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Enduring increases in anxiety-like behavior and rapid nucleus accumbens dopamine signaling in socially isolated rats

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Abstract

Social isolation (SI) rearing, a model of early life stress, results in profound behavioral alterations, including increased anxiety-like behavior, impaired sensorimotor gating and increased self-administration of addictive substances. These changes are accompanied by alterations in mesolimbic dopamine function, such as increased dopamine and metabolite tissue content, increased dopamine responses to cues and psychostimulants, and increased dopamine neuron burst firing. Using voltammetric techniques, we examined the effects of SI rearing on dopamine transporter activity, vesicular release and dopamine D2-type autoreceptor activity in the nucleus accumbens core. Long–Evans rats were housed in group (GH; 4/cage) or SI (1/cage) conditions from weaning into early adulthood [postnatal day (PD) 28–77]. After this initial housing period, rats were assessed on the elevated plus-maze for an anxiety-like phenotype, and then slice voltammetry experiments were performed. To study the enduring effects of SI rearing on anxiety-like behavior and dopamine terminal function, another cohort of similarly reared rats was isolated for an additional 4 months (until PD 174) and then tested. Our findings demonstrate that SI rearing results in lasting increases in anxiety-like behavior, dopamine release and dopamine transporter activity, but not D2 activity. Interestingly, GH-reared rats that were isolated as adults did not develop the anxiety-like behavior or dopamine changes seen in SI-reared rats. Together, our data suggest that early life stress results in an anxiety-like phenotype, with lasting increases in dopamine terminal function.

Keywords

fast-scan cyclic voltammetry; release; social; stress; uptake

Introduction

Early life stress (Heidbreder *et al.*, 2000) increases vulnerability to a variety of affective mental health disorders, including anxiety, schizophrenia and substance abuse (for review, see Scheller-Gilkey *et al.*, 2004; Nugent *et al.*, 2011). Social isolation (SI) rearing is commonly used as an animal model of early life stress, where post-weanling animals are raised in single-housed conditions (Lapiz *et al.*, 2003; Fone & Porkess, 2008). SI produces several behavioral outcomes similar to those observed in humans with early life stress. For example, SI rats display anxiety/depressive-like behaviors, including decreased time spent

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on open arms of the elevated plus-maze (EPM; Da Silva *et al.*, 1996), decreased mobility during the Porsolt forced swim test and decreased social interaction (Kokare *et al.*, 2010). Consistent with reports of increased rates of schizophrenia in people with early life stress (Scheller-Gilkey *et al.*, 2004), SI rats demonstrate schizophrenia-like impairments, including reduced pre-pulse inhibition (PPI) of the acoustic startle reflex, indicating sensorimotor gating deficits, and disrupted latent inhibition in associative learning (Shao *et al.*, 2009; Han *et al.*, 2012). Lastly, similar to the increased risk for drug addiction observed in people with early life stress (Enoch, 2012), SI animals display increased self-administration of drugs of abuse (Schenk *et al.*, 1987; Bozarth *et al.*, 1989; Yajie *et al.*, 2005; McCool & Chappell, 2009; but see Phillips *et al.*, 1994).

Many SI rearing-induced behavioral alterations are long-lasting and may be permanent. For instance, resocialization of SI-reared rats fails to ameliorate anxiety-like behaviors and PPI deficits (Einon & Morgan, 1977; Wright *et al.*, 1991; but see Kokare *et al.*, 2010). Additionally, PPI impairments are not present in adult isolated group-reared rats (McCool & Chappell, 2009), suggesting that there is a critical period for developing impaired PPI (Liu *et al.*, 2011; but see Varty *et al.*, 1999).

SI-induced increases in drug self-administration, hyperlocomotor activity, impaired PPI, and latent inhibition may be related to changes in dopamine signaling, as each of these behavioral assays can be modulated by dopamine activity (Domeney & Feldon, 1998; Pierce & Kumaresan, 2006; Sora *et al.*, 2009; Weiner & Arad, 2009). SI rearing produces robust alterations in mesolimbic dopamine systems (for review, see Lukkes *et al.*, 2009), including increased ventral tegmental area dopamine neuron phasic bursting activity (Fabricius *et al.*, 2010), nucleus accumbens (NAc) tissue dopamine levels (Miura *et al.*, 2002), dopamine turnover (Hall *et al.*, 1998b; Heidbreder *et al.*, 2000), and dopamine responses to cocaine, amphetamine and footshock stress (Jones *et al.*, 1992; Fulford & Marsden, 1998; Hall *et al*, 1998b; Howes *et al.*, 2000). Together, these various neurobiological studies suggest that SI animals have overall increased NAc dopaminergic activity. However, while many different mechanisms may contribute to dopamine increases, the specific dopamine changes in SI animals remain unknown. NAc dopamine is primarily regulated by a balance of release and uptake, and knowing whether release or uptake is affected will help define the mechanisms involved for the observed increased dopamine activity and drug responsivity. For example, previous studies have shown that higher dopamine transporter levels are associated with increased sensitivity to stimulants (Chen & Reith, 2007), and that increased dopamine uptake rates are associated with augmented stimulant-induced hyperlocomotion (Salahpour *et al.*, 2008). In short, understanding specific neurochemical changes that occur after early life stress may help us better understand the observed behavioral impairments. Therefore, to further explore the effects of SI rearing on NAc dopamine transmission, using voltammetric methods, we examined dopamine release and uptake kinetics, as well as dopamine D2-type autoreceptor activity, in brain slices of SI and group-housed (GH) rats.

Materials and methods

Animal housing

Previous studies have reported robust sex differences in measures related to the present studies, including greater NAc dopamine release, uptake and tissue levels in females than males (Walker *et al.*, 2000; Duchesne et al., 2009), and increased anxiety-like behavior in males, although the behavioral differences are inconsistent (for review, see Simpson & Kelly, 2012). In the current study, male rats were chosen to avoid hormonal fluctuations in females and complex sex interactions, and to compare with previous SI studies examining dopamine parameters and anxiety-like behavior in males. Male Long–Evans rats (Harlan Laboratories, Indianapolis, IN, USA) were procured on postnatal day (PD) 21, and housed

for 1 week under standard conditions (4 rats/cage, food/water *ad libitum*, 12/12 h light/dark). On PD 28, rats were randomly assigned to two groups: SI (1 rat/cage; 20 × 27-cm cages; Allentown, Allentown, NJ, USA); and GH (4 rats/cage; 33 × 60-cm cages; Ancare, Bellmore, NY, USA) for 6 weeks (Fig. 1). Experimental protocols adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

Young adult rats

Preceding the end of the initial housing period (PD 77), SI and GH rats were tested for anxiety-like behavior on the EPM (PD 74). Afterward, *in vitro* voltammetry experiments were performed. To reduce isolation stressor effects in GH-reared rats for voltammetry experiments, GH animals were killed in pairs, so that each cage of four rats was examined across 2 days (2 rats/day).

