Experimental Brain Research (2022) 240:2803–2815
https://doi.org/10.1007/s00221-022-06449-w

RESEARCH ARTICLE

Age-dependent effects of social isolation on mesolimbic dopamine release

Megan A. McWain[1] · Rachel L. Pace[1] · Patricia A. Nalan[1] · Deranda B. Lester[1]

Received: 22 April 2022 / Accepted: 21 August 2022 / Published online: 3 September 2022

Abstract

In humans, social isolation is a known risk factor for disorders such as substance use disorder and depression. In rodents, social isolation is a commonly used environmental manipulation that increases the occurrence of behaviors related to these disorders. Age is thought to influence the effects of social isolation, but this predictive relationship is not well-understood. The present study aimed to determine the effects of social isolation on mesolimbic dopamine release at different developmental age points in mice. The experimental ages and their corresponding comparison to human age stages are as follows: 1 month = adolescence, 4 months = mature adulthood, 12 months = middle adulthood, and 18 months = older adult. Mice were socially isolated for 6 weeks during these developmental stages, then in vivo fixed potential amperometry with recording electrodes in the nucleus accumbens was used to measure stimulation-evoked dopamine release, the synaptic half-life of dopamine, dopamine autoreceptor functioning, and the dopaminergic response to cocaine. Isolation altered dopamine functioning in an age-dependent manner. Specifically, isolation increased dopamine release in the adult ages, but not adolescence, potentially due to increased inhibitory effects of dopamine autoreceptors following adolescent social isolation. Regarding the cocaine challenge, isolation increased dopaminergic responses to cocaine in adolescent mice, but not the adult mice. These findings have implications for clinical and experimental settings. Elucidating the relationship between age, social isolation, and neurochemical changes associated with substance use disorder and depression may lead to improvements in preventing and treating these disorders.

Keywords Dopamine · Aging · Nucleus accumbens · Isolation · Amperometry · Development

Introduction

Social isolation has been repeatedly associated with poor mental health and increased prevalence of substance use disorder (SUD) and depression (see Global Council on Brain Health 2017; Holt-Lunstad et al. 2015), and both objective and subjective types of isolation have increased in prevalence due to the COVID-19 pandemic (Loades et al. 2020). Although time will further elucidate the impact of pandemic-induced isolation on mental health, early studies showed particularly detrimental mental health effects on adolescent and elderly populations (Berg-Weger and Morley 2020; Loades et al. 2020). In the current study, we used mice to examine the effects of social isolation on neurochemical functioning related to SUD and depression in different age groups.

In rodents, social isolation is a commonly used environmental manipulation to increase the expression of stress hormones, drug-seeking, and the occurrence of behaviors related to anxiety and depression (Brenes et al. 2008; Fone and Porkess 2008; Ieraci et al. 2016; Kokare et al. 2010; Walker et al. 2019). In these studies, social isolation was applied during adolescence or young adulthood, developmental stages in which the brain is still plastic and considered more vulnerable to environmental stressors (Arakawa 2018). Social isolation during adulthood has been shown to induce similar behaviors related to substance use, anxiety, and depression, but often to a lesser degree than isolation during adolescence (Fone and Porkess 2008; Ieraci et al. 2016; Zorzo et al. 2019). Although several studies suggest that late adulthood may be another age at which rodents and

Communicated by Sreedharan Sajikumar.

✉ Deranda B. Lester
dbrewer@memphis.edu

[1] Department of Psychology, The University of Memphis, Memphis, TN 38152-6400, USA

humans become more vulnerable to the detrimental effects of social isolation (Arranz et al. 2009; Sullens et al. 2021), no related studies have compared isolation-induced effects in multiple developmental stages that span from adolescence to late adulthood.

The mesolimbic dopamine system is one modulator of behaviors related to SUD and depression. The mesolimbic dopamine pathway consists of dopamine cell bodies in the ventral tegmental area (VTA) that project to limbic nuclei, most notably the nucleus accumbens (NAc) (Baik 2013; Ikemoto and Panksepp 1999; Wise 2008). Social isolation during adolescence or young adulthood has been shown to increase dopamine release, dopamine uptake rates, dopamine transporter (DAT) expression levels, and psychostimulant-induced dopamine transmission (Han et al. 2012; Yorgason et al. 2013, 2016). Isolation-induced hyperdopaminergic profiles fit with behavioral studies that show increased self-administration and conditioned place preference to substances of abuse, such as ethanol, morphine, cocaine, and other psychostimulants (Ding et al. 2005; Fone and Porkess 2008; McCool and Chappell 2009; Whitaker et al. 2013; Schenk et al. 1987; Zakharova et al. 2009). On the other end of the spectrum, reduced functioning in this pathway is associated with anhedonia and decreased motivation, both symptoms associated with depression (Perona et al. 2008; Scheggi et al. 2018; Wise 2008). Accordingly, several antidepressants function either fully or partly as dopamine agonists. A functional mesolimbic dopamine system is crucial for the healthy regulation of reward-seeking, motivation, and mood.

The present study aimed to determine the effects of social isolation on dopamine release at different developmental age points. Dopamine functioning is known to alter with age alone, and dopamine neurons are thought to be particularly vulnerable to the aging process. Dopamine receptor expression in the NAc reaches its highest point during mid-adolescence, declining during adulthood, following an inverted U-shape of rising and falling (Burke and Miczek 2013; Karkhanis et al. 2019). Similarly, dopamine release and neuronal activity also seem to fit this developmental inverted U-shape with dopamine release and activity being the greatest in young adulthood (Huang et al. 1995; Pitts et al. 2020; Santiago et al. 1993; Stamford 1989). Little is known about the effects of social isolation on dopamine functioning across these ages, especially during mid to late adulthood. Such information is particularly important given the commonality of reduced social interaction during old age and health-related quarantines (Banerjee and Rai 2020; Singh and Misra 2009).

In the current study, mice from four age groups (adolescence, young adulthood, middle-aged adulthood, and old age) were placed in one of two housing conditions (group-housed or socially isolated) for 6 weeks. See Fig. 1 for a comparison of mouse and human age ranges. In vivo fixed potential amperometry was used to measure stimulation-evoked dopamine efflux in the NAc of anesthetized mice. Measures included quantification of dopamine release and two mechanisms that govern extracellular dopamine: dopamine transporter (DAT) and dopamine autoreceptor (DAR) functioning. Also, to examine the effects of aging and social isolation on the dopaminergic response to a commonly researched psychostimulant, all mice received an in-test injection of cocaine, which serves as a dopamine agonist by inhibiting DAT. Overall, we expected that social isolation would have the greatest effects on dopamine functioning when applied during adolescence and late adulthood. Such studies are important for elucidating the relationship

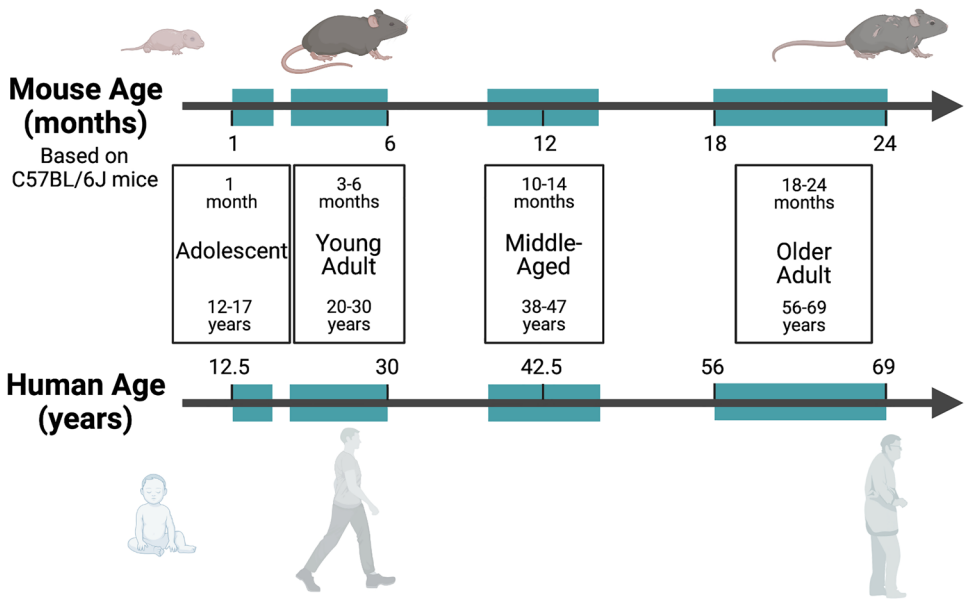

Fig. 1 A comparison between the rates at which mice and humans age. Mouse ages are based on the developmental rates of C57BL/6J mice with the assumption of healthy aging in a laboratory setting. Created with BioRender.com

between social isolation, aging, and neurochemical changes associated with SUD and depression. An improved understanding of these contributing factors may help in prevention and treatment of these disorders.

