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Early Social Experience Is Critical for the Development of Cognitive Control and Dopamine Modulation of Prefrontal Cortex Function

Petra JJ Baarendse[1], Danielle S Counotte[2], Patricio O'Donnell[2] and Louk JMJ Vanderschuren*[,1,3]

[1]Department of Neuroscience and Pharmacology, Rudolf Magnus Institute of Neuroscience, UMC Utrecht, Utrecht, The Netherlands; [2]Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA; [3]Faculty of Veterinary Medicine, Division of Behavioural Neuroscience, Department of Animals in Science and Society, Utrecht University, Utrecht, The Netherlands

Social experiences during youth are thought to be critical for proper social and cognitive development. Conversely, social insults during development can cause long-lasting behavioral impairments and increase the vulnerability for psychopathology later in life. To investigate the importance of social experience during the juvenile and early adolescent stage for the development of cognitive control capacities, rats were socially isolated from postnatal day 21 to 42 followed by re-socialization until they reached adulthood. Subsequently, two behavioral dimensions of impulsivity (impulsive action in the five-choice serial reaction time task (5-CSRTT) and impulsive choice in the delayed reward task) and decision making (in the rat gambling task) were assessed. In a separate group of animals, long-lasting cellular and synaptic changes in adult medial prefrontal cortex (PFC) pyramidal neurons were determined following social isolation. Juvenile and early adolescent social isolation resulted in impairments in impulsive action and decision making under novel or challenging circumstances. Moreover, socially isolated rats had a reduced response to enhancement of dopaminergic neurotransmission (using amphetamine or GBR12909) in the 5-CSRTT under challenging conditions. Impulsive choice was not affected by social isolation. These behavioral deficits were accompanied by a loss of sensitivity to dopamine of pyramidal neurons in the medial PFC. Our data show long-lasting deleterious effects of early social isolation on cognitive control and its neural substrates. Alterations in prefrontal cognitive control mechanisms may contribute to the enhanced risk for psychiatric disorders induced by aberrations in the early social environment.
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INTRODUCTION

Humans and many other mammals are social species. Marked changes in social behavior take place during post-weaning development (ie, childhood and adolescence in humans, equivalent to the juvenile and adolescent stages in rodents), including increased complexity of the social repertoire and a profound increase in peer-directed social interactions, signified by an abundance of social play behavior (Blakemore, 2008; Nelson *et al*, 2005; Spear, 2000; Vanderschuren *et al*, 1997). Social play behavior is thought to facilitate neural and behavioral development to equip the individual with a flexible and adaptive behavioral repertoire (Pellis and Pellis, 2009; Špinka *et al*, 2001). Conversely, disruptions in the early social environment can result in persistent neurobiological changes that may increase the vulnerability for psychiatric diseases later in life (Cacioppo and Hawkley, 2009; Paus *et al*, 2008).

The juvenile and adolescent stages are critical periods of neural maturation (Casey *et al*, 2005; Counotte *et al*, 2010; Paus *et al*, 2008). In particular, the prefrontal cortex (PFC) continues to develop until early adulthood in humans (Casey *et al*, 2005; Paus *et al*, 2008) and rodents (Counotte *et al*, 2010; Tseng and O'Donnell, 2005, 2007). As adverse events are likely to maximally impact on brain regions that are in transition (Andersen, 2003), early environmental insults, such as social isolation, may profoundly affect PFC function (Leussis *et al*, 2008; Makinodan *et al*, 2012). Indeed, isolation rearing studies, in which animals are socially isolated after weaning, have consistently shown changes in PFC function, including disrupted synaptic plasticity, as well as decreases and increases in PFC dopamine and serotonin signaling, respectively (for review see Fone and Porkess, 2008). However, isolation rearing comprises continuous social isolation from weaning onwards, so that the specific period of social isolation that is critical for inducing these neurobehavioral changes is largely unknown. As the PFC is essential for cognitive control functions, such as impulse control and decision

*Correspondence: Professor LJMJ Vanderschuren, Faculty of Veterinary Medicine, Division of Behavioural Neuroscience, Department of Animals in Science and Society, Utrecht University, Yalelaan 2, Utrecht 3584 CM, The Netherlands. Tel: +31 30 2535239, Fax: +31 30 2537997, E-mail: l.j.m.j.vanderschuren@uu.nl
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making (Miller and Cohen, 2001; Robbins and Arnsten, 2009), we hypothesized that social play behavior facilitates the development of these functions. To test this hypothesis, we assessed the long-term cognitive effects of social isolation during postnatal day (PND) 21–42. This age range is comparable to childhood and early adolescence in humans (McCutcheon and Marinelli, 2009; Spear, 2000), and it is the period when social play behavior peaks in rats (Panksepp, 1981). Impulsive action was studied using the five-choice serial reaction time task (5-CSRTT; Robbins, 2002), and the delayed reward task (DRT) was used to measure impulsive choice (Evenden and Ryan, 1996). Decision making was measured using the rat gambling task (rGT; Zeeb *et al*, 2009), a rodent analog of the human Iowa gambling task (Bechara *et al*, 1994). To characterize the role of monoamine neurotransmission in the long-term behavioral effects of early adolescent social isolation, we investigated the effects of amphetamine, as well as selective inhibitors of the reuptake of dopamine, serotonin, and noradrenaline on impulsivity and decision making. Moreover, we used slice electrophysiology to determine long-lasting cellular and synaptic changes in medial PFC (mPFC) pyramidal neurons in adult animals following social isolation.

