and .001, respectively) and no differences between SE and isolated mice (p = 1.00).

There was a significant interaction between housing and strain [$F(2,48) = 3.50$, $p = .041$, $\eta_p^2 = .16$]. In B6 mice, housing significantly altered dopamine supply [$F(2,30) = 6.67$, $p = .004$, $\eta_p^2 = .33$], with Bonferroni post hoc test showing that EE mice had significantly reduced dopamine supply levels compared to isolated mice ($p = .004$) but no differences between EE and SE mice ($p = .541$) or SE and isolated mice ($p = .063$). However, in D2 mice, housing did not significantly alter dopamine supply [$F(2,18) = 2.64$, $p = .104$]. There were no significant interactions between sex and strain [$F(1,48) = 3.51$, $p = .069$], sex and housing [$F(2,48) = 1.95$, $p = .157$], or all 3 variables [sex, strain, and housing: $F(2,48) = 1.37$, $p = .266$] on dopamine supply (Figure 5B).

Dopaminergic Response to Cocaine

Mixed factorial ANOVAs were used to determine the effect of sex, strain, and housing (between-subject factors) on percent change of dopamine release and half-life following cocaine

administration over the 1-hour recording period (in 10 min intervals, within-subject factor) (Figure 6A). As expected, there was a significant main effect of time post cocaine injection on percent change in dopamine release [$F(6,288) = 90.52$, $p < .001$, $\eta_p^2 = 0.65$]. Neither sex nor strain altered the percent change in dopamine release over time following the cocaine injection [sex x time: $F(6,288) = .27$, $p = .949$; strain x time: $F(6,288) = .42$, $p = .868$]. There was a significant interaction between housing and time post cocaine injection [$F(12,288) = 1.85$, $p = .041$, $\eta_p^2 = .07$]; however, follow up ANOVAs indicated no significant differences in percent change in dopamine release between housing groups at specific time points post injection ($p = .089$ - $.957$) (Figure 6B and C). There were no significant interactions between sex x strain x time, sex x housing x time, or strain x housing x time on percent change in dopamine release following cocaine [$F(6,288) = .21$, $p = .993$; $F(12,288) = .95$, $p = .498$; $F(12,288) = .78$, $p = .689$], and no significant interactions were seen between all four variables [sex x strain x housing x time: $F(12,288) = .65$, $p = .800$].

Regarding percent change in dopamine half-life following cocaine injection, as with dopamine release, there was a significant main effect of time post cocaine injection [$F(6,288) = 83.70$, $p < .001$, $\eta_p^2 = 0.64$]. Sex did not alter percent change in dopamine half-life over time [$F(6,288) = .53$, $p = .787$]; however, there were significant effects of both strain and housing on percent change in dopamine half-life over time [$F(6,288) = 2.46$, $p = .024$, $\eta_p^2 = 0.05$; $F(12,288) = 3.34$, $p < .001$, $\eta_p^2 = .12$, respectively]. Regarding strain, follow-up analyses showed no significant differences in percent change in dopamine half-life between B6 and D2 mice at any specific time point ($p = .067$ - $.747$). Regarding housing, follow-up analyses with one-way ANOVAS and Bonferroni post hoc test revealed that isolated mice had a greater percent change in dopamine half-life compared to EE mice at 10 and 20 min ($p = .001$ and $.002$) and SE mice at

20 and 30 min (p = .002 and .043). All other housing comparisons at the various time points were not significantly different (p > .05) (Figure 6D and E).

No significant interactions between sex x strain x time or sex x housing x time were observed on percent change in dopamine half-life post cocaine [$F(6,288) = 1.94$, p = .074; $F(12,288) = 1.35$, p = .188 respectively]. There was a significant interaction of strain x housing x time on percent change in dopamine half-life post cocaine [$F(12,288) = 1.83$, p = .043, η_p^2 = .07], meaning strain and housing had a differing combined effect on dopamine half-life changes over time following cocaine administration. In B6 mice, follow-up ANOVAs revealed housing differences at all time points (p < .05), with specific group differences distinguished using Bonferroni post hoc tests. B6 isolated mice had increased percent change in dopamine half-life compared to EE mice at 10 and 20 min post cocaine and compared to SE mice at 10, 20, and 30 min post cocaine (p < .05). Surprisingly, towards the end of the recording period, at 50 and 60 min post cocaine, B6 EE mice had significantly increased percent change in dopamine half-life compared to SE mice. In D2 mice, ANOVAs at each time point revealed housing differences in percent change in dopamine half-life only at 10 min post injection (p = .014), with Bonferroni post hoc tests revealing that isolated and SE mice had increased percent changes in dopamine half-life compared to EE mice (p = .025 and .016, respectively) with no differences between isolated and SE mice (p = 1.00). No significant interactions were seen in percent change in dopamine half-life between all four variables [sex x strain x housing x time: $F(12,288) = .69$, p = .762].