Methods

All experiments were approved by the Institutional Animal Care and Use Committee at the University of Memphis and conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Efforts were made to reduce the number of animals used and to minimize pain and discomfort.

Animals and housing conditions

Fifty-five male C57BL/6J mice were obtained from Jackson Laboratory. All mice were housed in a temperature-controlled room at 21 °C with a 12 h light: 12 h dark cycle, with lights on at 0600. Food and water were available ad libitum. Mice were group-housed, 2–5 per cage, until 1, 4, 12, or 18 months. Age of mice roughly translates to human age stages as follows: 1 month = adolescence, 4 months = mature adulthood, 12 months = middle adulthood, and 18 months = older adult (see Fig. 1) (Flurkey et al. 2007; Hagan 2017; Lester 2021). At the appropriate age, mice were either isolated to a cage by themselves or were allowed to remain group-housed. The number of mice per experimental group were as follows: 1 month group-housed $n = 7$ and isolated $n = 7$, 4 months group-housed $n = 7$ and isolated $n = 8$, 12 months group-housed $n = 6$ and isolated $n = 7$, 18 months group-housed $n = 6$ and isolated $n = 7$. All mice were in transparent cages on racks in the same room so sensory cues were not limited, but they were inhibited from social isolation (for standard isolation methods see Walker et al. 2019). Mice remained in these housing conditions for 6 weeks, which is a commonly applied isolation period in related research (Yorgason et al. 2013, 2016).

Surgery and dopamine recordings

Dopamine recordings took place at the conclusion of the housing condition period. Mice were permanently anesthetized using urethane (1.5 g/kg, i.p.) and mounted in a stereotaxic frame (David Kopf Instruments), with body temperature maintained at approximately 37 °C. A stimulating electrode (SNE-100, MicroProbes) was inserted into the left VTA (coordinates: AP − 3.3 mm from bregma, ML + 0.3 mm from midline, DV − 4.0 from dura; Paxinos and Franklin 2001). A stainless-steel auxiliary and Ag/AgCl reference electrode combination was positioned in contact with cortical tissue on the contralateral side of the brain (− 2.0 mm from bregma). Finally, a carbon fiber recording electrode with an active recording surface of 500 µm (length) by 7 µm (o.d.) was implanted into the left NAc (coordinates: AP + 1.5 mm from bregma, ML + 1.0 mm from midline, and DV − 4.0 mm from dura) (Paxinos and Franklin 2001). All amperometric recordings were made within a Faraday cage to increase signal to noise ratio. A fixed potential (+ 0.8 V) was applied via the auxiliary electrode, and dopamine oxidation currents were continuously monitored at 10,000 samples per second via an electrometer filtered at 50 Hz (ED401 e-corder and EA162 picostat, eDAQ Inc.). Following surgical set-up, the initial stimulation protocol consisted of 20 monophasic 0.5 ms duration pulses (800 µAmps) at 50 Hz every 30 s to establish a dopamine response. Electrical stimulations were delivered to the stimulating electrode by an optical isolator and programmable pulse generator (Iso-Flex and Master-8, AMPI). In vivo fixed potential amperometry is confirmed as a reliable and valid measure of stimulation-evoked dopamine release in the NAc, providing a temporal resolution suitable to quantify phasic dopamine release, reuptake rates, and DAR functioning (Dugast et al. 1994; Holloway et al. 2018; Lester et al. 2010; Suaud-Chagny et al. 2002).

DAR sensitivity was assessed by applying paired test pulses (T1 and T2, 10 pulses each at 50 Hz, 10 s between T1 and T2) to the VTA every 30 s. Five sets of conditioning pulses (1, 5, 10, 20, and 40; 0.5 ms pulse duration at 15 Hz) were delivered prior to T2 such that there was 0.3 s between the end of the conditioning pulse train and the initiation of T2. These stimulation parameters are identical to those of Fielding et al. (2013), Holloway et al. (2018), and Mittleman et al. (2011) and similar in concept to studies by Benoit-Marand et al. (2000) and Kennedy et al. (1992). 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 (T2/T1 × 100). Low-to-high DAR functioning is represented as low-to-high percent inhibition of evoked dopamine efflux. In other words, increased DAR functioning results in more inhibition of dopamine release, observed as a reduced amplitude of T2 relative to T1.

Following the autoreceptor test, stimulation parameters were reset to 20 pulses at 50 Hz every 30 s. Baseline levels of VTA stimulation-evoked dopamine were monitored for 5 min before the drug challenge (cocaine administration, 10 mg/kg, i.p.). Stimulation parameters and dopamine recordings continued for 1-h post-injection. Dopamine release was quantified as the magnitude of the response and dopamine uptake, an indication of DAT functioning, was measured by the synaptic half-life of dopamine (the time required for 50% decrease from the maximum evoked increase to the pre-stimulus baseline level) (Benoit-Marand et al. 2000; Estes et al. 2019; Holloway et al. 2018;

Mittleman et al. 2011). Given that cocaine is a DAT inhibitor, specifically target proteins that regulate dopamine uptake kinetics and not necessarily release, analysis of changes in dopamine half-life is commonly used as an indication of the influence of a DAT inhibitor (Holloway et al. 2018; Mittleman et al. 2011; Siciliano et al. 2014). Thus, the synaptic half-life of dopamine will be extracted every 10 min following the cocaine injection. Following dopamine recordings, in vitro electrode calibrations were conducted 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 et al. 1994; Prater et al. 2018), allowing conversion of raw current data (nAmp) to dopamine concentration (μM).

Histology

At the conclusion of each amperometric recording, a direct anodic current (100 μAmps for 10 s) was applied to the stimulating electrode to mark electrode placement. Mice were then euthanized with intracardial urethane (0.345 g/ml), and brains were removed and stored in a solution of 10% formalin with 0.1% potassium ferricyanide for at least 1 week then 30% sucrose for at least 1 additional week. Coronal sectioning of each brain was performed using a cryostat at − 20 °C, and electrode placements were identified using a light microscope and recorded on coronal diagrams (Paxinos and Franklin 2001).

Drugs

All chemicals used were obtained from MilliporeSigma. Urethane (U2500) and cocaine hydrochloride (C5776) were dissolved in 0.9% saline. Dopamine hydrochloride (H8502) was dissolved in phosphate-buffered saline (PBS) at pH 7.4.

Data analysis

Baseline dopamine release and half-life

Two-way between-subjects analysis of variances (ANOVAs) were used to determine the effect of age at time of isolation (1, 4, 12, and 18 months old) and housing (isolated and group-housed) on baseline dopamine release and the baseline synaptic half-life of dopamine. When appropriate, independent t tests were used to compare means between housing conditions in each age group.

Dopamine autoreceptor functioning

Autoreceptor-mediated inhibition of evoked dopamine release was expressed as percentage change between test stimulations (T1 and T2) for each set of pre-pulses. A mixed three-way ANOVA was used to determine the effect of age and housing (between-subjects factors) on DAR functioning across the different pre-pulse settings (within-subjects factor). When appropriate, independent t tests were used to compare autoreceptor-mediated dopamine release between housing conditions in each age group.

Dopaminergic response to cocaine

The synaptic half-life of dopamine following the cocaine injection was expressed as percent change of baseline dopamine half-life (with baseline half-life being 100%). The timing of dopamine efflux following drug administration has been associated with abuse liability, with drugs that increase dopamine release quicker being more reinforcing (Volkow and Morales 2015). Thus, we were interested in determining whether age or housing altered the dopaminergic response over time. A mixed three-way ANOVA was used to determine the effects of age and housing (between-subjects factors) on percent change of dopamine half-life following cocaine administration over the 1-h recording period in 10 min intervals (within-subject factor). To assess differences at the peak effect time of cocaine, a two-way between-subjects ANOVA was used to determine the effect of age and housing on percent change in dopamine half-life at 20 min post-injection. When appropriate, independent *t* tests were used to compare percent change in dopamine half-life at 20 min post-injection between housing conditions in each age group.