MATERIALS AND METHODS

Animals

Male Lister Hooded (behavioral experiments; Harlan, The Netherlands) or Long-Evans rats (electrophysiology experiments; Charles Rivers Laboratories, USA) arrived at 21 days of age and were housed either socially (SOC) or individually (ISO) the day after arrival. Rats were housed under reversed lighting conditions (lights on at 1900 hours) for the behavioral experiments and normal light/dark conditions (lights on at 0700 hours) for the electrophysiology experiments. Rats of the ISO group were re-socialized, ie, housed together with an animal of the same treatment group, on day 43. Behavioral testing and electrophysiological recordings started at 12 weeks of age. A separate cohort of animals was used for every experiment (5-CSRTT, DRT, rGT, and mPFC recordings). During behavioral training and testing, rats were placed on a restricted diet of 14 g chow per day. All experiments were approved by the Animal Ethics Committee of the Utrecht University and the University of Maryland School of Medicine Institutional Animal Care and Use Committee and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996), European regulations (Guideline 86/609/EEC), and the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

Apparatus

Behavioral testing (5-CSRTT, DRT, and rGT) was conducted in operant conditioning chambers (Med Associates, USA) enclosed in sound-insulating, ventilated boxes. Set in the curved wall of each box was an array of five nose poke holes. Each nose poke unit was equipped with an infrared detector and a yellow stimulus light. Food pellets (45 mg, Formula P; Bio-Serv) could be delivered at the opposite wall via a dispenser. The chamber could be illuminated by a white house light, mounted in the center of the roof. Online control of the apparatus and data collection was performed using MEDPC (Med Associates).

Five-Choice Serial Reaction Time Task

Rats were trained to respond to a brief visual stimulus presented randomly in one of the five nose poke units to obtain a food reward (Baarendse and Vanderschuren, 2012; Robbins, 2002; Van Gaalen *et al*, 2006a). Each session consisted of 100 trials or 30 min, whichever occurred first. A trial started with an intertrial interval (ITI) of 5 s, followed by 1 s illumination of one of the five apertures and a 2 s limited hold. A nose poke in the illuminated aperture (correct response) was rewarded with one food pellet. Nose pokes made during the ITI (premature responses) were recorded as a measure of impulsivity. An incorrect response, failure to respond (error of omission) and premature response, resulted in no food delivery and a time-out period with the house light illuminated for 5 s. After training to baseline performance, rats were exposed to various task manipulations, such as increasing the ITI to 7 s (long ITI), decreasing the stimulus duration to 0.5 s (short stimulus duration), and increasing the performance load by decreasing the ITI to 2.5 s (high event rate). The following measures were recorded: (1) premature responses (number of responses into one of the holes during the ITI); (2) accuracy (the percentage of correct responses [(number correct responses)/(correct + incorrect responses) $\times$ 100]); and (3) omission errors (the total number of omitted trials during a session).

Delayed Reward Task

Each session was divided into five blocks of 12 trials. Nose poking into one illuminated nose poke hole resulted in the immediate delivery of a small reward (one food pellet), whereas nose poking into one other illuminated hole resulted in the delivery of a large, delayed reward (four food pellets) (Baarendse and Vanderschuren, 2012; Evenden and Ryan, 1996; Van Gaalen *et al*, 2006b). The delays to the large reward increased within a session from 0 to 10, 20, 40, and 60 s per block. After delivery of the reward or the choice phase time elapsed, an ITI commenced. The holes associated with the small and large reward were counterbalanced between animals. Following baseline performance, rats were exposed to a task manipulation that consisted of increased delays, ie, 0, 20, 40, 60, and 80 s per block. The percentage choice for the large reward as a function of delay was calculated as the [(number of choices for the large reinforcer)/(number choices large + small reinforcers) $\times$ 100].

Rat Gambling Task

In this task, rats are confronted with four choices differing in the probability and magnitude of reward (food) and punishment (time-out; Baarendse *et al*, 2013; Zeeb *et al*, 2009). Rats were tested once daily in a 30-min session. A trial started with a 5-s ITI followed by illumination of holes 1, 2, 4, and 5 for 10 s. A response in an illuminated hole turned off all stimulus lights, and led to either the delivery

of reward or the start of a punishment time-out. Animals were first tested in 10 free choice sample sessions, in which the first two choices for each option were rewarded, after which the reward and punishment contingencies associated with the four response options were introduced. The free sample sessions were followed by five forced-choice sessions before moving on to the full free choice task. Premature responses were punished by a 5-s time-out period, signaled by illumination of the house light. The reinforcement schedules were designed such that the optimal strategy, in terms of maximal number of food pellets earned per session, was to select the two-pellet option (P2), associated with a 10-s time-out period that occurs 20% of the time (80% chance of reward). The next best option is P1 (5 s time-out, 90% chance of reward). The two disadvantageous options were both associated with larger immediate gain (three or four pellets), but also longer time-out periods (P3: 30 s time-out, 50% chance of reward; P4: 40 s time-out; 40% chance of reward). The spatial locations of the pellet choice options were counterbalanced across subjects. Choice scores were calculated as the sum of the two advantageous options (average percentage choice of P1 and P2) and the sum of the two disadvantageous options (average percentage choice of P3 and P4).