Open Field Activity

From the open field testing results, three dependent variables were analyzed: locomotor activity, number of rears, and time in center. Three-way between-subject ANOVAs were used to determine the effect of sex, strain, and housing on each of these dependent variables.

Locomotor Activity: No main effect of sex was observed on locomotor activity [$F(1,199) = .81$, $p = .370$]; however, strain and housing both significantly altered locomotor activity [$F(1,199) = 39.50$, $p < .001$, $\eta_p^2 = .17$; $F(2,199) = 15.53$, $p < .001$, $\eta_p^2 = .14$, respectively]. Regarding the main effect of strain, B6 mice displayed higher overall locomotor activity than D2 mice. Regarding the main effect of housing, Bonferroni post hoc test revealed that isolated mice had higher locomotor activity than both EE and SE mice ($p < .001$ and $p = .001$, respectively) with no difference between EE and SE mice ($p = .086$).

There were no significant interactions between sex and strain or sex and housing on locomotor activity [$F(1,199) = .35$, $p = .558$; $F(2,199) = .87$, $p = .421$, respectively]. However, a significant interaction between housing and strain was observed [$F(2,199) = 3.61$, $p = .029$, $\eta_p^2 = .04$]. In B6 mice, isolated mice had higher locomotor activity than both EE and SE mice ($p < .001$ and $p = .004$, respectively) with no difference between EE and SE mice ($p = .733$). In D2 mice, isolated mice had higher locomotor activity than EE mice (p = .017) but not SE mice (p = 1.000) with no difference between EE and SE mice ($p = .075$). No interaction was observed between all 3 factors on locomotor activity [sex x strain x housing: $F(2,199) = .24$, $p = .788$] (Figure 7A).

Rearing: No main effect of sex was observed on rearing [$F(1,199) = .01$, $p = .926$]; however, strain and housing both significantly altered rearing behavior [$F(1,199) = 31.92$, $p < .001$, $\eta_p^2 = .15$; $F(2,199) = 9.27$, $p < .001$, $\eta_p^2 = .09$, respectively]. Regarding the main effect of strain, B6 mice had significantly higher number of rears than D2 mice. Regarding the main effect

of housing, Bonferroni post hoc test revealed that EE mice had fewer rears than isolated mice ($p < .001$) and SE mice (near significant, $p = .050$) with no difference between SE and isolated mice ($p = .064$).

There were no significant interactions between sex and strain or sex and housing on rearing [$F(1,199) = .27$, $p = .601$; $F(2,199) = .81$, $p = .448$, respectively]. However, a significant interaction between housing and strain was observed [$F(2,199) = 4.70$, $p = .010$, $\eta_p^2 = .05$]. In B6 mice, isolated mice had an increased number of rears compared to both EE and SE mice ($p < .001$ and $p = .025$, respectively) with no difference between EE and SE mice ($p = .191$). In D2 mice, no significant differences in rearing were observed between any housing conditions ($p > .05$). No interaction was observed between all 3 factors on number of rears [sex x strain x housing: $F(2,199) = 2.40$, $p = .094$] (Figure 7B).

Time in Center: In mice, reduced time spent in the center of the open field chamber is considered an anxiety-related behavior (Seibenhener & Wooten, 2015). Main effects of sex, strain, and housing were observed on time spent in the center [$F(1,199) = 8.61$, $p = .004$, $\eta_p^2 = .04$; $F(1,199) = 100.13$, $p < .001$, $\eta_p^2 = .35$; $F(2,199) = 6.33$, $p = .002$, $\eta_p^2 = .06$, respectively]. Regarding the sex effect, female mice spent less time in the center than males. Regarding the strain effect, D2 mice spent less time in the center than B6 mice. Regarding the main effect of housing, Bonferroni post hoc tests revealed no significant differences between specific housing groups on time spent in the center ($p > .05$).

There were no significant interactions between sex and strain or sex and housing on time in center [$F(1,199) = 2.63$, $p = .107$; $F(2,199) = 1.10$, $p = .336$, respectively]. However, a significant interaction between housing and strain was observed [$F(2,199) = 5.95$, $p = .003$, $\eta_p^2 = .06$]. In B6 mice, isolated mice spent less time in the center than EE and SE mice ($p = .001$ and

.033, respectively) with no difference between EE and SE mice (p = .973). In D2 mice, no significant differences in time spent in the center were observed between any housing conditions (p > .05). No interaction was observed between all 3 factors on number of rears [sex x strain x housing: $F(2,199) = 2.76$, p = .066] (Figure 7C).