Adult rats

Adult GH- and SI-reared rats followed similar housing procedures to young adult rats until PD 77. At this time GH and SI rats were single-housed for about 4 months, and were involved in additional behavioral experiments that were unrelated to this study (Chappell *et al.*, 2012). At the end of this period (PD 174), adult rats were reexamined in the EPM, and dopamine function was characterized using *in vitro* voltammetric experiments. Two adult animals were removed from the study due to technical difficulties during brain slice voltammetric experiments.

EPM

Anxiety-like behavior was assessed using a standard EPM (Med Associates, St Albans, VT, USA). The maze consisted of four radial arms (10.2 × 50.8 cm) elevated 72.4 cm above the floor. Two opposing arms were enclosed by black polypropylene walls (40.6 cm high), and the other two arms were open and illuminated by incandescent light (approximately 40 lux). Infrared sensors were positioned at the opening of each arm to score an animal's entry and/or exit from each arm, and data acquisition was performed using a personal computer interfaced with control units and programmed with MED-PC (Med Associates). Subjects were placed at the central junction, facing an open arm, and activity measures were recorded for 5 min. Decreases in open-arm time, and increases in closed arm time, were used as a measure of anxiety-like behavior, and closed-arm entries were used as a measure of locomotor activity.

In vitro slice preparation

Rats were killed, and their brains rapidly removed and prepared as previously described (John & Jones, 2007). Coronal slices (400 μm) of the striatum were maintained at 32 °C in oxygen-perfused (95% O_2–5% CO_2) artificial cerebrospinal fluid, which consisted of (in mM): NaCl, 126; $NaHCO_3$, 25; D-glucose, 11; KCl, 2.5; $CaCl_2$, 2.4; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; L-ascorbic acid, 0.4; pH adjusted to 7.4. A capillary glass-based carbon-fiber electrode was positioned approximately 75 μm below the surface of the slice in the NAc core. Dopamine release was evoked every 5 min by a 4-ms, one-pulse stimulation (monophasic, 350 μA)from a bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) placed 100–200 μm from the carbon-fiber electrode.

Fast-scan cyclic voltammetry

Fast-scan cyclic voltammetry recordings were performed and analysed using recently developed in-house software (Demon Voltammetry and Analysis; Yorgason *et al.*, 2011). The electrode potential was linearly scanned as a triangular waveform from −0.4 to 1.2 V

and back to −0.4 V (Ag vs. AgCl) using a scan rate of 400 V/s. Cyclic voltammograms were recorded at the carbon-fiber electrode every 100 ms by means of a potentiostat (Dagan, Minneapolis, MN, USA). Once the stimulated dopamine response was stable for three successive collections, baseline measurements were taken and evaluated using a Michaelis–Menten-based kinetic model (Wightman *et al.*, 1988; Yorgason *et al.*, 2011). Michaelis–Menten-based changes in release and uptake were obtained by setting baseline apparent affinity (K_m) values to 0.16 μM, and establishing a baseline maximal uptake rate (V_{max}) and stimulated dopamine release ([DA_p]) individually for each subject (Wightman *et al.*, 1988; Wightman & Zimmerman, 1990; Wu *et al.*, 2001). Extracellular concentrations of dopamine were assessed by comparing the current at the peak oxidation potential for dopamine with electrode calibrations of known concentrations of dopamine (1–3 μM).

For dopamine D2 autoreceptor studies, the selective D2-type receptor agonist (–)-quinpirole hydrochloride (Sigma-Aldrich, St Louis, MO, USA) was used to induce autoreceptor activation. Quinpirole-induced decreases in electrically stimulated dopamine release were compared with pre-drug values (each animal served as its own control) to obtain a percent change in stimulated dopamine release. The dose–response curve was then plotted as log concentration (*M*) of quinpirole vs. percent of control dopamine response, and the data were fit using a non-linear regression curve fit (sigmoidal dose–response curve) to determine EC_{50} concentrations.

Statistical analyses

Behavioral measures on the EPM were analysed using unpaired *t*-tests or the Mann–Whitney Rank Sum *U*-test when variance differences were detected. Anxiety-like behavior was assessed using open- and closed-arms times, and closed-arm entries were used as a measure of general locomotor activity (Holmes & Rodgers, 1998).

All dopamine release voltammetric assessments are reported as μM concentration, or as percentage of baseline. To examine whether dopamine uptake and release measures differ across housing conditions in young and adult rats, a two-way omnibus analysis of variance (ANOVA) was performed with age (adult vs. young) and housing (SI vs. GH) as the between-subject variables, followed by Tukey's *post hoc* tests on statistically significant effects. Correlation analyses examining relationships between EPM behavior and dopamine function were performed using Pearson's correlation. For correlation analysis, all data were collapsed across housing and age groups. Autoreceptor sensitivity comparisons were performed using a three-way mixed-measures ANOVA or analysis of covariance (ANCOVA), with age and housing as the between-subject variables, drug concentration as the within-subject variable, and baseline stimulated release as the covariant. ANCOVA was performed using the Delaney and Maxwell method (Delaney & Maxwell, 1981) to prevent the covariate from changing the main effect of the repeated measures. Statistical analyses were performed using SPSS 20 (IBM, New York, NY, USA) and SIGMAPLOT (Systat Software, San Jose, CA, USA).