Results

Baseline dopamine release and half-life

The tips of the stimulating and recording electrodes were positioned within the anatomical boundaries of the VTA and NAc core, respectively (Fig. 2). Baseline stimulation-evoked dopamine release and synaptic half-life were assessed in each mouse prior to cocaine administration. Regarding baseline dopamine release, no main effect of age was observed [$F(3,47) = 0.74$, $p = 0.533$, $\eta_p^2 = 0.05$]; however, a main effect of housing was observed [$F(1,47) = 9.15$, $p = 0.004$, $\eta_p^2 = 0.17$], with isolated mice displaying greater dopamine release than group-housed mice. There was not a significant interaction between age and housing on baseline dopamine release [$F(3,47) = 2.24$, $p = 0.096$, $\eta_p^2 = 0.13$]; however, the effect size for this interaction was large enough to warrant further exploration of these effects (Cohen 1988). Follow-up tests concluded that in the youngest age group (isolated at 1 month old), no differences were observed between isolated and group-housed mice, $t(12) = 0.58$, $p = 0.570$, $\eta_p^2 = 0.03$. However, housing did alter baseline dopamine

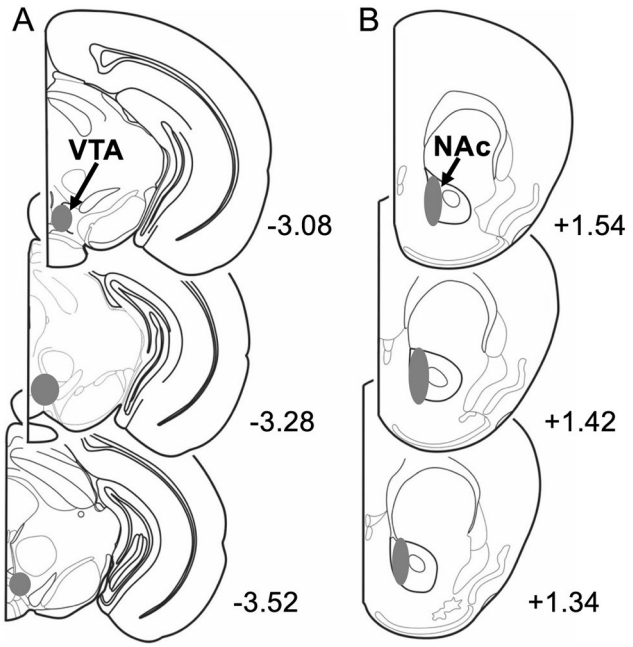

Fig. 2 Representative coronal sections of the mouse brain (adapted from the atlas of Paxinos and Franklin 2001), with gray shaded areas indicating the placements of (A) stimulating electrodes in the ventral tegmental area (VTA) and amperometric recording electrodes in the nucleus accumbens (NAc). Numbers correspond to mm from bregma

release in the young adult mice (isolated at 4 months old) and the middle adult mice (isolated at 12 months old), with isolated mice displaying increased dopamine release concentrations compared to group-housed mice (young adults: $t(13) = -2.60$, $p = 0.022$, $\eta_p^2 = 0.34$; middle-age adults: $t(11) = -2.42$, $p = 0.034$, $\eta_p^2 = 0.35$). In the older mice (isolated at 18 months), no differences in baseline dopamine release were observed between isolated and group-housed mice, $t(11) = -1.04$, $p = 0.319$, $\eta_p^2 = 0.09$ (Fig. 3a, b).

Regarding baseline dopamine half-life, no main effects of age or housing were observed [age: $F(3,47) = 1.16$, $p = 0.337$, $\eta_p^2 = 0.07$; housing: $F(1,47) = 1.23$, $p = 0.273$, $\eta_p^2 = 0.03$], and there was not a significant interaction between age and housing [$F(3,47) = 0.56$, $p = 0.643$, $\eta_p^2 = 0.04$]. Thus, none of the experimental manipulations altered the time required for the stimulation-evoked dopamine to be cleared from the synapse (Fig. 3a, c).

Dopamine autoreceptor functioning

Autoreceptor-mediated inhibition of evoked dopamine release was expressed in terms of percent change of dopamine release between test stimulations (T2/T1 × 100) for each set of conditioning pre-pulses. A lower percentage indicates greater autoreceptor-mediated inhibition of dopamine release. As expected, there was a significant main effect of the number of pre-pulses on autoreceptor-mediated dopamine release [$F(6, 270) = 250.28$, $p < 0.001$, $\eta_p^2 = 0.85$], with indications that autoreceptor-mediated dopamine release decreased as the number of pre-pulses increased. In other words, as the number of pre-pulses increases, DAR functioning increases also, resulting in greater inhibition of dopamine release. Neither age nor housing altered this pattern of autoreceptor-mediated dopamine release across the conditioning pre-pulses (pre-pulses × age: $F(18,270) = 0.46$, $p = 0.971$, $\eta_p^2 = 0.03$; pre-pulses × housing: $F(6,270) = 0.21$, $p = 0.972$, $\eta_p^2 = 0.01$) However, there was a significant three-way interaction between age, housing, and number of pre-pulses [$F(18,270) = 1.713$, $p = 0.037$, $\eta_p^2 = 0.10$], indicating that isolation altered DAR functioning differently depending on the age of the mice. Follow-up analyses assessed differences in autoreceptor-mediated dopamine release between group-housed and isolated mice in each age group. The only significant differences were found in the youngest age group (isolated at 1 month old), with isolated mice displaying reduced dopamine release following 40 and 80 pre-pulses compared to group-housed mice (40 pre-pulses: $t(10) = 3.47$, $p = 0.006$, $\eta_p^2 = 0.55$; 80 pre-pulses: $t(10) = 4.21$, $p = 0.002$, $\eta_p^2 = 0.64$). These differences indicate greater DAR functioning in the isolated mice at this age group (Fig. 4).

Dopaminergic response to cocaine

The synaptic half-life of dopamine was measured in 10 min intervals following the cocaine challenge and was converted into percent change, with baseline representing 100%. As expected, there was a significant main effect of time post injection on percent change in dopamine half-life [$F(6,270) = 77.26$, $p < 0.001$, $\eta_p^2 = 0.63$]. Neither age nor housing altered the percent change in dopamine half-life over time following the cocaine injection [time × age: $F(18,270) = 1.09$, $p = 0.361$, $\eta_p^2 = 0.07$; time × housing: $F(6,270) = 0.92$, $p = 0.481$, $\eta_p^2 = 0.02$], and there was no significant three-way interaction between time, age, and housing [$F(18,270) = 0.72$, $p = 0.786$, $\eta_p^2 = 0.05$]. Thus, neither age nor housing nor the interaction of the two variables altered the timing of the dopaminergic response to cocaine.

Next, we compared group differences in the dopaminergic response to cocaine at the time of cocaine's peak effect (20 min post injection). A significant main effect of age was observed [$F(3,45) = 3.52$, $p = 0.022$, $\eta_p^2 = 0.19$], with the following percent changes in dopamine half-life averaged across housing groups: adolescent $M = 222.41\%$, SEM = 15.81; young adult $M = 220.65\%$, SEM = 14.41; middle-age adult $M = 175.22\%$, SEM = 8.25; old adult $M = 186.67\%$, SEM = 16.12. No main effect of housing was observed on percent change in dopamine half-life 20 min post cocaine [$F(1,45) = 0.80$, $p = 0.376$, $\eta_p^2 = 0.02$]; however, a significant interaction between age × housing was observed [$F(3,45) = 3.10$, $p = 0.036$, $\eta_p^2 = 0.17$]. Follow-up analyses were conducted to compare percent change