Discussion

This study utilized fixed-potential amperometry for quantification of stimulation-evoked phasic dopamine release and open field testing for measurement of exploratory behavior in B6 and D2 mice that had been in one of three housing conditions (EE, SE, or isolated) throughout their adolescence (for 10-12 weeks starting at 3 weeks of age). The housing conditions were designed to parse apart the influence of physical and social environmental stimuli in relation to their abilities to protect against isolation-induced phenotypes. EE conditions included both physical and social stimuli, SE conditions included only social stimuli, and isolated condition included neither physical nor social stimuli. The variables measured in this study include baseline (pre-drug) dopamine release, baseline dopamine synaptic half-life, dopamine autoreceptor functioning, available dopamine supply, dopamine release following cocaine, dopamine half-life following cocaine, and open field behaviors (locomotor activity, rearing, and time in the center). Given that isolated mice have been shown to be more likely to seek and show preference for drugs of abuse (Howes et al., 2000; Schenk et al., 1987), specific interest was given to the effects these housing conditions had on nucleus accumbens dopamine efflux in response to a drug challenge (cocaine), this being used as a marker for addiction risk.

Sex and Strain Effects

Sex and strain are 2 factors that have been shown to influence isolation-induced phenotypes; therefore, the impact of these factors alone will be considered first, prior to the impact of these factors in combination with housing conditions. Sex did not affect any of the measured variables associated with dopamine functioning. Sex also did not affect exploratory behaviors (locomotor activity or rearing), but did influence the time the mice spent in the center of the open field chamber. Time spent in the center of the testing chamber was used as a brief measure of anxiety-like-behaviors since thigmotaxis, remaining in the corners and sides of an enclosure, is related to anxiety (Seibenhener & Wooten, 2015). Female mice spent less time in the center, a behavior associated with increased anxiety, compared to males, supporting the findings of Bondar et al. (2018). Sex differences did not influence strain- or housing-related phenotypes, as no sex x strain, sex x housing, or sex x strain x housing interactions were observed in any of the measured variables.

B6 and D2 strains are commonly compared in behavioral neuroscience research and often show opposing results in behavioral tasks related to drug reward. B6 mice are typically characterized as being more prone to self-administer drugs of abuse including cocaine. Alternately, and as demonstrated in the present study, D2 mice are characterized as being less active and more prone to display anxiety-related behaviors (Crawley et al., 1997; Morse et al., 1993). The present results indicated no differences in baseline dopamine release or half-life between the 2 strains. This mirrors prior studies which found no significant differences between B6 and D2 strains in phasic dopamine dynamics (Yorgason, et al., 2015; Brodie and Appel, 2000). However, the present study did reveal strain differences in dopamine autoreceptor functioning and available neuronal supply levels, with B6 mice displaying increased autoreceptor functioning and decreased dopamine supply compared to D2 mice. This negative relationship

between autoreceptor function and dopamine supply has been observed previously (Holloway et al., 2018). Autoreceptor functioning may increase to compensate for reduced dopamine supply, or dopamine supply may decrease in response to increased autoreceptor functioning.

When examining the dopaminergic response of these strains to cocaine, we expected cocaine to have a greater effect on dopamine transmission in B6 mice relative to D2 mice, given previously mentioned behavioral studies. No strain-related differences were observed in cocaine-induced dopamine release patterns, but with cocaine being a DAT inhibitor, specifically targeting proteins regulating dopamine uptake kinetics, not necessarily release, analysis of dopamine half-life along with the magnitude of dopamine efflux is necessary. Dopamine half-life is commonly used as an indication of the influence of a DAT inhibitor (Mittleman et al., 2011; Siciliano et al., 2014). Strain did significantly influence change in dopamine half-life following cocaine, with the biggest differences seen 10 min post injection. Strain differences at the individual time points were not significant, but it is possible that these were masked by housing effects. In the SE condition, which is considered standard housing conditions in most laboratory settings, the B6 mice appeared to have greater percent change in dopamine half-life following cocaine administration compared to D2, although this specific analysis was not run given the nature of the project intent.

Housing Effects in Each Strain

Examining housing group differences on dopamine functioning, no discernable differences occurred in baseline dopamine release, half-life, or autoreceptor functioning. However, available neuronal dopamine supply was significantly altered housing, with SE and isolated mice displaying increased dopamine supply relative to EE mice. Regarding post-cocaine dopamine half-life, as expected, housing groups were significantly different at the early stages

post injection (10-30 min), isolated mice exhibiting a greater percent change in dopamine half-life versus EE and SE mice.