Results

EPM

We first assessed the effect of juvenile SI on anxiety-like behavior using the EPM on PD 74 (Fig. 2). SI rats ($n = 7$) spent significantly less time in the open arms of the EPM than GH ($n = 7$) animals ($t_{12} = 2.29$, $P < 0.04$), and more time in the closed arms ($t_{12} = 2.231$, $P < 0.05$), suggesting increased anxiety-like behavior in these rats. In contrast, no differences in closed-arm entries, a measure of general locomotor activity, were noted ($t_{12} = 1.18$, $P = 0.26$). To determine the extent to which anxiety-like behavior endured into adulthood, we repeated the EPM assay 4 months later in the same animals (PD 174). Despite the fact that

both cohorts had been housed singly during this 4-month period, SI-reared rats still displayed decreased open-arm time ($U = 7$, $P < 0.03$) and increased closed-arm time ($U = 7$, $P < 0.04$) relative to the GH-reared subjects. Again, no group differences in closed-arm entries were observed on this second test of anxiety-like behavior ($t_{12} = 1.86$, $P = 0.09$).

Stimulated dopamine release and uptake

To examine the enduring effects of SI rearing on dopamine neurochemistry, electrically evoked dopamine release was measured in the NAc core of young (PD = 84 ± 7; GH $n = 8$; SI $n = 8$) and adult (PD = 181 ± 7; GH $n = 7$; SI $n = 7$) GH and SI animals (Fig. 3). The averaged dopamine traces display clear differences, with increased overall amplitude in dopamine signals from SI animals ($t_{28} = 3.273$, $P = 0.0028$). As shown in Fig. 4, two-way ANOVA indicated that there was a significant effect of SI on μM DA release (Housing, $F_{1,26} = 11.78$, $P < 0.002$), but no effect of age ($F_{1,26} = 0.370$, $P = 0.548$) or housing × age interaction ($F_{1,26} = 0.374$, $P = 0.546$). For V_{max}, ANOVA indicated a significant effect of housing ($F_{1,26} = 5.034$, $P < 0.001$) and age (V_{max} Age, $F_{1,26} = 5.034$, $P = 0.034$), but no housing × age interaction ($F_{1,26} = 1.837$, $P = 0.187$). Further analysis of these dopamine signals using Tukey's *post hoc* tests revealed that dopamine release and uptake V_{max} are both increased in SI rats regardless of age (Fig. 4B; [DA_p]: Young, $q_{1,16} = 3.91$, $P < 0.05$; Adult, $q_{1,14} = 5.23$, $P < 0.01$; V_{max}: Young, $q_{1,16} = 5.21$, $P < 0.01$; Adult, $q_{1,14} = 4.79$, $P < 0.01$), suggesting that observed dopamine changes are long-lasting, occurring both in young and adult SI rats. To examine relationships between anxiety-like behavior and dopamine measures, open-arm time from previous EPM experiments in young (PD 84 ± 7) and adult (PD 181 ± 7) rats was compared with dopamine release and V_{max} values using correlation analysis. As shown in Fig. 5, these analyses revealed that changes in EPM behavior are highly correlated with dopamine signaling ([DA_p], $r_{30} = -0.423$, $P = 0.0099$; V_{max}, $r_{30} = -0.3905$, $P = 0.0164$).

Dopamine autoreceptor activity

Previous studies indicate that NAc dopamine release and uptake are both regulated by dopamine D2-type autoreceptor activity (Benoit-Marand *et al.*, 2001; Kramer *et al.*, 2011). Therefore, in another set of voltammetry experiments in young (PD = 84 ± 7; GH $n = 5$; SI $n = 5$) and adult (PD = 181 ± 7; GH $n = 4$; SI $n = 4$) rats, we tested whether presynaptic autoreceptor activity was different in SI and GH animals. Increasing concentrations of the D2-type receptor agonist, quinpirole, were bath-applied to NAc core brain slices, and electrically stimulated dopamine release was measured (Fig. 6; Mateo *et al.*, 2005; Maina & Mathews, 2010). Given that the variations observed in baseline dopamine release between SI and GH rats could potentially affect autoreceptor activity, we conducted a three-way ANCOVA with housing and age as the between-subject variables, quinpirole concentration as the repeated-measures variable, and baseline levels of dopamine release as the covariate. As demonstrated previously (Mateo *et al.*, 2005), quinpirole reduced stimulated dopamine release in a concentration-dependent manner (Fig. 6A; Quinpirole, $F_{4,52} = 284.6$, $P < 0.001$). Although ANCOVA revealed that the baseline covariate significantly interacted with the effects of quinpirole (Baseline × Quinpirole, $F_{4,52} = 33.034$, $P < 0.001$), there were no effects of age or housing on quinpirole-induced reductions in dopamine release (Age, $F_{1,13} = 2.00$, $P = 0.18$; Housing, $F_{1,13} = 0.436$, $P = 0.521$; Age × Housing, $F_{1,13} = 0.106$, $P = 0.749$). A similar set of findings was observed when we conducted a three-way ANOVA on the effects of quinpirole with dopamine release expressed as a percent baseline. In these analyses, quinpirole significantly reduced dopamine release, but again there were no significant effects of age or housing (Fig. 6B; quinpirole, $F_{4,56} = 373.836$, $P < 0.001$; Age, $F_{1,14} = 4.16$, $P = 0.061$; Housing, $F_{1,14} = 0.031$, $P = 0.863$; Age × Housing, $F_{1,14} = 0.031$, $P = 0.863$). When taken together, these results suggest that autoreceptor activity is the same regardless of housing condition or age.

Discussion

In the current study, we tested the long-term effects of SI rearing on anxiety-like behavior and on NAc dopamine terminal function. Using the EPM assay in young and adult animals, we demonstrated that SI rearing decreases time spent on the open arms, consistent with greater anxiety-like behavior. Additionally, voltammetric studies in these animals provided evidence that juvenile SI rearing increases dopamine terminal release and reuptake rates. Additional groups of SI and GH animals were isolation housed at PD 77 and tested 4 months later (PD 181). At the later time point, GH rats had lower levels of anxiety-like behavior and decreased dopamine activity compared with their SI counterparts, despite several months of adult social isolation. This suggests that the protective effects of GH rearing on dopamine transmission and anxiety-like behavior are long lasting. Alternatively, SI housing may only have these effects when performed during a critical period of development. Future studies specifically examining critical periods' effects on dopamine and anxiety-like behavior may provide insight into this possibility.