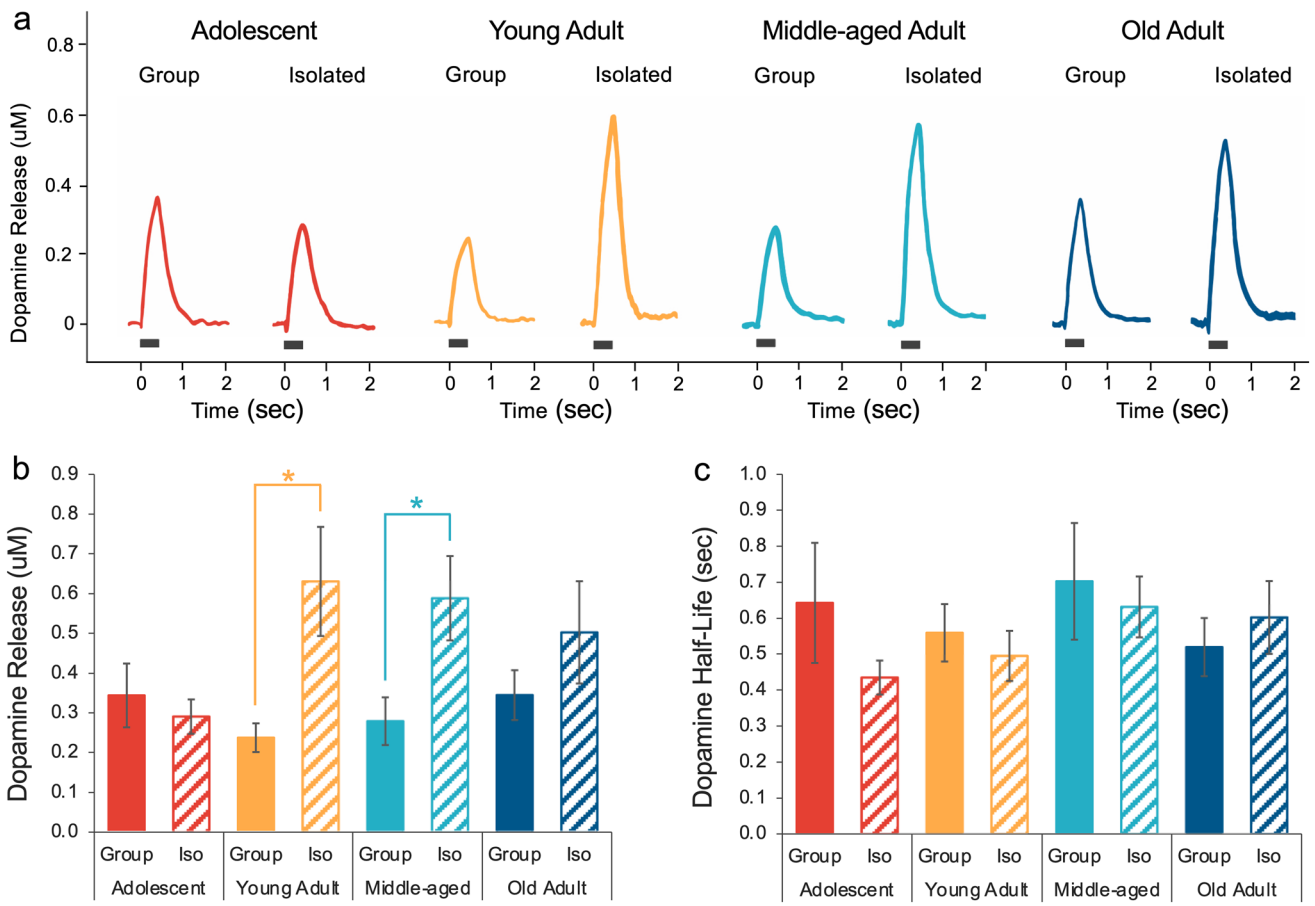

Fig. 3 Baseline (pre-cocaine) dopamine release and half-life. Profiles indicate representative responses from each age and housing group (**a**). Significant differences in mean (±SEM) dopamine release for housing were observed, with isolated (Iso) mice displaying increased dopamine release compared to group-housed mice in the young adult and middle-aged adult groups (**b**). No significant differences in mean (±SEM) dopamine half-life were observed between age or housing groups (**c**). *Indicates $p < 0.05$

in dopamine half-life at 20 min post injection between housing conditions from each age group. In the youngest group (isolated as adolescents at 1 month old), the percent change in dopamine half-life following cocaine was significantly greater in isolated mice compared to group-housed mice [$t(12) = -2.30$, $p = 0.040$, $\eta_p^2 = 0.31$], indicating that social isolation during adolescence resulted in a greater dopaminergic response to cocaine. No significant differences in dopamine half-life following cocaine were observed between isolated and group-housed mice in the other age groups [young adult: $t(13) = -0.30$, $p = 0.771$, $\eta_p^2 = 0.01$; middle-aged adult: $t(11) = -1.91$, $p = 0.083$, $\eta_p^2 = 0.25$; older adult: $t(9) = 1.82$, $p = 0.103$, $\eta_p^2 = 0.27$] (Fig. 5).

Discussion

Social isolation is a prevalent public health risk factor that, although certainly not new, has been cited as part of a "loneliness epidemic of modern society" within the last twenty years (Hämmig 2019; Killeen 1998). Social isolation is a risk factor for many psychiatric disorders including SUD and depression, with studies indicating particular vulnerabilities in adolescent and elderly populations (Berg-Weger and Morley 2020; Loades et al. 2020). The present study aimed to examine the effect of social isolation on mesolimbic dopamine functioning in mice across 4 age groups. The mesolimbic dopamine system modulates behaviors related to reward, motivation, and mood (Baik 2013; Berg-Weger and Morley 2020; Ikemoto and Panksepp 1999; Wise 2008), and dysfunction of this pathway is implicated in SUD and depression. Overall, we found that age by itself altered the measured aspects of dopamine functioning and that age also influenced the effects of social isolation on mesolimbic dopamine functioning.

Age effects

The mesolimbic dopamine system is thought to alter with development/aging; however, in the present study, no main

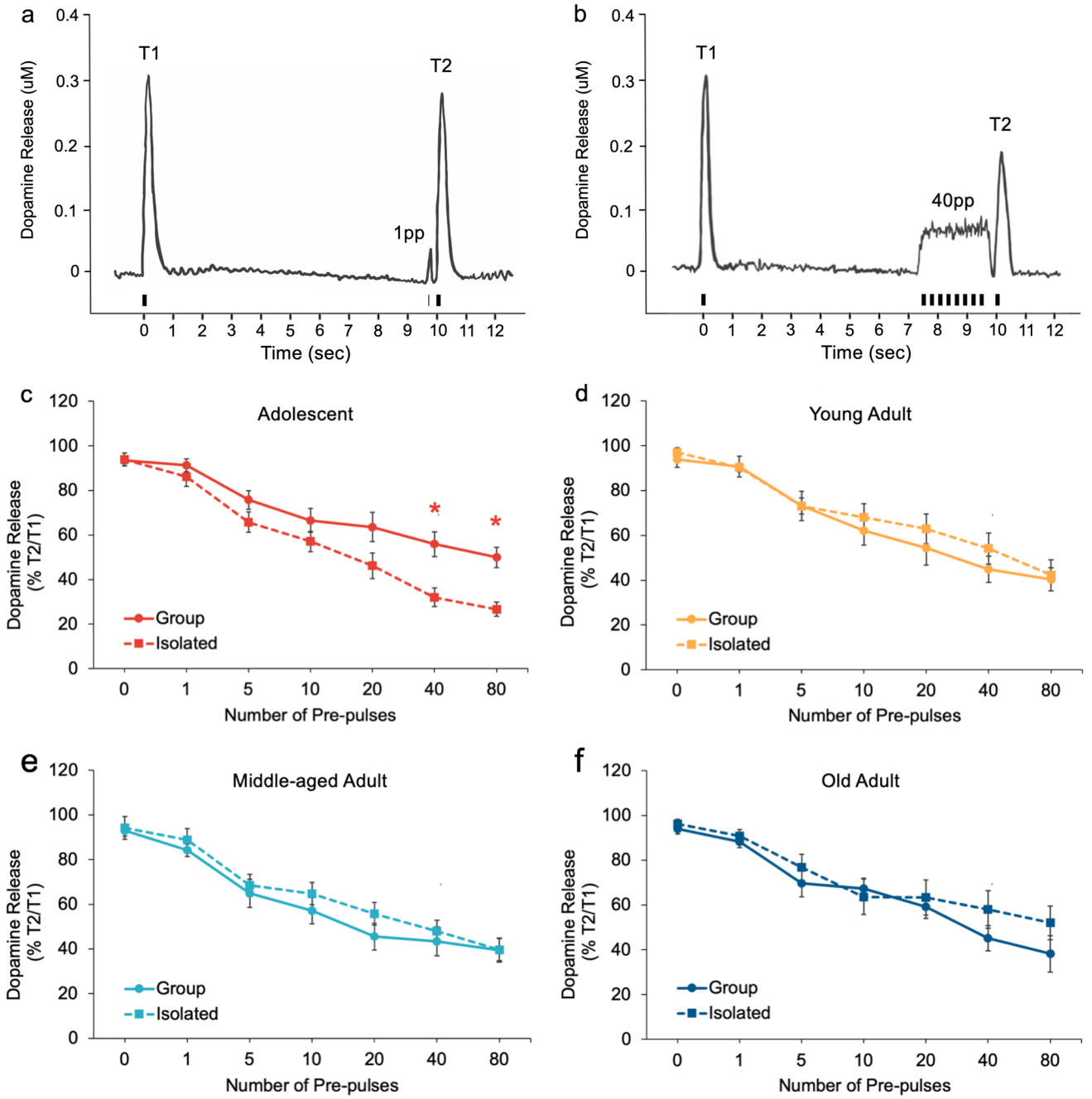

Fig. 4 Autoreceptor-mediated inhibition of dopamine release. Stimulation parameters were set to include two test stimulations (T1, T2) and a varying number of conditioning pre-pulses (pp). Two example responses are depicted (**a**, **b**). Greater decreases in dopamine release (% of T2/T1) indicates increased autoreceptor functioning. The only significant effect of isolation on mean (±SEM) differences in autoreceptor functioning were found in adolescent age mice (**c**). Isolation did not alter dopamine autoreceptor functioning in the other age groups (**d**–**f**). *Indicates $p < 0.05$

effect of age was observed on baseline (pre-cocaine) phasic release. Endogenous dopamine release and neuronal activity have been shown to exhibit an inverted U-shape pattern across the lifespan of rodents and humans, with dopamine release and activity being the greatest towards the end of adolescence and declining with old age (Burke and Miczek 2013; Karkhanis et al. 2019; Huang et al. 1995; Pitts et al. 2020; Santiago et al. 1993; Stamford 1989). The present study measured VTA stimulation-evoked dopamine release. Electrical stimulation negates dopamine release differences that are due to changes in neuronal activity; therefore, the present findings suggest that previously observed