Previous studies have shown that mouse strains respond differently to stress and housing condition manipulations. Interactions between strain and housing were significant in several of the measured variables, including percent change in dopamine half-life following cocaine. In B6 mice, isolated mice had a greater percent change in dopamine half-life following cocaine compared to both EE and SE at the initial time points (10-30 min). Alternately, in D2 mice, significant differences were seen early post-injection, with isolated and SE mice exhibiting significantly increased changes in half-life versus EE groups. Thus, in B6 mice the SE condition seemed to have a similar effect as the EE condition, but in D2 mice the SE condition seemed to have a more similar effect to the isolated condition.

Increased exploratory behaviors have been shown to correlate with increased drug self-administration (Belin et al., 2015; Mitchell, et al., 2016). Similar grouping patterns from the dopaminergic responses to cocaine were also observed in the current exploratory behavior results. B6 isolated mice had significantly higher locomotor activity and rearing compared to EE and SE mice, while D2 SE and isolated mice showed significantly higher locomotor activity and rearing compared to EE mice. These results suggest that for the B6 mice, social enrichment (group housing) may be enough to protect against the effects of isolation. The added physical stimuli did not appear to make any differences. However, in the D2 mice, the physical stimuli seemed to make more difference than the social stimuli.

Regarding time spent in the center of the testing chamber, an interactive effect of strain and housing was observed. In B6 mice, EE and SH groups spent significantly more time in center than isolated mice, indicating increased anxiety in isolated mice. This behavioral pattern

again mirrors the locomotor activity data and dopaminergic response to cocaine data showing that social stimuli alone has the same effect as the combination of social and physical stimuli in B6 mice. Surprisingly, housing did not alter the time spent in the center for D2 mice. However, due to D2 mice exhibiting higher anxiety overall, with little movement toward the center of the chamber, there is the potential of a floor effect masking further anxiety responses due to housing. Further measurements of anxiety, such as elevated plus maze or light/dark box testing, could have elucidated different results. Another factor to be considered, in our isolation housing condition mice were still able to see and smell other mice, as they were in transparent cages placed on a rack side by side. This may have detracted from the overall stress level enough to negate the possibility of overcoming any such floor effects.

Given that the present housing variable did not alter anxiety-related behaviors in D2 mice but did affect the dopaminergic response of these mice to cocaine, anxiety is likely not the only causative factor in the measured relationship between housing and dopamine transmission. Instead of focusing on the impact of isolation, an alternate explanation may be that exposure to physical and social stimuli condition the mesolimbic dopamine systems to respond to dopamine agonists (or rewards) differently than if these stimuli were not present. Physical and social stimuli can elicit dopamine release, resulting in compensatory changes in the mesolimbic dopamine system over time (Gunaydin et al., 2014; Torquet et al., 2018). Behavioral studies suggesting that B6 mice exhibit increased social interaction compared to D2 mice (Mineur et al., 2006; An et al., 2011)) may explain the current findings that in B6 mice the SE groups were more similar to the EE groups, both differing from isolated mice (at least in regards to locomotor activity and dopaminergic response to cocaine), while in D2 mice the SE groups were more similar to isolated mice, both differing from the EE groups. A comparison of dopamine release

during social interaction in both strains and across housing conditions is needed to explore this idea.

Conclusions

The results of the proposed study provide further insight into the effects of early-life isolation versus enriched environmental conditions on anxiety-like behavior and drug abuse vulnerability. Animal models allow us to control variables and determine cause/effect relationships not possible with human studies. Thus, these results may potentially aid in illuminating the importance of early interventions to combat drug abuse in vulnerable human populations by showing the potential effects of neglect or impoverished environment on abuse liability. Along with this, the study provides a further characterization of the behavioral and neurochemical profile of two widely used mouse strains. Further characterization of the C57BL/6J and DBA/2J mouse strains allows these strains to be utilized to their full potential in future research studies and help provide a foundation for addiction-related genetic studies.