As mentioned earlier, early life stress is associated with increased risk for developing disorders of anxiety, schizophrenia and substance abuse (for review, see Scheller-Gilkey *et al.*, 2004; Nugent *et al.*, 2011). However, it is important to note that long-lasting changes in dopamine function and behavior may occur after only mild stress in early life, which may fulfill a behaviorally protective role. For example, increases in dopamine function may serve to increase awareness to environmental cues related to natural rewards. Also, increased anxiety-like behavior may be appropriate for animals raised in more threatening environments, where it may be beneficial to spend more time in enclosed areas.

Enduring behavioral changes after SI rearing

As mentioned earlier, SI rearing has been shown to produce behavioral phenotypes that are similar to the sequelae observed in people who have experienced severe early life stress (Fone & Porkess, 2008; Lukkes *et al.*, 2009). A myriad studies have shown increases in anxiety-like behavior after SI rearing using both the EPM and open-field assays (Wright *et al.*, 1991; Da Silva *et al.*, 1996;Hall *et al.*, 1998a; McCool & Chappell, 2009), increased aggression (Wongwitdecha & Marsden, 1996), impulsivity/reactivity to novelty (Gentsch *et al.*, 1988; Hall *et al.*, 1997), impaired sensorimotor gating (Powell *et al.*, 2003) and attenuated memory function (Hellemans *et al.*, 2004; Bianchi *et al.*, 2006). Some of these behavioral alterations are permanent, whereas others can be reversed through resocialization and enrichment. For example, SI rearing-induced locomotor hyperactivity, hypoalgesia and increased latency for emergence into a novel environment are all reversible through resocialization (Einon & Morgan, 1977; Gentsch *et al.*, 1988; Cilia *et al.*, 2001; Liu *et al.*, 2011). In contrast, PPI impairment, increased novel object reactivity, decreased open-arm time on the EPM and increased anxiety-like vocalizations are not reversed through resocialization (Einon & Morgan, 1977; Wright *et al.*, 1991; Bassi *et al.*, 2007; Liu *et al.*, 2011).

GH rearing also produces enduring behaviors. In addition to showing that resocialization does not reverse SI-induced reductions in EPM open-arm time, Wright *et al.* (1991) also examined the effects of 30-day isolation in adulthood on GH-reared animals in the EPM assay, and found that GH rearing blocks the anxiogenic-like effect of single housing on EPM behavior. We have confirmed these previous findings, demonstrating that GH rearing protects against isolation-induced impairments in EPM behavior. Additionally, we have extended these studies by showing that the protective effects of GH rearing on EPM behavior endure for at least 4 months of isolation, further demonstrating the long-term nature of these behavioral alterations. Because the present results are documented in older animals than in the previous study (PD 174 vs. PD 81), this confirms that SI-induced

anxiety-like effects are very long lasting. In contrast to these results, Wallace *et al.* (2009) demonstrated that adult isolation increases anxiety-like behavior on the EPM. However, there are several important differences between these studies, including strain, age and length of isolation, and early life housing conditions, which may affect EPM behavior. Our present results suggest that GH rearing makes animals less susceptible to the stress associated with single-housing conditions.

Enduring dopamine terminal changes after SI rearing

We showed that SI rearing produces robust increases in electrically evoked dopamine release in the NAc. Similar to our findings, other experimental paradigms have also shown results consistent with greater dopamine release. For instance, electrophysiology and tissue content studies have revealed that SI rearing increases the number of ventral tegmental area dopamine neurons that burst fire, and levels of dopamine and its metabolites in the NAc (Fabricius *et al.*, 2010; Han *et al.*, 2011). Also, CDCrel-1, a presynaptic septin protein that inhibits dopamine release through interactions with the SNARE-protein, syntaxin (Beites *et al.*, 1999), is downregulated in the striatum of SI rats, suggesting that NAc dopamine release should be increased after SI (Barr *et al.*, 2004). Lastly, psychostimulants such as cocaine and amphetamine have a greater increasing effect on extracellular dopamine levels in SI animals (Hall *et al.*, 1998b; Howes *et al.*, 2000). Together, these previous studies have given a clear picture of increased dopamine activity after early life stress. In addition to validating these previous studies, by using voltammetric methods to study dopamine function in an early life stress model, we have demonstrated that increases in dopaminergic activity are not due to increased cell firing alone (Fabricius *et al.*, 2010), but also due to increased dopamine terminal activity.

We have documented increased dopamine transporter function in isolates. These results may be surprising as extracellular dopamine levels are regulated by transporter activity (Salahpour *et al.*, 2008; Owesson-White *et al.*, 2012), and microdialysis studies measuring basal dopamine levels in SI and GH cohorts have shown either no difference (Wilkinson *et al.*, 1994; Fulford & Marsden, 1998; Howes *et al.*, 2000) or increases in SI rats (Hall *et al.*, 1998b; Bortolato *et al.*, 2011; Han *et al.*, 2011). However, microdialysis techniques measuring extracellular dopamine levels are influenced by both release and uptake, and the increased release found in SI animals may partially offset the increased uptake rates. This balance between release and uptake may help explain the seemingly paradoxical lack of effect of isolation on basal dopamine levels reported previously.

Dopamine activity in the NAc is thought to play an important role in cue-related learning for positive and negative reinforcers (for review, see Wheeler & Carelli, 2009). Considering the aforementioned increases in dopamine activity in isolates, it is notable that SI rearing also produces increases in acquisition of Pavlovian conditioning. For example, SI animals show enhanced cue-conditioned responding for food and discriminative learning for a sucrose reward (Jones *et al.*, 1990, 1992; Harmer & Phillips, 1998; Lapiz *et al.*, 2003). Footshock-conditioned contextual cues also induce greater increases in dopamine levels in the NAc of SI rats compared with GH cohorts (Fulford & Marsden, 1998; Lapiz *et al.*, 2003). The increased dopamine release and dopamine transporter activity observed in this study may be partly responsible for the increased sensitivity to Pavlovian conditioning, and greater dopamine responses to cues (Fulford & Marsden, 1998; Lapiz *et al.*, 2003).