Drugs

(+)-Amphetamine sulphate (0.5 mg/kg; O.P.G., The Netherlands), GBR12909 dihydrochloride (10 mg/kg), atomoxetine hydrochloride (3 mg/kg), and citalopram hydrobromide (1 mg/kg; Tocris Bioscience, UK) were dissolved in 0.9% saline (amphetamine, atomoxetine, and citalopram) or sterile water (GBR12909). Animals received five daily test sessions per week until stable patterns of performance were observed. Next, the effects of the task manipulations were assessed (5-CSRTT, DRT). Subsequently, the effects of the four drugs on performance in the 5-CSRTT, DRT, and rGT were tested. Drug tests were conducted on Tuesdays and Fridays with baseline training sessions on the other weekdays. Before the first drug test, all animals had been habituated twice to i.p. saline injections. Every animal in a cohort received a challenge with every drug, according to a Latin square design. Injections were given i.p., 30 min (5-CSRTT, rGT) or 10 min (DRT) before behavioral testing. We have previously demonstrated effects on impulsivity of these drugs using these doses and pretreatment intervals (Baarendse and Vanderschuren, 2012).

Whole-Cell Recordings

At 12 weeks of age, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) 15 min before decapitation. Brains were quickly removed and placed into ice-cold artificial CSF oxygenated with 95% O_2-5% CO_2 and containing the following (in mM): 125 NaCl, 25 $NaHCO_3$, 10 glucose, 3.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, and 3 $MgCl_2$, pH 7.45 (295–300 mOsm). Coronal slices (300 μm) containing the PFC were obtained with a vibratome in ice-cold aCSF and incubated in warm (~35 °C) aCSF solution constantly oxygenated with 95% O_2-5% CO_2 for at least 45 min before recording. Whole-cell recordings from layer V pyramidal neurons were made using standard electrophysiological techniques (Tseng *et al*, 2008). Patch electrodes (7–10 MΩ) were filled with a solution containing 0.125% neurobiotin and the following (in mM): 115 K-gluconate, 10 HEPES, 2 $MgCl_2$, 20 KCl, 2 MgATP, 2 Na2-ATP, and 0.3 GTP, pH 7.25–7.30 (280–285 mOsm). All experiments were conducted at 33–35 °C.

Synaptic responses were tested in pyramidal neurons with electrical stimulation of layers I–II with a bipolar electrode made from a pair of twisted Teflon-coated tungsten wires (tips separated by ~200 μm) and placed ~500 μm lateral to the vertical axis of the recorded neuron. Stimulation pulses (0.02–0.4 mA; 0.5 ms) were delivered every 15 s. Throughout the experiment, input resistance was measured with a single hyperpolarizing step and the cell was discarded when the input resistance changed more than 20% during the course of the experiment. The amplitude of evoked postsynaptic potentials (EPSPs) was measured with Clampfit 9.0 and averaged over 10 sweeps before and after 10 min of application of a mixture of SKF38393 and quinpirole. At the end of each experiment, the slices were placed in 4% paraformaldehyde and processed for DAB staining using standard histochemical techniques to verify morphology and location of the neurons.

Data Analysis

In the behavioral experiments, the initial group size was $n = 12$. However, due to experimental errors and computer failure, the data of some drug challenges were excluded from the analysis. The final group size in the behavioral experiments was therefore $n = 10$–12. An arcsine transformation was performed before analysis of the percentage of choice in the DRT and rGT to limit the effect of an artificially imposed ceiling. In the DRT, animals that did not show delay-dependent choice behavior (0% choice for large reward at 0 s delay, 100% choice for large reward at 40 and 60 s delay) were excluded from the experiment. Data were analyzed by one- (5-CSRTT, EPSP amplitude) or two-factor (DRT, rGT) repeated-measures ANOVAs with task challenge (5-CSRTT), dopamine (EPSP amplitude), or drug treatment (5-CSRTT, DRT, rGT) and delay to large reinforcer (DRT) or choice (rGT) as within-subjects variables, and rearing condition (SOC-ISO) as between-subjects variables. In case of statistically significant main effects, *post-hoc* comparisons were conducted using Paired samples or Student's *t*-tests. The level of probability for statistically significant effects was set at 0.05.

RESULTS

Impulsive Action (5-CSRTT): Baseline, Behavioral, and Pharmacological Challenges

ISO and SOC rats acquired the 5-CSRTT at comparable rates (sessions needed to reach baseline performance: SOC 36.3 ± 2.0, ISO 33.3 ± 1.7; $F[group](1,22) = 0.47$, $p = 0.26$), and there were no differences in behavior under baseline conditions between ISO and SOC rats (Figure 1a–c). Exposure to task challenges in the 5-CSRTT significantly affected premature responses, ie, impulsive action (Figure 1a; task challenge: $F(3,66) = 107.51$, $p < 0.001$),