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Appendix

Table 1. Experimental Groups and Sample Sizes

Strain	*N*	Sex	*N*	Housing	*Behavioral N*	*Amperometry N*
C57/BL6J	98	Female	47	Enriched housing	13	5
				Standard housing	13	4
				Isolation housing	21	6
		Male	51	Enriched housing	12	6
				Standard housing	12	6
				Isolation housing	27	4
DBA/2J	101	Female	55	Enriched housing	14	5
				Standard housing	20	7
				Isolation housing	21	4
		Male	46	Enriched housing	15	4
				Standard housing	13	5
				Isolation housing	18	4
Total					199	60

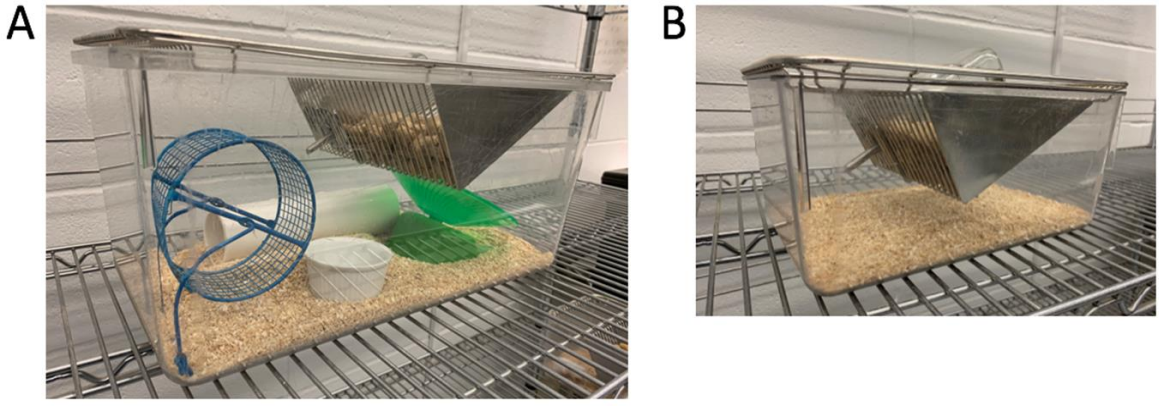

Figure 1. Housing Conditions. (A) EE cages are larger and include 2 running wheels, a tunnel, and cage mates (5 per cage). (B) SE and isolated conditions (3 and 1 per cage, respectively) are standard cages with no EE objects.

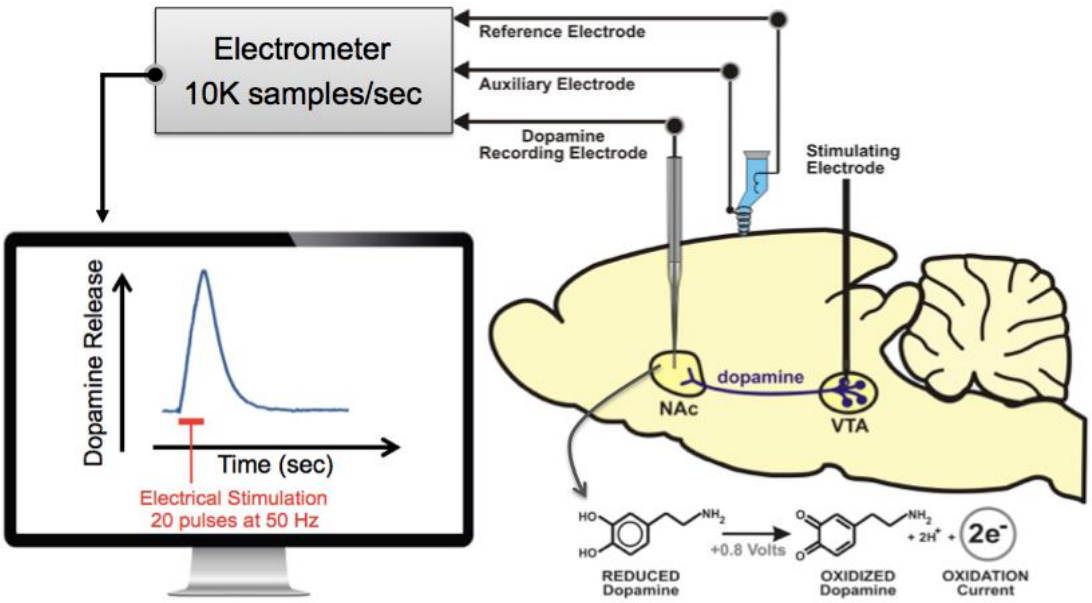

Figure 2. Amperometric Set-up. In vivo fixed potential amperometry was used to quantify nucleus accumbens (NAc) dopamine release evoked by stimulation of dopamine cell bodies within the ventral tegmental area (VTA).