Relationship between anxiety and dopamine

Although the amygdala, hippocampus and prefrontal cortex are the focus of many anxiety studies in humans and rodents, the mesolimbic dopamine system also appears to play a critical role in anxiety-like behavior. The relationship between anxiety and dopamine

activity is somewhat inconsistent, however, and may vary by brain region. For example, while intra-amygdala infusion of either D1-like or D2-like dopamine receptor agonists results in increased anxiety-like behaviors in rats (Bananej *et al.*, 2012), systemic administration of dopamine agonists decreases anxiety-like behavior, an effect that can be blocked by D2-like receptor antagonists (Bartoszyk, 1998; Garcia *et al.*, 2005). Also, dopamine is thought to have opposing roles in the central vs. the basolateral amygdala (Perez de la Mora *et al.*, 2012). Although dopamine interactions with anxiety-like behavior are still being elucidated, it has been established that acute stressors increase dopamine in the nucleus accumbens (Abercrombie *et al.*, 1989; Pei *et al.*, 1990; Imperato *et al.*, 1992), possibly through corticotrophin-releasing factor and/or glucocorticoid receptors (Angulo & McEwen, 1994; Piazza *et al.*, 1996; Piazza & Le Moal, 1997; Lemos *et al.*, 2012), and injections of dopamine receptor antagonists or temporary inactivation of VTA dopamine cell bodies prior to footshock stress prevent the later expression of anxiety-related behaviors (Corral-Frias *et al.*, 2013). In addition, administration of L-DOPA, the dopamine precursor, in an animal model of Parkinson's disease increases anxiety-like behavior (Eskow Jaunarajs *et al.*, 2012). Because of the growing body of literature suggesting that circuits involved in anxiety-like and reinforcing behaviors are highly interconnected (Pezze & Feldon, 2004; Price & Drevets, 2010), we examined the relationship between our accumbal dopamine function and anxiety measures. Our finding of significant negative correlations between EPM open-arm time and both dopamine release and uptake, regardless of housing condition, reinforces the notion that elevated accumbal dopamine signaling is associated with increased anxiety-like behavior.

SI rearing and dopamine D2-type autoreceptors

Dopamine terminal release and reuptake in the NAc are highly regulated processes, and both are modulated by feedback mechanisms such as dopamine D2-type autoreceptor activity (Joseph *et al.*, 2002). D2 autoreceptors regulate dopamine activity by decreasing vesicular release through inhibition of dopamine synthesis and inhibition of exocytosis by hyperpolarization of dopamine cell bodies and terminals (Cubeddu & Hoffmann, 1982; Wolf & Roth, 1990; Mercuri *et al.*, 1997). Additionally, presynaptic D2 receptor activation can affect uptake by increasing dopamine transporter surface expression as well as increasing the rate of uptake for each dopamine transporter by hyperpolarization of the presynaptic membrane (Meiergerd *et al.*, 1993; Sonders *et al.*, 1997; Dickinson *et al.*, 1999; Bolan *et al.*, 2007). Although SI has consistently produced increases in general dopamine activity, studies examining D2 levels have shown mixed results in the NAc core. For example, SI rearing has been reported to increase D2 receptor numbers (King *et al.*, 2009) and the proportion of D2 receptors in a high-affinity state (Han *et al.*, 2012), suggesting overall increases in striatal D2 activity. In contrast, several other studies have shown decreases (Hall *et al.*, 1998b) or no changes in striatal D2 levels after SI rearing (Jones *et al.*, 1992; Del Arco *et al.*, 2004; Djouma *et al.*, 2006). To further complicate the interpretation, these previous studies do not distinguish between pre- and post-synaptic D2 receptor expression, nor are the assays utilized capable of measuring changes in dopamine terminal function. To overcome these problems, in the current study, we examined presynaptic D2 receptor activity after SI rearing using voltammetry, which can be used to measure autoreceptor activity as agonist-induced (quinpirole) inhibition of evoked dopamine release (Joseph *et al.*, 2002; Mateo *et al.*, 2005; Maina & Mathews, 2010). Our results suggest that there are no SI rearing-induced differences in presynaptic D2 dopamine autoreceptor activity.

Conclusions

In the present study, we demonstrated that SI rearing results in long-lasting increases in anxiety-like behavior, and that these behavioral alterations are accompanied by changes in NAc dopamine kinetics, including increased evoked dopamine overflow and increased reuptake rates. These functional increases may produce greater dopamine responses in SI-reared rats, compared with GH animals, in the presence of a stimulus or challenge, such as during cue-conditioned learning or stimulant drug administration. Because SI rearing is a model of schizophrenia (Scheller-Gilkey *et al.*, 2004; Nugent *et al.*, 2011), the increased dopamine responses observed in SI rats may be related to the impaired latent inhibition and reduced PPI observed in humans with schizophrenia. Our finding that increased dopamine function in isolates is associated with increased anxiety-like behavior suggests that individuals who have experienced early life stress, and suffer from anxiety disorders, may also have disrupted dopamine system function. These differences in dopamine terminal activity are enduring, lasting well into adulthood. Additionally, there appears to be a protective effect of juvenile GH rearing conditions, such that adult isolation in GH animals does not engender an SI-like behavioral and neurochemical phenotype, further supporting the concept that in humans, stressors may be more deleterious in early life.

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Abbreviations

[DA_p]	stimulated dopamine release
EPM	elevated plus-maze
GH	group housed
K_m	apparent affinity
NAc	nucleus accumbens
PD	postnatal day
PPI	pre-pulse inhibition
SI	socially isolated
V_{max}	maximal rate of uptake