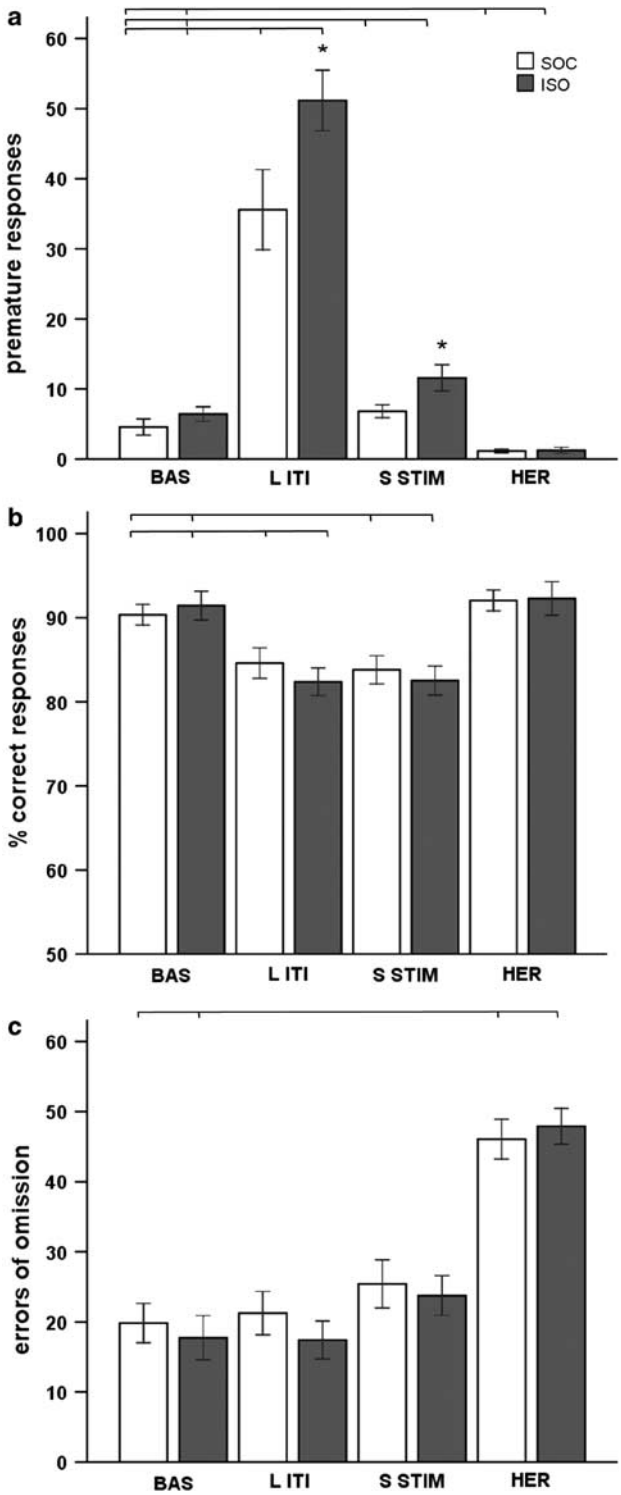

Figure 1 Effect of social isolation during postnatal day 21–42 followed by re-socialization on adult performance in the 5-choice serial reaction time task. (a) Amount of premature responses, ie, impulsive action, (b) percentage of correct responses, ie, accuracy and (c) errors of omission, under baseline conditions (BAS: visual stimulus presented 5 s after trial initiation, 1 s stimulus duration) or long intertrial interval (L ITI: visual stimulus presented 7 s after trial initiation), short stimulus duration (S STIM: 0.5 s stimulus duration), high event rate (HER: intertrial interval 2.5 s). Group size was $n = 12$. SOC = socially housed rats during PND 21–42, ISO = socially isolated rats during PND 21–42. Asterisk indicates $p < 0.05$ compared with SOC. Line on top of bar indicates $p < 0.05$ compared with baseline. All data are expressed as mean ± SEM.

percentage of correct responses (Figure 1b; task challenge: $F(3,66) = 27.41$, $p < 0.001$), and errors of omission (Figure 1c; task challenge: $F(3,66) = 72.95$, $p < 0.001$) in both groups. However, when the ITI was increased to 7 s or the stimulus duration decreased to 0.5 s, ISO rats made more premature responses (challenge × isolation: $F(3,66) = 3.48$, $p < 0.03$; Figure 1a). These task challenges did not differentially affect task accuracy (Figure 1b) or errors of omission (Figure 1c) in ISO *vs* SOC rats (challenge × isolation: F[% correct responses](3,66) = 0.66, NS; F[errors of omission](3,66) = 0.58, NS). No difference between groups was observed when rats were exposed to a high event rate (decrease of ITI to 2.5 s; $p > 0.05$).

Pharmacological challenges under baseline conditions significantly affected premature responses (Figure 2a; drug: $F(4,80) = 18.05$, $p < 0.001$), percentage of correct responses (Figure 2b; drug: $F(4,88) = 17.44$, $p < 0.001$), and errors of omission (Figure 2c; drug: $F(4,88) = 34.3$, $p < 0.001$), without an effect of early adolescent social isolation (drug × isolation: F[premature responses](4,80) = 1.14, NS; F[% correct responses](4,88) = 2.14, NS; F[errors of omission](4,88) = 0.22, NS). Under baseline conditions, amphetamine and the selective dopamine reuptake inhibitor GBR12909 increased premature responding, and the selective noradrenaline reuptake inhibitor atomoxetine decreased the amount of premature responses in both groups ($p < 0.05$). The percentage of correct responses was decreased by amphetamine and GBR12909, whereas atomoxetine increased errors of omission ($p < 0.05$). Although no overall effect of social isolation was observed (drug × isolation: F[premature responses](4,80) = 1.14, NS; F[% correct responses](4,88) = 2.14, NS; F[errors of omission](4,88) = 0.22, NS), the selective serotonin reuptake inhibitor citalopram decreased premature responding in ISO rats, and decreased accuracy in the SOC group (drug: F[premature responses](4,80) = 18.05, $p < 0.001$; F[% correct responses](4,88) = 17.44, $p < 0.001$; *post hoc* $p < 0.05$).

Under a long ITI, amphetamine and GBR12909 increased premature responding (Figure 2d; drug: $F(4,88) = 26.67$, $p < 0.001$), but this effect was found in the SOC group only (drug × isolation: $F(4,88) = 2.42$, $p < 0.05$), as no enhancement of premature responding by amphetamine and GBR12909 was observed in ISO rats ($p > 0.05$). Citalopram and atomoxetine decreased premature responding in both groups (both *post-hoc* $p < 0.001$; Figure 2d). Atomoxetine increased errors of omission, whereas both citalopram and atomoxetine increased the percentage of correct responses under the long ITI (drug: F[% correct responses](4,88) = 31.99, $p < 0.001$; F[errors of omission](4,88) = 9.92, $p < 0.001$), but not differentially between groups (drug × isolation F[% correct responses](4,88) = 2.1, NS; F[errors of omission](4,88) = 0.21, NS; Figure 2e and f).