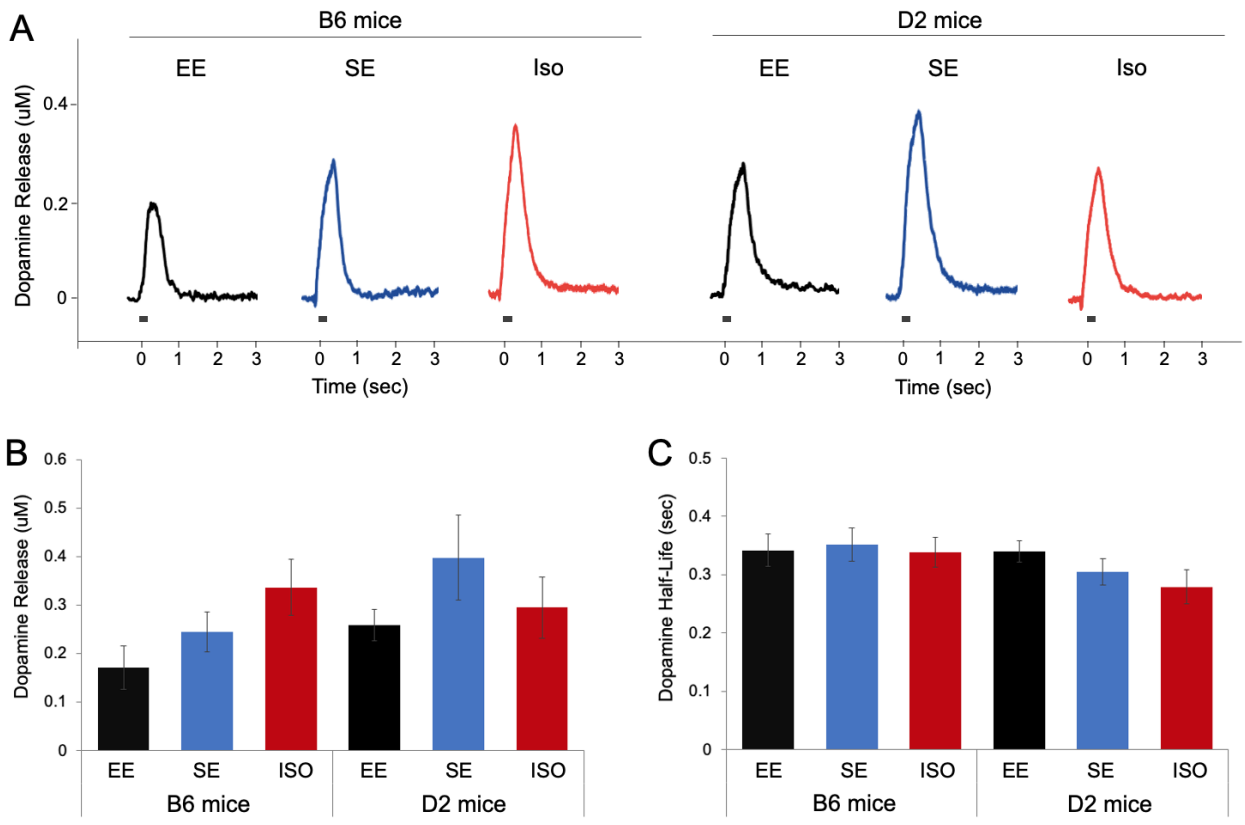

Figure 3. Baseline (pre-drug) stimulation-evoked dopamine release in the nucleus accumbens. (A) Profiles indicate example responses from each strain and housing group. (B, C) No differences in mean (± SEM) differences in dopamine release or half-life between strain or housing groups (n = 4-7 per group). EE: environmental enrichment, SE: social enrichment, ISO: isolation housed.