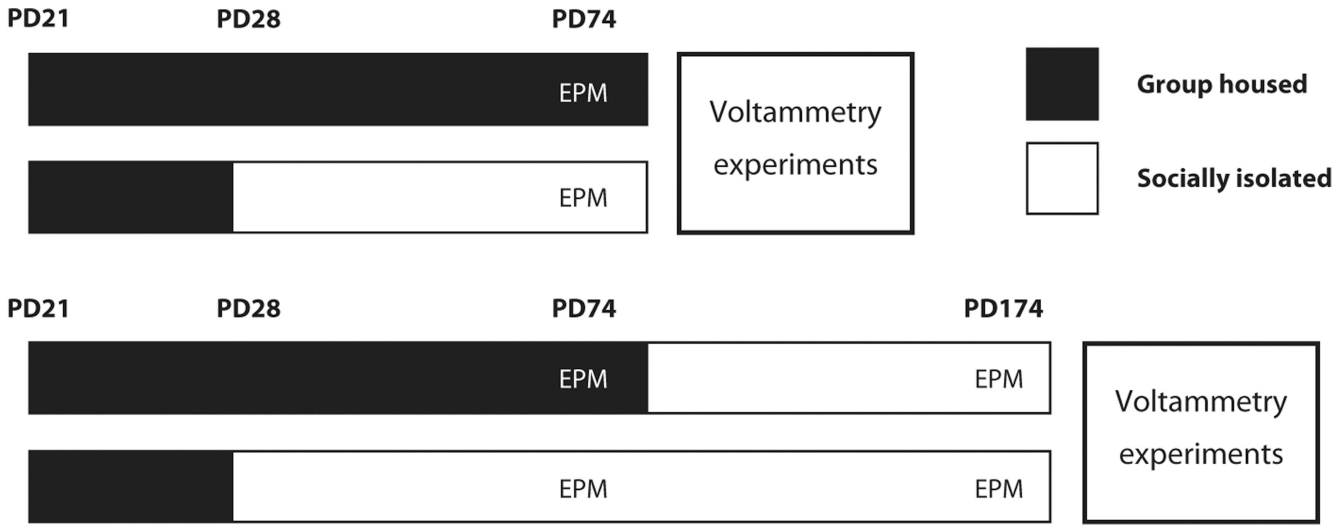

Fig. 1.

Schematic model of housing paradigm and experimental timeline. Rats were obtained on postnatal day (PD) 21 and placed in group-housed (GH) conditions until PD 28. Two groups maintained GH, while another two groups were socially isolated (SI) for the remainder of the study. At PD 74 all rats were tested on the elevated plus-maze (EPM). A set of SI and GH rats were killed, and slice voltammetry experiments were performed to examine release, uptake and autoreceptor activity (PD = 84 ± 7). The second set of SI and GH rats were isolated for four additional months (PD = 77–174), and examined again on the EPM and in similar voltammetry experiments to the previous group (PD = 181 ± 7).

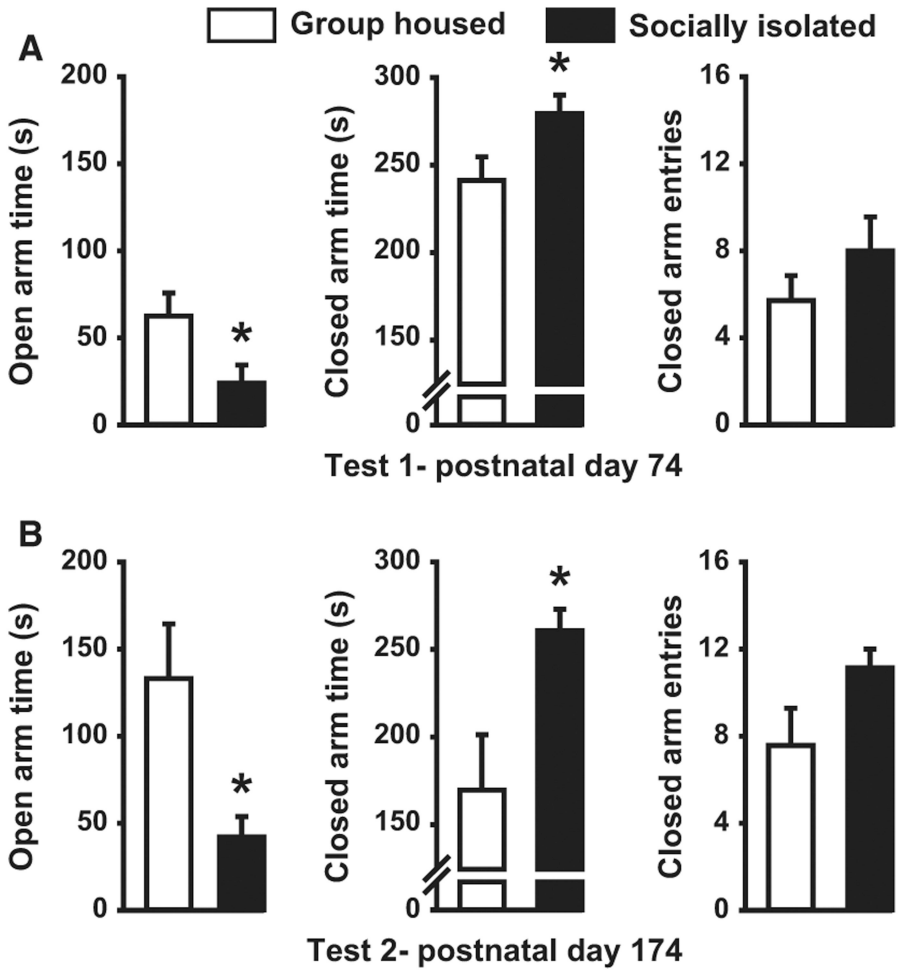

Fig. 2.

Effect of isolation rearing on anxiety-like behavior on the EPM. Bar graph illustrating the effect of adolescent rearing condition on mean (± SEM) open- and closed-arm time, and number of closed-arm entries in the EPM at two separate time points, PD 74 (A) and PD 174 (B) in group-housed (GH $n = 7$) and socially isolated (SI $n = 7$) rats. $^{*}P < 0.05$.