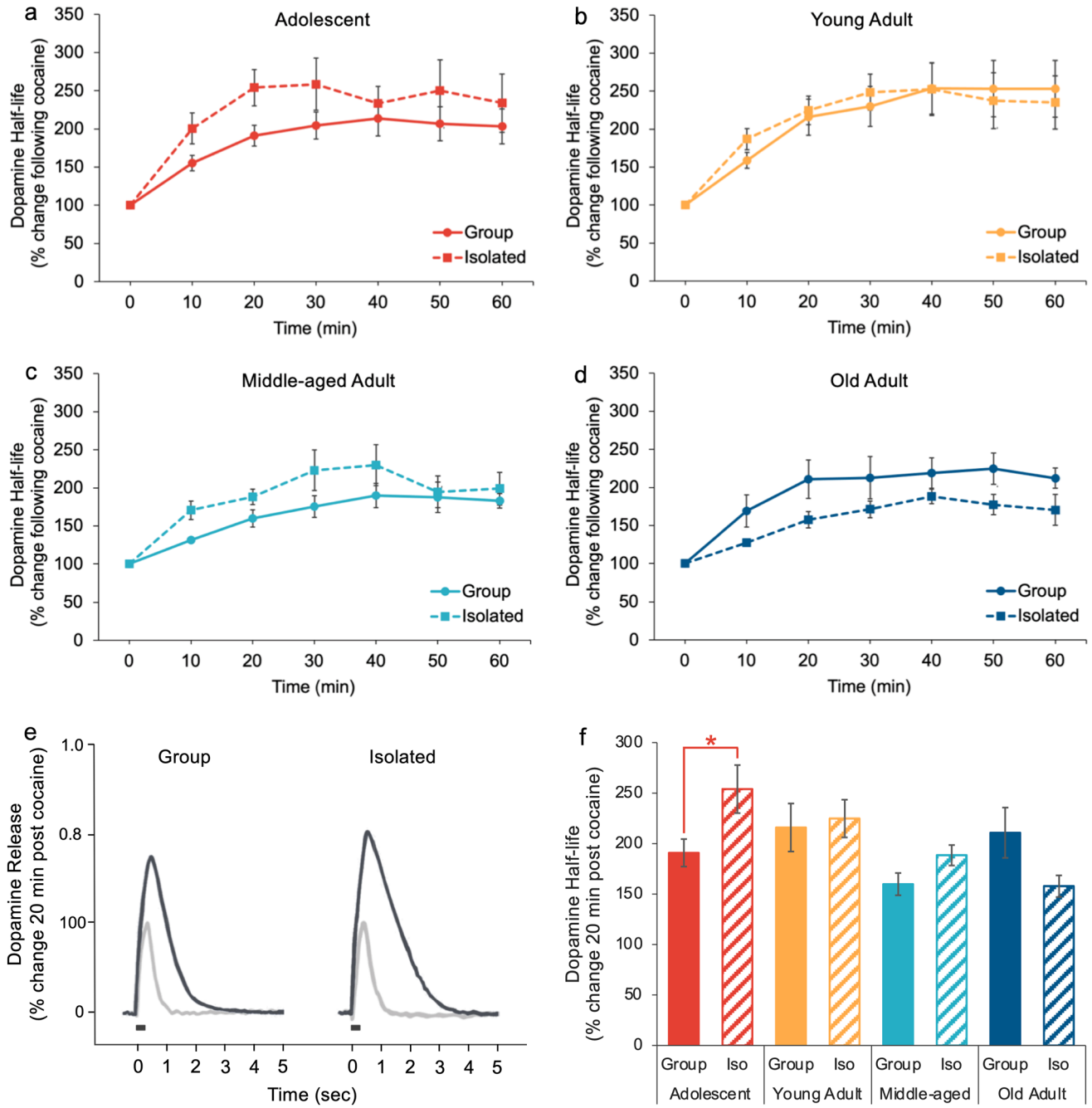

Fig. 5 Dopaminergic response to cocaine. Neither age nor housing altered the pattern of percent change in dopamine half-life over the 1-h recording period following cocaine administration (**a**–**d**). Profiles indicate example responses from group-housed and isolated (Iso) adolescent mice 20 min post cocaine, with the gray line representing pre-drug (**e**). A significant interaction between age and housing was observed in mean (±SEM) percent change in dopamine half-life at 20 min post injection. In adolescents, isolated mice displayed a greater dopaminergic response to cocaine compared to group-housed mice (**f**). *Indicates $p < 0.05$

age-dependent differences in dopamine transmission are related more to neuronal activity levels or firing rate rather than properties of dopamine release. Another possibility is that our study design missed the adolescent peak in dopamine release, as the youngest group of mice were 9 weeks old at the time of dopamine recordings (placed in housing conditions at 3 weeks old and remained for 6 weeks). The end of adolescence in mice is defined by researchers as being between post-natal day 55–65 (7–9 weeks old) (see Brust et al. 2015). Thus, our "adolescent" group was adolescent at the time of housing condition onset but was transitioning into young adulthood by the time of dopamine recordings.

Comparing dopamine responses with mice in the earlier stages of adolescence would address these questions.

A main effect of age was observed in the percent change in dopamine half-life following cocaine, with the adolescent and young adults displaying a greater dopaminergic response to cocaine compared to the older age groups. These results fit with previous studies that have shown enhanced behavioral sensitization to and preference for cocaine and amphetamine in adolescent mice (Caster et al. 2007; Kameda et al. 2011; Schramm-Sapyta et al. 2004). Age-related differences in dopaminergic responses to psychostimulants are inconsistent in the literature. Psychostimulants have been shown to elicit less (Cao et al. 2007; Laviola et al. 2001), more (Stansfield and Kirstein 2005), or equal (Camarini et al. 2008) dopamine in adolescent rodents compared to adults. Discrepancies are likely due to the differences in applied research techniques and the type of dopamine transmission being quantified (phasic vs tonic dopamine). It should also be noted that given the purpose of this project separate analyses of non-isolated mice were not run. Instead, main effects of age and interactive effects of age were assessed. It is possible that the inclusion of the isolation factor masked potential age-effects.

Housing effects at each age group

Adolescent mice

In the present study, social isolation altered dopamine functioning in an age-dependent manner. Stressors such as social isolation are thought to have greater long-term impacts on neural functioning during developmental stages of increased plasticity, with adolescence being a potential sensitive period (Fuhrmann et al. 2015; McEwen 2017; Ver Hoeve et al. 2013). In the adolescent mice, social isolation did not alter baseline (pre-cocaine) dopamine release. These results fit with other studies showing similar baseline dopamine levels in isolated and group-housed rodents (Fabricius et al. 2010; Karkhanis et al. 2014; Wang et al. 2011); however, in some studies, social isolation during adolescence increased dopamine release (Han et al. 2012; Yorgason et al. 2013, 2016). In the present study, the isolated adolescent mice displayed increased DAR functioning compared to the group-housed controls. DARs, which are generally comprised of D2 type receptors, regulate dopamine release by inhibiting dopamine synthesis and inducing hyperpolarization (Cubeddu and Hoffman 1982; Mercuri et al. 1997; Wolf and Roth 1990). Adolescent social isolation has been shown to increase the number of D2 receptors (Han et al. 2012; King et al. 2009), supporting our findings of increased DAR functioning in isolated adolescents. This observed increase in DAR functioning may have counteracted an increase in baseline dopamine release in the adolescent isolated mice.

All mice received an injection of cocaine (10 mg/kg, ip) during NAc dopamine recordings. In adolescent mice, social isolation increased the dopaminergic response to cocaine, as measured by the percent change in the synaptic half-life of dopamine at the peak effect time of cocaine (20 min post injection). These results were expected as others have also shown that social isolation during adolescence leads to a greater effect on extracellular dopamine levels following psychostimulant administration (Fulford and Marsden 2002; Jones et al. 1992; Yorgason et al. 2016) and increased self-administration of psychostimulants (Bozarth et al. 1989; Ding et al. 2005; Howes et al. 2000; Schenk et al. 1987). Karkhanis et al. (2019) suggest that this increased release following dopamine agonists may be due to higher levels of tyrosine hydroxylase, the rate limiting enzyme for dopamine synthesis, in rodents isolated during adolescence. Overall, our findings suggest that social isolation during adolescence increases DAR functioning, which may be a compensatory mechanism to inhibit excess dopamine release, but that the cocaine challenge overpowered the inhibitory control of DARs, resulting in an increased dopaminergic response to cocaine in isolated adolescents compared to group-housed controls. Initial exposure to cocaine has been shown to decrease D2 receptor signaling (Navarro et al. 2013), and repeated cocaine administration attenuates DAR-mediated inhibition of dopamine release (Jones et al. 1996; Pierce et al. 1995). Thus, the effects of cocaine are dependent on D2 functioning, and the present data suggests that mice with more sensitive DAR (i.e., dopamine systems more tightly controlled by DAR), such as the isolated adolescents, may respond more dramatically to cocaine.