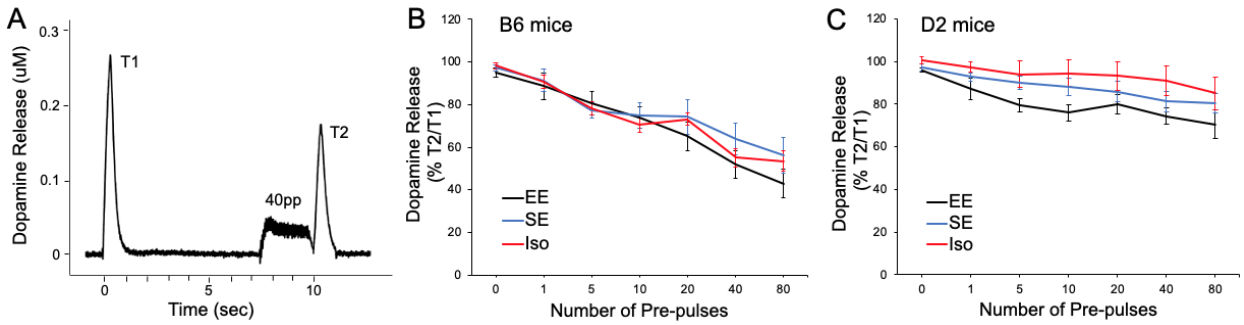

Figure 4. Autoreceptor-mediated inhibition of dopamine release. (A) Example amperometric recording of autoreceptor test stimulations (T1,T2) separated by 40 pre-pulses (pp). Greater decreases in dopamine release (% of T2/T1) indicates increased autoreceptor functioning. (B,C) The only significant effect on mean (± SEM) differences in autoreceptor functioning was that of strain, with B6 mice displaying increased autoreceptor functioning versus D2 mice (n = 4-7 per group). EE: environmental enrichment, SE: social enrichment, ISO: isolation housed.

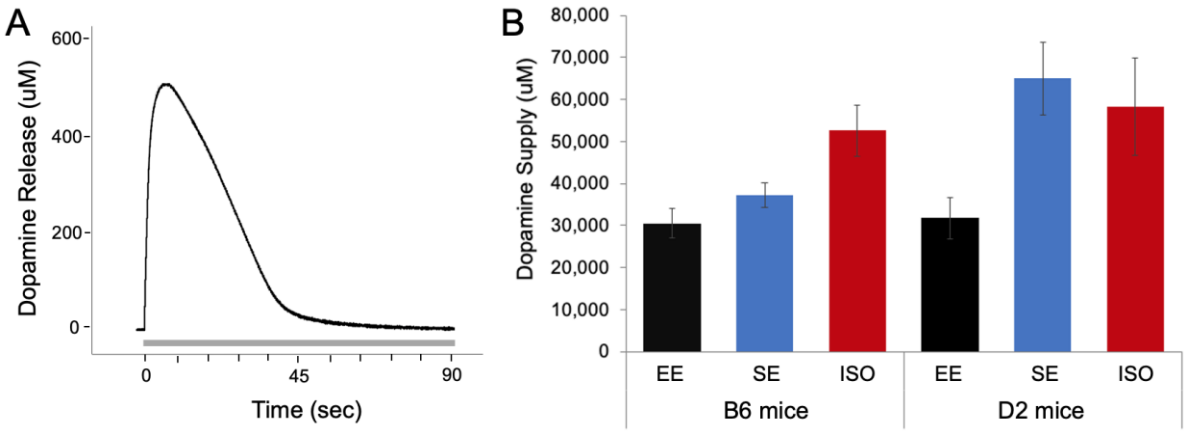

Figure 5. Available neuronal supply of dopamine. (A) Dopamine supply was determined by continuously stimulating the VTA and summing NAc dopamine release across the first 90 sec. (B) A significant main effect of strain and housing plus an interactive effect of strain x housing was observed on mean (± SEM) dopamine supply levels (n = 4-7 per group). EE: environmental enrichment, SE: social enrichment, ISO: isolation housed.