Impulsive Choice (DRT): Baseline, Increased Delay, and Pharmacological Challenges

Overall, both groups showed a delay-dependent decline in their choice for the large, delayed reward under baseline conditions (delay: $F(4,80) = 69.02$, $p < 0.001$) without an effect of early adolescent social isolation (delay × isolation: $F(4,80) = 1.70$, NS; Figure 3a). As the behavioral deficits in the ISO animals in the 5-CSRTT were especially apparent

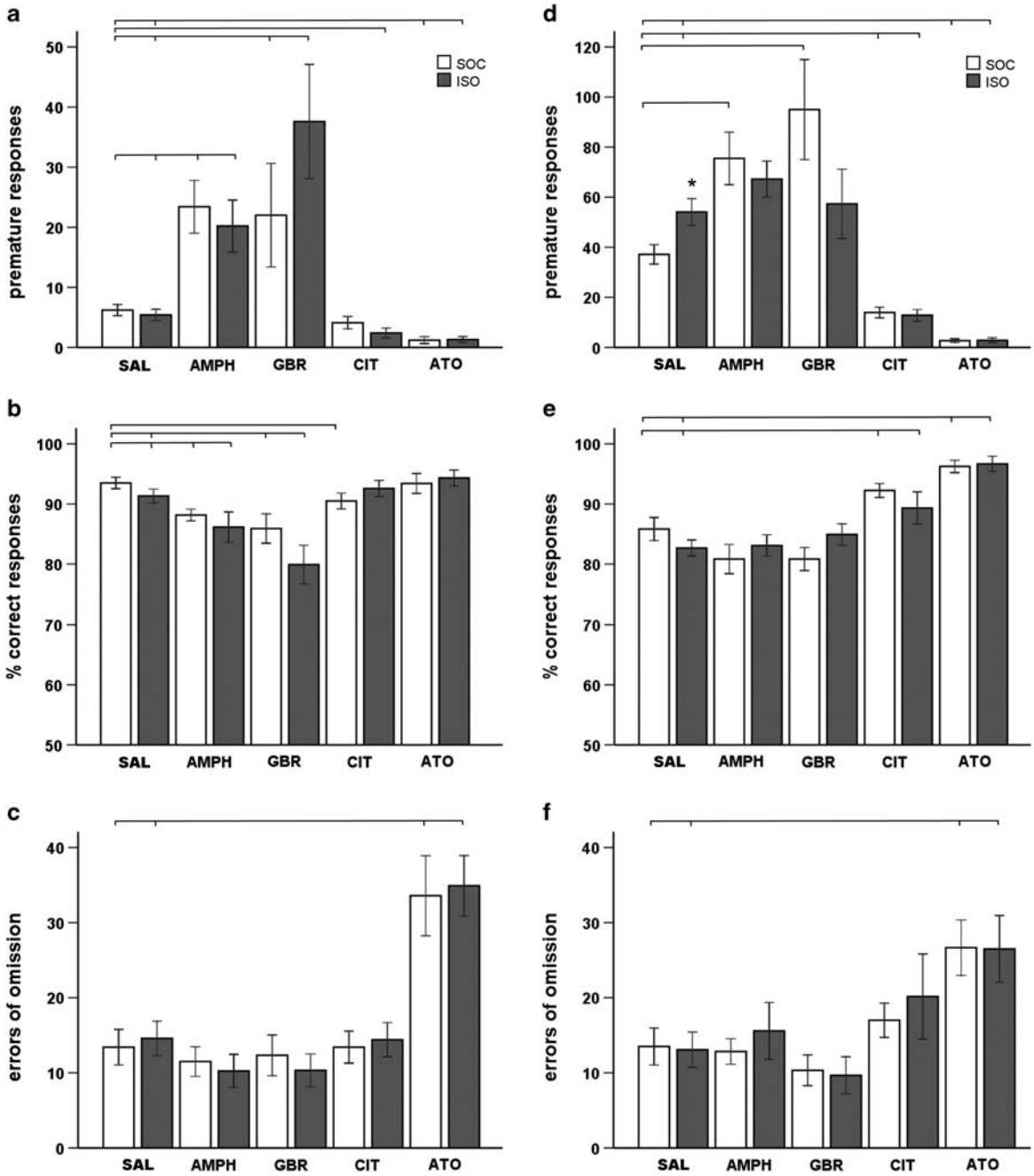

Figure 2 Effect of social isolation during postnatal day 21–42 followed by re-socialization on adult performance in the 5-choice serial reaction time task. (a,d) Amount of premature responses, ie, impulsive action; (b,e) percentage of correct responses, ie, accuracy; (c,f) errors of omission, under (a–c) baseline conditions (visual stimulus presented 5 s after trial initiation) or (d–f) long ITI conditions (visual stimulus presented 7 s after trial initiation). Pharmacological challenges consisted of treatment with 0.5 mg/kg of amphetamine (AMPH), 10 mg/kg of the selective dopamine reuptake inhibitor GBR12909 (GBR), 1 mg/kg of the selective serotonin reuptake inhibitor citalopram (CIT), and 3 mg/kg of the selective noradrenaline reuptake inhibitor atomoxetine (ATO). In total, $n = 10$–12 animals per treatment group were included in the analysis. SOC = socially housed rats during PND 21–42, ISO = socially isolated rats during PND 21–42, SAL = saline treatment. Asterisk indicates $p < 0.05$ compared with SOC group. Line on top of bar indicates $p < 0.05$ compared with baseline or saline treatment. All data are expressed as mean ± SEM.