The current study did not measure the duration of isolation-induced effects, but recent evidence suggests that social isolation and other stressful experiences during adolescence may result in long-term, even permanent changes in neural function (Bendersky et al. 2021; Burke et al. 2017). In the current study, the mice were isolated for 10–12 weeks, which is a relatively long isolation period, but it is possible that we could have seen more dopaminergic phenotypes develop had we tested later into adulthood. Most studies examining the effects of isolation during adolescence do not resocialize the rodents before testing; however, some effects of adolescent isolation, such as altered novelty-seeking and VTA activity, have been shown to persist long-term, even after resocialization (Einon and Morgan 1977; Whitaker et al. 2013).

Adult mice

The adult mice used in this study ranged in ages from 4 to 18 months at the onset of the housing condition. Young and middle-aged adult mice (4 and 12 months, respectively) exhibited similar isolation-induced dopaminergic phenotypes. Isolation in these age groups increased

baseline dopamine release but did not alter the synaptic half-life of dopamine, DAR functioning, or the dopaminergic effect of cocaine. Although dopamine autoreceptor functioning was enhanced in socially isolated adolescent mice, young and middle-aged adult mice did not exhibit this effect. Therefore, the increased inhibitory control on dopamine release seen in adolescents was not present in the adult age cohort. Few studies have examined the effect of social isolation on dopamine release in adult mice. Yorgason et al. (2013) found that social isolation during adulthood does not alter NAc dopamine signaling, while Gomes et al. (2019) found that environmental stressors in adulthood may instead induce a depression-like hypodopaminergic state. The findings of Gomes et al. (2019) are particularly interesting given the inverted U-shape of the development of the dopamine system with receptor expression and activity declining during adulthood (Burke and Miczek 2013). Furthermore, social isolation, especially during late adulthood, may increase the vulnerability of neurons to age-related degeneration (Zigmond and Smeyne 2019).

In the present study, the old mice (isolated at 18 months) did not exhibit isolation-induced baseline dopamine release, DAR functioning, or dopaminergic response to cocaine. Although no phenotypes were significantly different between isolated and group-housed older mice, the pattern across phenotypes appears to be trending in a direction opposite than that of the adolescent mice. Isolated old mice seemed to display a reduced dopaminergic response to cocaine, although not at the significant level ($p = 0.075$). Similar to the results of Gomes et al. (2019), an isolation-induced hypodopaminergic state may result in reduced motivation, lack of internal drive for rewards, and increased occurrence of depressive episodes. Older populations have already shown to exhibit this vulnerability to social isolation (Perona et al. 2008; Scheggi et al. 2018; Wise 2008), and more research is needed to understand the underlying neural mechanisms. It is important to note that the social isolation paradigm used in the present study was not complete isolation. Mice were in ventilated, transparent cages on racks with other mice; therefore, theoretically, mice could see, hear, and smell other mice even while being "isolated" in a cage with no cage-mates. While this isolation may be enough to alter dopaminergic functioning on adolescent and young adult mice, it may not provide enough of a stressor to alter the dopaminergic system once it has fully matured. Social defeat stress exposure in adulthood has been shown to alter NAc dopamine functioning (Deal et al. 2018); thus, varying degrees of social stress may alter this system differently.

Conclusions

Isolation altered dopamine release measurements in an age-dependent manner. Specifically, isolation increased dopamine release in the adult ages, but not adolescence, potentially due to increased inhibitory effects of DARs following social isolation during adolescence. Regarding the cocaine challenge, isolation increased the dopaminergic response to cocaine in adolescent mice, but not the adult mice. Thus, in some measurements, isolation seemed to have the opposite effect in adolescents compared to the old mice. The mechanisms controlling these differences may provide insight on the relationship between age, social interaction, and behaviors related to motivation and reward. It is important to note that the mice in this study were all male. Further studies are needed to assess these interactive effects of age and isolation in females. Previous studies suggest that dopamine receptor populations fluctuate at different rates across development in males vs females (Andersen et al. 1997; Trainor 2011) and that social isolation may be more anxiogenic (Trainor 2011) or more anxiolytic (Guo et al. 2004) in female rodents compared to males. Others, however, have suggested that males and females do not differ in basal or drug-induced dopamine levels (Egenrieder et al. 2019) and that males and females are affected similarly by isolation (see Mrackova 2020). Understanding the influence of social isolation on dopaminergic functioning across the lifespan is important for both clinical and experimental settings. Animal researchers should consider mouse age and housing conditions when examining dopamine-related behaviors and neural functioning. Elucidating the relationship between age, social isolation, and neurochemical changes associated with SUD and depression may lead to improvements in preventing and treating these disorders.

Author contributions All authors contributed to the study conception and design, with MAM serving as the project lead. Material preparation, data collection, and analysis were performed by all authors. The first draft of the manuscript was written by MAM and DBL. All authors were involved in editing and reviewing subsequent drafts, and all authors have read and approved the final manuscript.

Funding No funding was received to assist with the preparation of this manuscript.

Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval All experiments were approved by the Institutional Animal Care and Use Committee at the University of Memphis and

conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

References

Andersen SL, Rutstein M, Benzo JM et al (1997) Sex differences in dopamine receptor overproduction and elimination. NeuroReport 8:1495–1497. https://doi.org/10.1097/00001756-199704140-00034

Arakawa H (2018) Ethological approach to social isolation effects in behavioral studies of laboratory rodents. Behav Brain Res 341:98–108. https://doi.org/10.1016/j.bbr.2017.12.022

Arranz L, Giménez-Llort L, De Castro NM et al (2009) El Aislamiento Social Durante La Vejez empeora el Deterioro Cognitivo, conductual e inmunitario. Rev Esp Geriatr Gerontol 44:137–142. https://doi.org/10.1016/j.regg.2008.12.001

Baik JH (2013) Dopamine signaling in reward-related behaviors. Frontiers in neural circuits 7:152. https://doi.org/10.3389/fncir.2013.00152

Banerjee D, Rai M (2020) Social isolation in covid-19: the impact of loneliness. Int J Soc Psychiatry 66:525–527. https://doi.org/10.1177/0020764020922269

Bendersky CJ, Milian AA, Andrus MD, De La Torre U, Walker DM (2021) Long-Term Impacts of Post- weaning Social Isolation on Nucleus Accumbens Function. Frontiers in psychiatry 12:745406. https://doi.org/10.3389/fpsyt.2021.745406

Benoit-Marand M, Jaber M, Gonon F (2000) Release and elimination of dopamine in vivo in mice lacking the dopamine transporter: Functional consequences. Eur J Neurosci 12:2985–2992. https://doi.org/10.1046/j.1460-9568.2000.00155.x

Berg-Weger M, Morley JE (2020) Loneliness and social isolation in older adults during the COVID-19 pandemic: implications for gerontological social work. J Nutr Health Aging 24:456–458. https://doi.org/10.1007/s12603-020-1366-8

Bozarth MA, Murray A, Wise RA (1989) Influence of housing conditions on the acquisition of intravenous heroin and cocaine self-administration in rats. Pharmacol Biochem Behav 33:903–907. https://doi.org/10.1016/0091-3057(89)90490-5

Brenes JC, Rodríguez O, Fornaguera J (2008) Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. Pharmacol Biochem Behav 89:85–93. https://doi.org/10.1016/j.pbb.2007.11.004

Brust V, Schindler PM, Lewejohann L (2015) Lifetime development of behavioural phenotype in the house mouse (*Mus musculus*). Front Zool. https://doi.org/10.1186/1742-9994-12-s1-s17

Burke AR, Miczek KA (2013) Stress in adolescence and drugs of abuse in rodent models: role of dopamine, CRF, and Hpa axis. Psychopharmacology 231:1557–1580. https://doi.org/10.1007/s00213-013-3369-1

Burke AR, McCormick CM, Pellis SM, Lukkes JL (2017) Impact of adolescent social experiences on behavior and neural circuits implicated in mental illnesses. Neurosci Biobehav Rev 76(Pt B):280–300. https://doi.org/10.1016/j.neubiorev.2017.01.018

Camarini R, Griffin WC, Yanke AB et al (2008) Effects of adolescent exposure to cocaine on locomotor activity and extracellular dopamine and glutamate levels in nucleus accumbens of DBA/2J mice. Brain Res 1193:34–42. https://doi.org/10.1016/j.brainres.2007.11.045