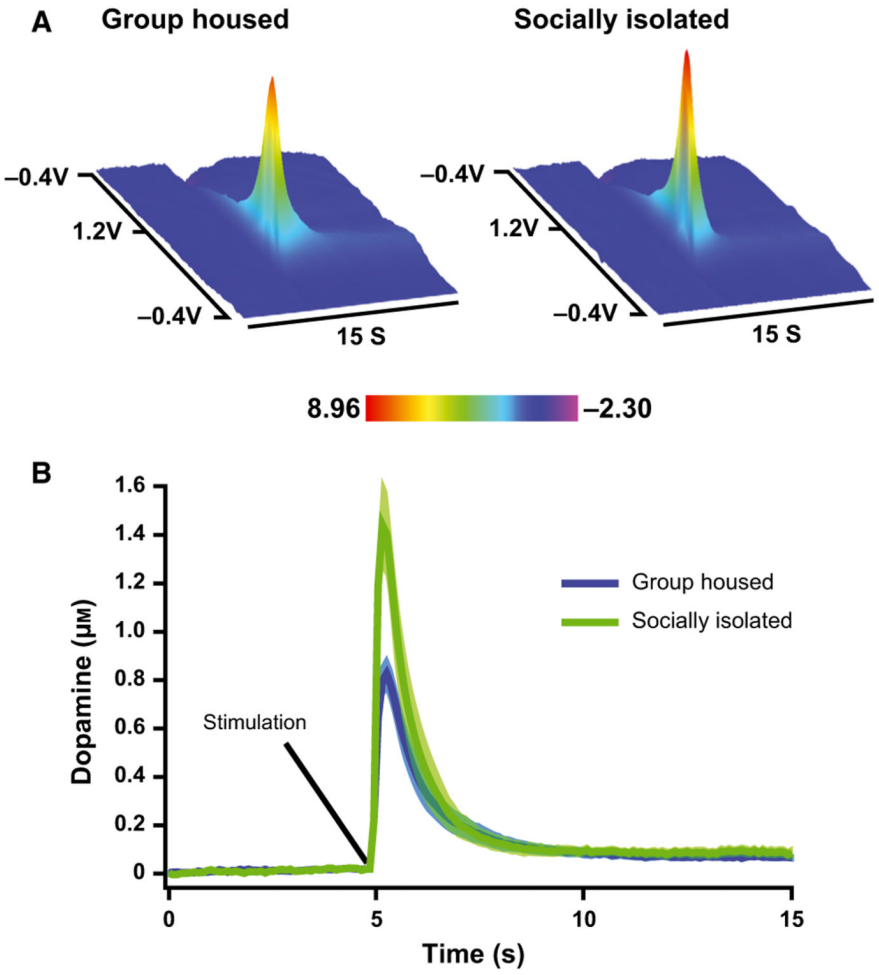

Fig. 3.

NAc core stimulated dopamine overflow in socially isolated (SI; young $n = 8$; adult $n = 7$) and group-housed (GH; young $n = 8$; adult $n = 7$) rats. (A) Averaged background subtracted voltammetric color plots for SI and GH rats, with applied potential (y-axis) plotted against time (x-axis) and current (z-axis). (B) Mean (± SEM) concentration vs. time traces for GH- and SI-reared rats. Solid line is mean data, whereas the lighter outline represents the SEM.

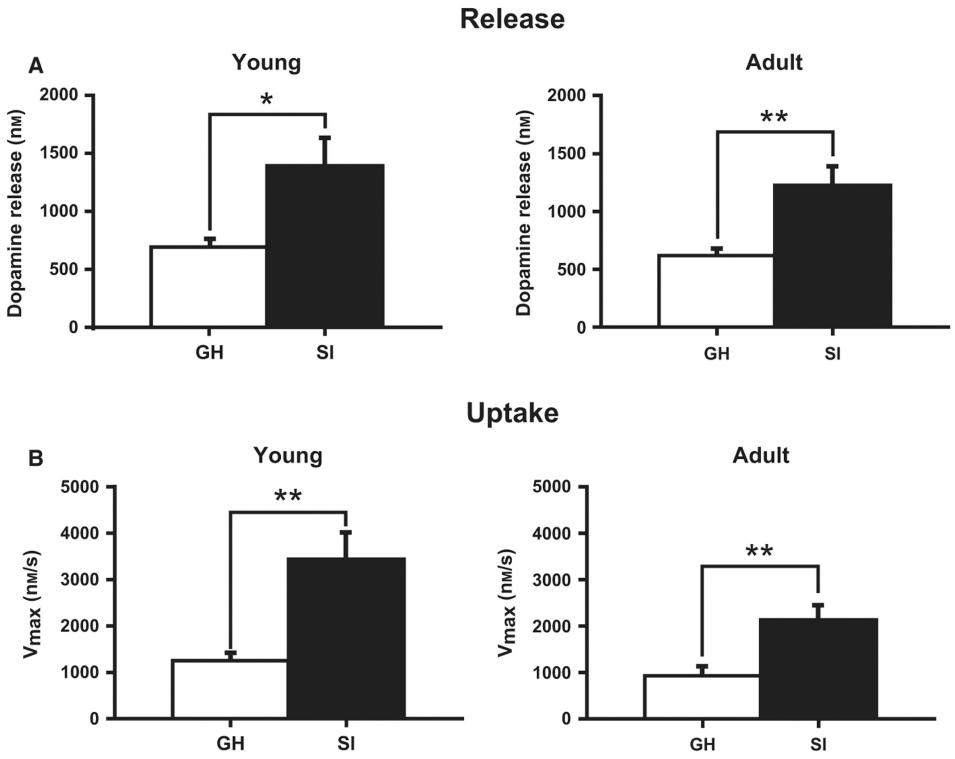

Fig. 4.

Effect of isolation rearing on stimulated dopamine release and uptake rate within the NAc. (A) Bar graph of mean (± SEM) stimulated dopamine release in socially isolated (SI) and group-housed (GH) rats at young (left; PD = 84 ± 7; GH $n = 8$; SI $n = 8$) and adult (right; PD = 181 ± 7; GH $n = 7$; SI $n = 7$) time points. (B) Bar graph of mean (± SEM) dopamine uptake rates (V_{max}) in SI and GH rats at young (left; PD = 84 ± 7; GH $n = 8$; SI $n = 8$) and adult (right; PD = 181 ± 7; GH $n = 7$; SI $n = 7$) time points. *$P < 0.05$, **$P < 0.01$.