when the animals were unexpectedly challenged with more demanding task conditions, we also assessed their behavior in the DRT when the delays were unexpectedly increased. Under these circumstances, there was no difference between ISO and SOC animals (delay: $F(4,84) = 42.29$, $p < 0.001$; delay × isolation: $F(4,84) = 0.77$, NS; Figure 3b). Early social isolation did not affect the effects of amphetamine, GBR12909, citalopram, and atomoxetine on impulsive choice in the DRT (delay × drug × isolation: $F(4,84) = 0.61$, NS). The effects of amphetamine, which enhanced the preference for the large reward (drug: $F(1,21) = 22.03$, $p < 0.001$; delay × drug: $F(4,84) = 3.56$, $p < 0.02$), are shown in Figure 3c. Consistent with previous findings (Baarendse and Vanderschuren, 2012), GBR12909

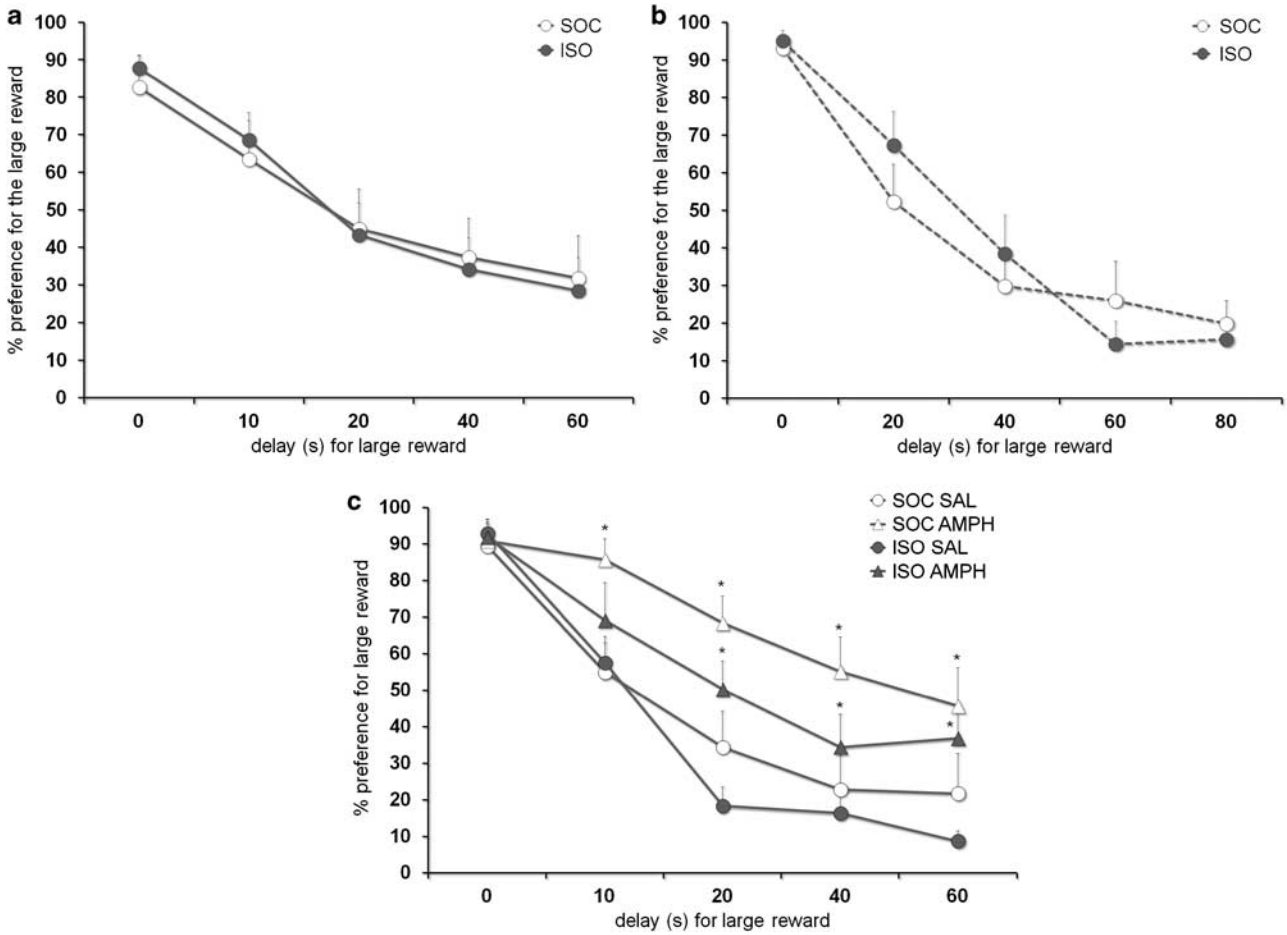

Figure 3 Effect of social isolation during postnatal day 21–42 followed by re-socialization on impulsive choice in the DRT during adulthood. Percentage of choice for the large reward expressed per block of trials with increasing delays under (a) baseline, (b) increased delays, and (c) 1 mg/kg amphetamine challenge. In total, $n = 10$–12 animals per treatment group were included in the analysis. AMPH = amphetamine, SOC = socially housed rats during PND 21–42, ISO = socially isolated rats during PND 21–42. Asterisk indicates $p < 0.05$ compared with saline treatment. All data are expressed as mean ± SEM.

increased the percentage of choice for the large reward, ie, improved impulsive choice, whereas atomoxetine and citalopram did not influence choice behavior in the DRT (drug: GBR12909: $F(1,19) = 8.34$, $p < 0.01$; atomoxetine: $F(1,21) = 0.08$, NS; citalopram: $F(1,21) = 3.66$, NS; data not shown).