Cao J, Lotfipour S, Loughlin SE, Leslie FM (2007) Adolescent maturation of cocaine-sensitive neural mechanisms. Neuropsychopharmacology 32:2279–2289. https://doi.org/10.1038/sj.npp.1301349

Caster JM, Walker QD, Kuhn CM (2007) A single high dose of cocaine induces differential sensitization to specific behaviors across adolescence. Psychopharmacology 193:247–260. https://doi.org/10.1007/s00213-007-0764-5

Cohen J (1988) Statistical power analysis for the behavioral sciences. L. Erlbaum Associates, Hillsdale

Cubeddu LX, Hoffman IS (1982) Operational characteristics of the inhibitory feedback mechanism for regulation of dopamine release via presynaptic receptors. J Pharmacol Exp Ther 223:496–501

Deal AL, Konstantopoulos JK, Weiner JL, Budygin EA (2018) Exploring the consequences of social defeat stress and intermittent ethanol drinking on dopamine dynamics in the rat nucleus accumbens. Sci Rep. https://doi.org/10.1038/s41598-017-18706-y

Ding Y, Kang L, Li B, Ma L (2005) Enhanced cocaine self-administration in adult rats with adolescent isolation experience. Pharmacol Biochem Behav 82:673–677. https://doi.org/10.1016/j.pbb.2005.11.007

Dugast C, Suaud-Chagny MF, Gonon F (1994) Continuousin vivo monitoring of evoked dopamine release in the rat nucleus accumbens by amperometry. Neuroscience 62:647–654. https://doi.org/10.1016/0306-4522(94)90466-9

Egenrieder L, Mitricheva E, Spanagel R, Noori HR (2019) No basal or drug-induced sex differences in striatal dopaminergic levels: a cluster and meta-analysis of rat microdialysis studies. J Neurochem 152:482–492. https://doi.org/10.1111/jnc.14911

Einon DF, Morgan MJ (1977) A critical period for social isolation in the rat. Dev Psychobiol 10:123–132. https://doi.org/10.1002/dev.420100205

Estes MK, Freels TG, Prater WT, Lester DB (2019) Systemic oxytocin administration alters mesolimbic dopamine release in mice. Neuroscience 408:226–238. https://doi.org/10.1016/j.neuroscience.2019.04.006

Fabricius K, Helboe L, Fink-Jensen A et al (2010) Pharmacological characterization of social isolation-induced hyperactivity. Psychopharmacology 215:257–266. https://doi.org/10.1007/s00213-010-2128-9

Fielding J, Rogers T, Meyer A et al (2013) Stimulation-evoked dopamine release in the nucleus accumbens following cocaine administration in rats perinatally exposed to polychlorinated biphenyls. Toxicol Sci 136:144–153. https://doi.org/10.1093/toxsci/kft171

Flurkey K, Brandvain Y, Klebanov S et al (2007) POHNB6F1: a cross of wild and domestic mice that is a new model of extended female reproductive life span. J Gerontol A Biol Sci Med Sci 62:1187–1198. https://doi.org/10.1093/gerona/62.11.1187

Fone KCF, Porkess MV (2008) Behavioural and neurochemical effects of post-weaning social isolation in rodents—relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 32:1087–1102. https://doi.org/10.1016/j.neubiorev.2008.03.003

Fuhrmann D, Knoll LJ, Blakemore S-J (2015) Adolescence as a sensitive period of brain development. Trends Cogn Sci 19:558–566. https://doi.org/10.1016/j.tics.2015.07.008

Fulford AJ, Marsden CA (2002) Effect of isolation-rearing on conditioned dopamine release in vivo in the nucleus accumbens of the rat. J Neurochem 70:384–390. https://doi.org/10.1046/j.1471-4159.1998.70010384.x

Global Council on Brain Health (GCBH) (2017) AARP. https://www.aarp.org/health/brain-health/global-council-on-brain-health/?cmp=RDRCT-GCBH_Main_10_26_015

Gomes FV, Zhu X, Grace AA (2019) The pathophysiological impact of stress on the dopamine system is dependent on the state of the critical period of vulnerability. Mol Psychiatry 25:3278–3291. https://doi.org/10.1038/s41380-019-0514-1

Guo M, Wu CF, Liu W et al (2004) Sex difference in psychological behavior changes induced by long-term social isolation in mice. Prog Neuropsychopharmacol Biol Psychiatry 28:115–121. https://doi.org/10.1016/j.pnpbp.2003.09.027

Hagan C (2017) When are mice considered old? The Jackson Laboratory. https://www.jax.org/news-and-insights/jax-blog/2017/november/when-are-mice-considered-old

Hämmig O (2019) Health risks associated with social isolation in general and in young, middle, and old age. PLoS ONE 14:1–18. https://doi.org/10.1371/journal.pone.0219663

Han X, Li N, Xue X et al (2012) Early social isolation disrupts latent inhibition and increases dopamine D2 receptor expression in the medial prefrontal cortex and nucleus accumbens of adult rats. Brain Res 1447:38–43. https://doi.org/10.1016/j.brainres.2012.01.058

Holloway ZR, Freels TG, Comstock JF et al (2018) Comparing phasic dopamine dynamics in the striatum, nucleus accumbens, amygdala, and medial prefrontal cortex. Synapse. https://doi.org/10.1002/syn.22074

Holt-Lunstad J, Smith TB, Baker M et al (2015) Loneliness and social isolation as risk factors for mortality. Perspect Psychol Sci 10:227–237. https://doi.org/10.1177/1745691614568352

Howes SR, Dalley JW, Morrison CH et al (2000) Leftward shift in the acquisition of cocaine self-administration in isolation-reared rats: relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal fos expression. Psychopharmacology 151:55–63. https://doi.org/10.1007/s002130000451

Huang RL, Wang CT, Tai MY et al (1995) Effects of age on dopamine release in the nucleus accumbens and amphetamine-induced locomotor activity in rats. Neurosci Lett 200:61–64. https://doi.org/10.1016/0304-3940(95)12084-h

Ieraci A, Mallei A, Popoli M (2016) Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. Neural Plast 2016:1–13. https://doi.org/10.1155/2016/6212983

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Rev 31:6–41. https://doi.org/10.1016/s0165-0173(99)00023-5

Jones GH, Hernandez TD, Kendall DA et al (1992) Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and in vivo neurochemistry. Pharmacol Biochem Behav 43:17–35. https://doi.org/10.1016/0091-3057(92)90635-s

Jones SR, Lee TH, Wightman RM, Ellinwood EH (1996) Effects of intermittent and continuous cocaine administration on dopamine release and uptake regulation in the striatum: in vitro voltammetric assessment. Psychopharmacology 126:331–338. https://doi.org/10.1007/BF02247384

Kameda SR, Fukushiro DF, Trombin TF et al (2011) Adolescent mice are more vulnerable than adults to single injection-induced behavioral sensitization to amphetamine. Pharmacol Biochem Behav 98:320–324. https://doi.org/10.1016/j.pbb.2011.01.013

Karkhanis AN, Locke JL, McCool BA et al (2014) Social isolation rearing increases nucleus accumbens dopamine and norepinephrine responses to acute ethanol in adulthood. Alcoholism Clin Exp Res 38:2770–2779. https://doi.org/10.1111/acer.12555

Karkhanis AN, Leach AC, Yorgason JT et al (2019) Chronic social isolation stress during peri-adolescence alters presynaptic dopamine terminal dynamics via augmentation in accumbal dopamine availability. ACS Chem Neurosci 10:2033–2044. https://doi.org/10.1021/acschemneuro.8b00360

Kennedy RT, Jones SR, Wightman RM (1992) Dynamic observation of dopamine autoreceptor effects in rat striatal slices. J Neurochem 59:449–455. https://doi.org/10.1111/j.1471-4159.1992.tb09391.x

King MV, Seeman P, Marsden CA, Fone KCF (2009) Increased dopamine D2highreceptors in rats reared in social isolation. Synapse 63:476–483. https://doi.org/10.1002/syn.20624

Killeen C (1998) Loneliness: an epidemic in modern society. J Adv Nurs 28(4):762–770. https://doi.org/10.1046/j.1365-2648.1998.00703.x

Kokare DM, Dandekar MP, Singru PS et al (2010) Involvement of α-MSH in the social isolation induced anxiety- and depression-like behaviors in rat. Neuropharmacology 58:1009–1018. https://doi.org/10.1016/j.neuropharm.2010.01.006

Laviola G, Pascucci T, Pieretti S (2001) Striatal dopamine sensitization to D-amphetamine in periadolescent but not in adult rats. Pharmacol Biochem Behav 68:115–124. https://doi.org/10.1016/s0091-3057(00)00430-5