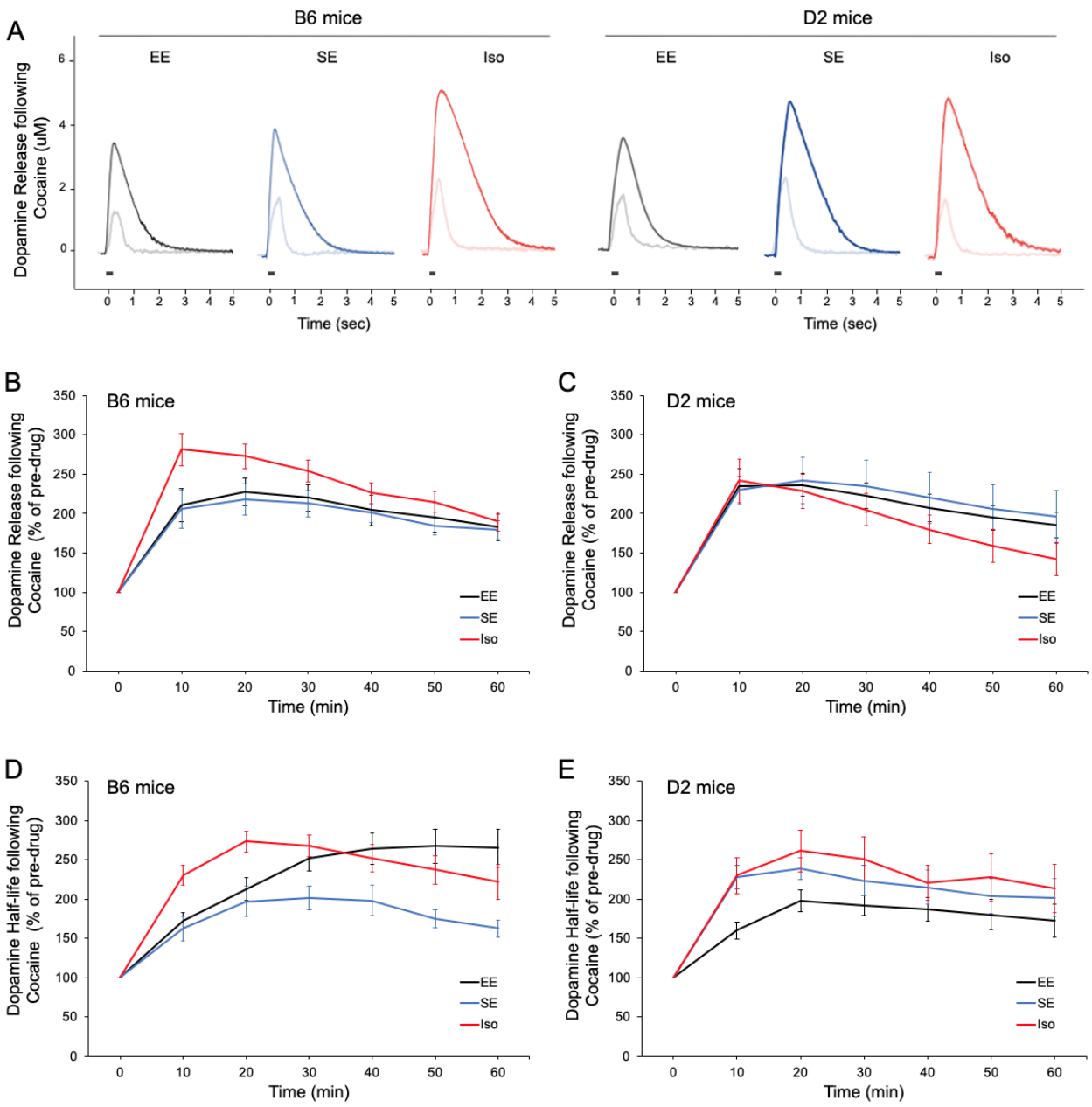

Figure 6. Dopaminergic response to cocaine (10 mg/kg, ip). (A) Profiles indicate example responses from each strain and housing group at 10 min post injection. Light lines represent pre-cocaine response. (B,C) Mean (± SEM) dopamine release over the 1 hour recording period following cocaine administration was altered by a main effect of housing with no main or interactive effects of strain. (D,E) Mean (± SEM) dopamine half-life over the 1 hour recording period following cocaine administration was altered by a main effect of strain and housing, plus an interactive effect of strain x housing (n = 4-7 per group). EE: environmental enrichment, SE: social enrichment, ISO: isolation housed.

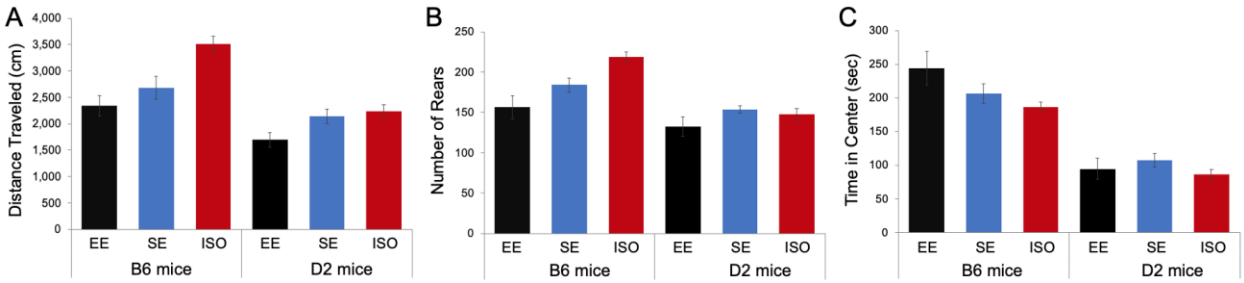

Figure 7. Open field behaviors. Data is shown in mean (± SEM). (A) Locomotor activity, (B) rearing, and (C) time spent in center were all altered by main effects of strain and housing, plus an interactive effect of strain x housing (n = 4-7 per group). EE: environmental enrichment, SE: social enrichment, ISO: isolation housed.