Decision Making (rGT): Acquisition, Baseline, and Pharmacological Challenges

A detailed analysis of choice behavior per quartile of trials during the first free sampling choice session is depicted in Figure 4a and b. SOC rats started with an equal preference for the advantageous *vs* disadvantageous options during the first quartile of trials (Q1), and developed a preference for the advantageous options within the next quartiles of trials (Figure 4a; quartile × choice: $F(3,33) = 5.22$, $p < 0.01$, *post-hoc* Q1: NS, Q2-4: $p < 0.02$). Remarkably, ISO rats did not show this acquisition curve in decision making in the first rGT session during adulthood (Figure 4b; quartile × choice: $F(3,33) = 0.28$, NS). This effect of social isolation was supported by a significant quartile by group interaction (quartile × group: $F(3,66) = 2.92$, $p < 0.05$). Analysis of choice behavior during the 10 free sampling choice sessions revealed that both groups acquired the task at comparable rates (F[group](1,21) = 0.89, NS; F[group × session] (9,189) = 1.85, $p = 0.06$), with no between-group differences in performance apparent from session 3 onwards (data not shown).

Neither amphetamine nor one of the monoamine reuptake inhibitors, ie, GBR12909, citalopram, atomoxetine, altered the choice for the advantageous *vs* disadvantageous options in the social or isolated group (drug: F[advantageous](4,36) = 0.73, p = NS; F[disadvantageous] (4,40) = 0.86, p = NS; Figure 4c). Moreover, there was no difference in the effect of pharmacological manipulations between both groups (drug × group, $F(4,76) = 0.51$, NS). Analysis of choice behavior for the separate pellet options showed that amphetamine increased choice for P1 and decreased choice for P2 (drug: $F(1,60) = 6.22$, $p = 0.02$), but this effect was not different between both groups. GBR12909, atomoxetine, and citalopram did not alter choice behavior for the four pellet options. This pattern of effects is consistent with our previous findings on the effects of amphetamine and monoamine reuptake inhibitors on behavior in the rGT (Baarendse *et al*, 2013).