Lester DB, Rogers TD, Blaha CD (2010) Acetylcholine-dopamine interactions in the pathophysiology and treatment of CNS disorders. CNS Neurosci Ther 16:137–162. https://doi.org/10.1111/j.1755-5949.2010.00142.x

Lester DB (2021) Mouse vs human aging. figshare. 10.6084/m9.figshare.14403179.v1. Accessed 22 Apr 2022

Loades ME, Chatburn E, Higson-Sweeney N et al (2020) Rapid systematic review: the impact of social isolation and loneliness on the mental health of children and adolescents in the context of covid-19. J Am Acad Child Adolesc Psychiatry. https://doi.org/10.1016/j.jaac.2020.05.009

McCool BA, Chappell AM (2009) Early social isolation in male long-Evans rats alters both appetitive and consummatory behaviors expressed during operant ethanol self-administration. Alcoholism Clin Exp Res 33:273–282. https://doi.org/10.1111/j.1530-0277.2008.00830.x

McEwen BS (2017) Neurobiological and systemic effects of chronic stress. Chronic Stress 1:247054701769232. https://doi.org/10.1177/2470547017692328

Mercuri NB, Saiardi A, Bonci A, Picetti R, Calabresi P, Bernardi G, Borrelli E (1997) Loss of autoreceptor function in dopaminergic neurons from dopamine D2 receptor deficient mice. Neuroscience 79:323–327. https://doi.org/10.1016/s0306-4522(97)00135-8

Mittleman G, Call SB, Cockroft JL et al (2011) Dopamine dynamics associated with, and resulting from, schedule-induced alcohol self-administration: analyses in dopamine transporter knockout mice. Alcohol 45:325–339. https://doi.org/10.1016/j.alcohol.2010.12.006

Mrackova E (2020) Effects of prolonged social isolation: sex differences in anxiety, depression, and sociability behavior in male and female rats. Lake Forest College. https://www.lakeforest.edu/news/effects-of-prolonged-social-isolation-sex-differences-in-anxiety-depression-and-sociability-behavior-in-male-and-female-rats

Navarro G, Moreno E, Bonaventura J, Brugarolas M, Farré D, Aguinaga D, Mallol J, Cortés A, Casadó V, Lluís C, Ferre S, Franco R, Canela E, McCormick PJ (2013) Cocaine inhibits dopamine D2 receptor signaling via sigma-1-D2 receptor heteromers. PLoS ONE 8:e61245. https://doi.org/10.1371/journal.pone.0061245

Paxinos G, Franklin KBJ (2001) The mouse brain in stereotaxic coordinates, 2nd edn. Academic Press, San Diego

Perona MTG, Waters S, Hall FS et al (2008) Animal models of depression in dopamine, serotonin, and norepinephrine transporter knockout mice: prominent effects of dopamine transporter deletions. Behav Pharmacol 19:566–574. https://doi.org/10.1097/fbp.0b013e32830cd80f

Pierce RC, Duffy P, Kalivas PW (1995) Sensitization to cocaine and dopamine autoreceptor subsensitivity in the nucleus accumbens. Synapse 20:33–36. https://doi.org/10.1002/syn.890200106

Pitts EG, Stowe TA, Christensen BA, Ferris MJ (2020) Comparing dopamine release, uptake, and D2 autoreceptor function across the ventromedial to dorsolateral striatum in adolescent and adult male and female rats. Neuropharmacology 175:108163. https://doi.org/10.1016/j.neuropharm.2020.108163

Prater WT, Swamy M, Beane MD, Lester DB (2018) Examining the effects of common laboratory methods on the sensitivity of carbon

fiber electrodes in amperometric recordings of dopamine. J Behav Brain Sci 08:117–125. https://doi.org/10.4236/jbbs.2018.83007
Santiago M, Machado A, Cano J (1993) Effects of age and dopamine agonists and antagonists on striatal dopamine release in the rat: an in vivo microdialysis study. Mech Ageing Dev 67:261–267. https://doi.org/10.1016/0047-6374(93)90004-b
Scheggi S, De Montis MG, Gambarana C (2018) Making sense of rodent models of anhedonia. Int J Neuropsychopharmacol 21:1049–1065. https://doi.org/10.1093/ijnp/pyy083
Schenk S, Lacelle G, Gorman K, Amit Z (1987) Cocaine self-administration in rats influenced by environmental conditions: implications for the etiology of drug abuse. Neurosci Lett 81:227–231. https://doi.org/10.1016/0304-3940(87)91003-2
Schramm-Sapyta NL, Pratt AR, Winder DG (2004) Effects of periadolescent versus adult cocaine exposure on cocaine conditioned place preference and motor sensitization in mice. Psychopharmacology 173:41–48. https://doi.org/10.1007/s00213-003-1696-3
Siciliano CA, Calipari ES, Jones SR (2014) Amphetamine potency varies with dopamine uptake rate across striatal subregions. J Neurochem 131:348–355. https://doi.org/10.1111/jnc.12808
Singh A, Misra N (2009) Loneliness, depression and sociability in old age. Ind Psychiatry J 18:51. https://doi.org/10.4103/0972-6748.57861
Stamford JA (1989) Development and ageing of the rat nigrostriatal dopamine system studied with fast cyclic voltammetry. J Neurochem 52:1582–1589. https://doi.org/10.1111/j.1471-4159.1989.tb09212.x
Stansfield KH, Kirstein CL (2005) Effects of novelty on behavior in the adolescent and adult rat. Dev Psychobiol 48:10–15. https://doi.org/10.1002/dev.20127
Suaud-Chagny MF, Dugast C, Chergui K et al (2002) Uptake of dopamine released by impulse flow in the rat mesolimbic and striatal systems in vivo. J Neurochem 65:2603–2611. https://doi.org/10.1046/j.1471-4159.1995.65062603.x
Sullens DG, Gilley K, Jensen K et al (2021) Social isolation induces hyperactivity and exploration in aged female mice. PLoS ONE. https://doi.org/10.1371/journal.pone.0245355
Trainor BC (2011) Stress responses and the mesolimbic dopamine system: social contexts and sex differences. Horm Behav 60:457–469. https://doi.org/10.1016/j.yhbeh.2011.08.013
Ver Hoeve ES, Kelly G, Luz S et al (2013) Short-term and long-term effects of repeated social defeat during adolescence or adulthood in female rats. Neuroscience 249:63–73. https://doi.org/10.1016/j.neuroscience.2013.01.073
Volkow ND, Morales M (2015) The brain on drugs: from reward to addiction. Cell 162:712–725. https://doi.org/10.1016/j.cell.2015.07.046
Walker DM, Cunningham AM, Gregory JK, Nestler EJ (2019) Long-term behavioral effects of post-weaning social isolation in males and females. Front Behav Neurosci. https://doi.org/10.3389/fnbeh.2019.00066
Wang Y-C, Ho U-C, Ko M-C et al (2011) Differential neuronal changes in medial prefrontal cortex, basolateral amygdala and nucleus accumbens after postweaning social isolation. Brain Struct Funct 217:337–351. https://doi.org/10.1007/s00429-011-0355-4
Whitaker LR, Degoulet M, Morikawa H (2013) Social deprivation enhances VTA synaptic plasticity and drug-induced contextual learning. Neuron 77:335–345. https://doi.org/10.1016/j.neuron.2012.11.022
Wise RA (2008) Dopamine and reward: the anhedonia hypothesis 30 years on. Neurotox Res 14:169–183. https://doi.org/10.1007/bf03033808
Wolf ME, Roth RH (1990) Autoreceptor regulation of dopamine synthesis. Ann N Y Acad Sci 604:323–343. https://doi.org/10.1111/j.1749-6632.1990.tb32003.x
Yorgason JT, España RA, Konstantopoulos JK et al (2013) Enduring increases in anxiety-like behavior and rapid nucleus accumbens dopamine signaling in socially isolated rats. Eur J Neurosci 37:1022–1031. https://doi.org/10.1111/ejn.12113
Yorgason JT, Calipari ES, Ferris MJ et al (2016) Social isolation rearing increases dopamine uptake and psychostimulant potency in the striatum. Neuropharmacology 101:471–479. https://doi.org/10.1016/j.neuropharm.2015.10.025
Zakharova E, Wade D, Izenwasser S (2009) Sensitivity to cocaine conditioned reward depends on sex and age. Pharmacol Biochem Behav 92:131–134. https://doi.org/10.1016/j.pbb.2008.11.002
Zigmond MJ, Smeyne RJ (2019) Solitary confinement: effects, practices, and pathways toward reform. Oxf Scholarsh Online. https://doi.org/10.1093/oso/9780190947927.001.0001
Zorzo C, Méndez-López M, Méndez M, Arias JL (2019) Adult social isolation leads to anxiety and spatial memory impairment: brain activity pattern of cox and c-fos. Behav Brain Res 365:170–177. https://doi.org/10.1016/j.bbr.2019.03.011