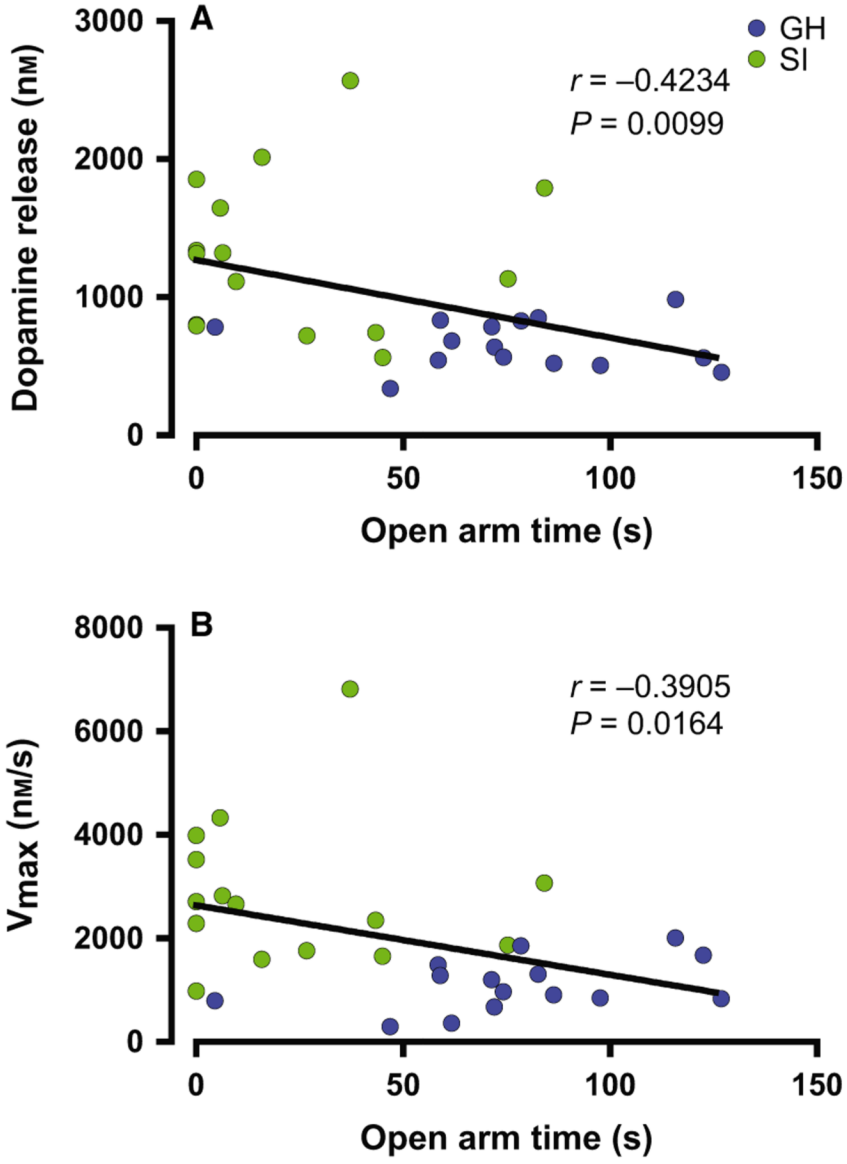

Fig. 5.

Relationship between dopamine function and anxiety-like behavior. NAc dopamine release (A) and rate of uptake (V_{max}; B) from voltammetric studies were significantly correlated with open-arm time on the EPM in data combined from young (PD 74) and adult (PD 174) group-housed (GH; blue) and socially isolated (SI; green) reared rats $n = 30$. Release $P = 0.0099$; Uptake $P = 0.0164$.

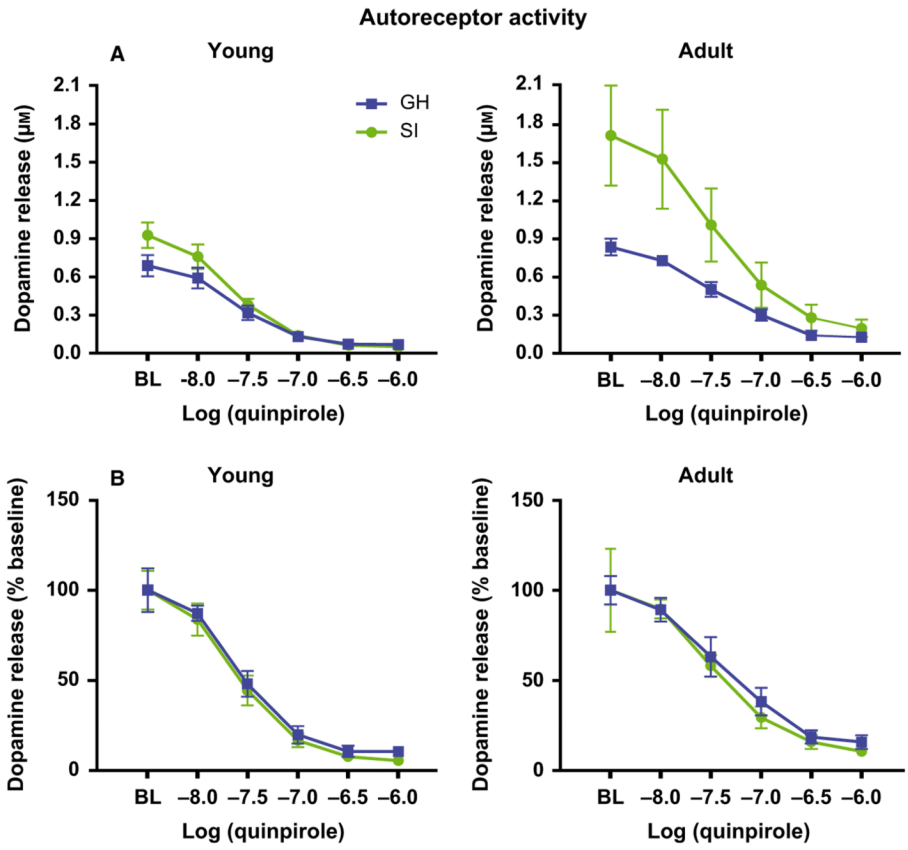

Fig. 6.

NAc dopamine D2 autoreceptor activity in group-housed (GH) and socially isolated (SI) reared rats. Electrically stimulated dopamine overflow was measured while bath-applying increasing concentrations of the D2-type dopamine receptor agonist quinpirole (10 nM–1 μM). (A) Mean μM dopamine release peak amplitude and (B) percent baseline stimulated dopamine in young (left; PD = 84 ± 7; GH *n* = 5; SI *n* = 5) and adult (right; PD = 181 ± 7; GH *n* = 4; SI *n* = 4) SI and GH rats. Quinpirole significantly reduced dopamine signals to the same extent in GH- and SI-reared rats for both young and adult cohorts.