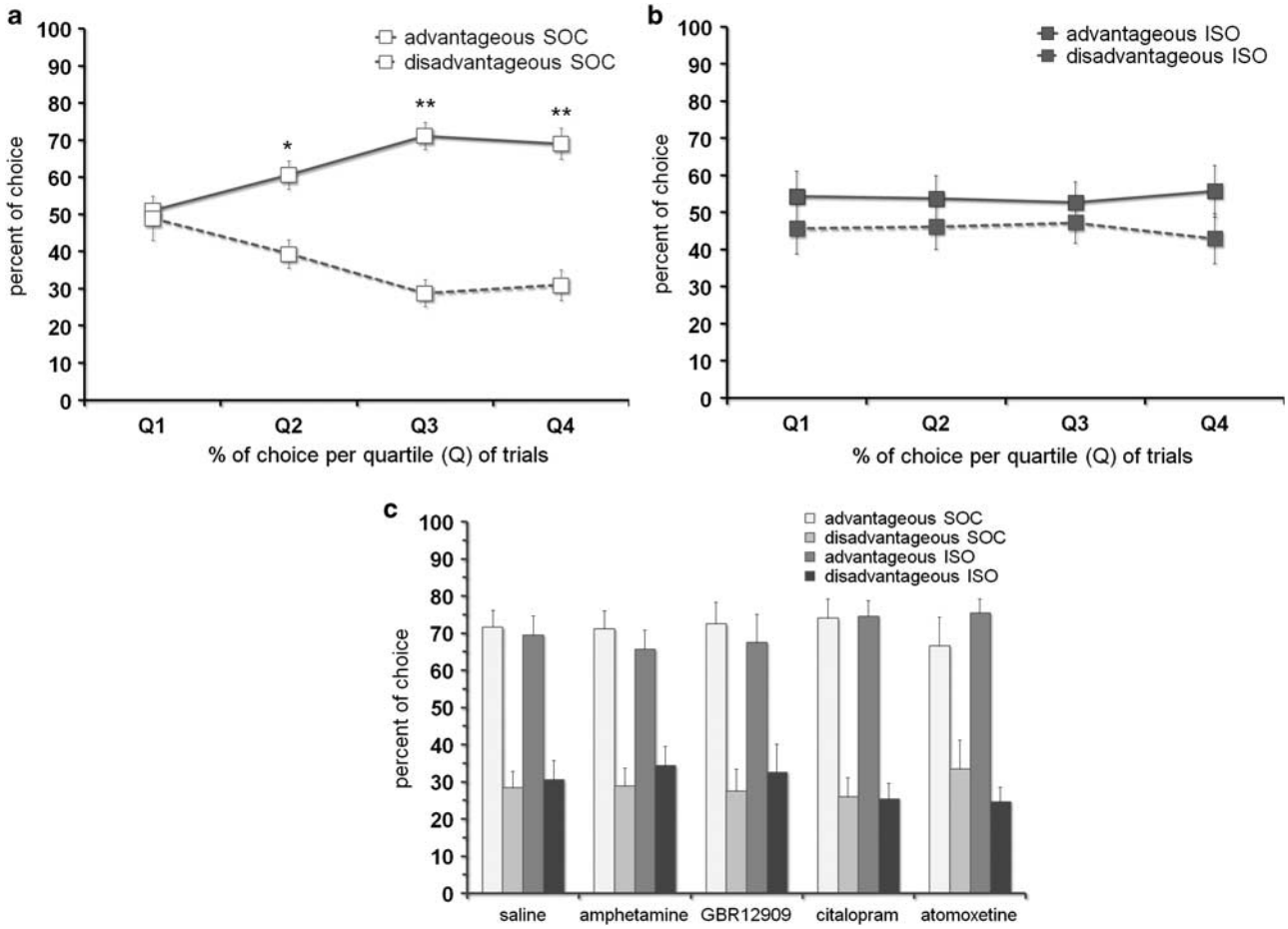

Figure 4 Effect of social isolation during postnatal day 21–42 followed by re-socialization on decision making in the rat gambling task during adulthood. Percentage of choice for the advantageous *vs* disadvantageous options during (a,b) the first free sample choice session, and (c) pharmacological challenges after stable baseline performance. Pharmacological challenges consisted of 1 mg/kg of amphetamine, 10 mg/kg GBR12909, 3 mg/kg citalopram, and 3 mg/kg atomoxetine. In total, $n = 10$–12 animals per treatment group were included in the analysis. SOC = socially housed rats during PND 21–42, ISO = socially isolated rats during PND 21–42. Asterisk indicates $p < 0.05$ and double asterisks indicate $p < 0.01$ difference between options. All data are expressed as mean ± SEM.

Whole-Cell Patch-Clamp Recordings

Functional differences following social isolation in early adolescence was studied using whole-cell recordings in layer V pyramidal cells in mPFC (Figure 5). Early adolescent social isolation did not change any of the basic membrane properties (resting membrane potential, rheobase (current to fire an action potential), and input resistance) of mPFC layer V pyramidal cells (data not shown). In SOC animals, stimulation of dopamine receptors with bath application of the D1 agonist SKF38393 (5 μM) and the D2 agonist quinpirole (1 μM) decreased the amplitude of synaptic responses, whereas this effect was absent in ISO rats (Figure 5; dopamine × group, $F(1,29) = 9.49$, $p = 0.004$; dopamine, $F(1,29) = 0.21$, NS). Locations of the recording sites are shown in Figure 5f. Most recorded cells were in infralimbic cortex, and a minority of recorded cells was located in the ventral prelimbic cortex.

DISCUSSION

Here, we show that early post-weaning social experience is critical for the development of cognitive capacities and PFC function in rats. Deprivation of social contact during a period in which marked changes in social behavior take place (PND 21–42), resulted in disrupted impulse control in the 5-CSRTT and impaired decision making in the rGT in adulthood, even after a prolonged period of re-socialization. ISO rats were less sensitive to amphetamine and GBR12909 under demanding conditions in the 5-CSRTT. In line with the reduced sensitivity to enhanced dopaminergic neurotransmission, whole-cell recordings in slices from adult animals showed that early social isolation caused mPFC pyramidal neurons to become insensitive to modulation of synaptic response amplitude by dopamine.

Early social life events have profound repercussions for the development of brain and behavior, which increase the vulnerability for neuropsychiatric disorders in adulthood (Cacioppo and Hawkley, 2009; Paus *et al*, 2008). However, only a limited number of studies have investigated the importance of social behavior during the juvenile and early adolescent stages, which is a period characterized by substantial changes in the structure of social behavior (Blakemore, 2008; Nelson *et al*, 2005; Spear, 2000). In particular, this period of life is characterized by the abundance of social play behavior, which is thought to

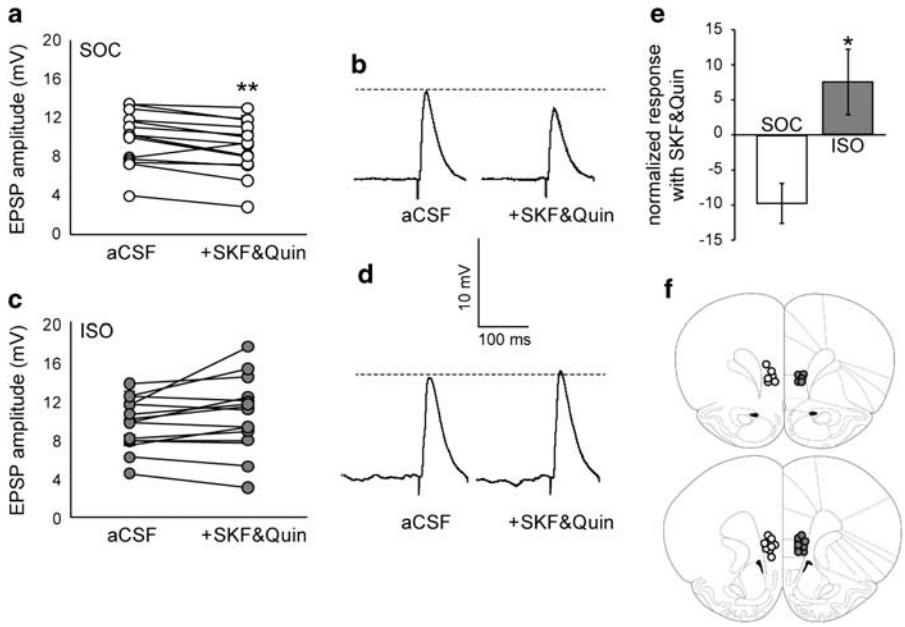

Figure 5 Effect of social isolation during postnatal day 21–42 followed by re-socialization on synaptic response size in mPFC. EPSP amplitude before and after bath-application of a combination of SK38393 (5 μM) and quinpirole (1 μM) in adult SOC animals (a; $n = 14$ cells from nine animals, double asterisks indicate $P < 0.01$ compared with aCSF; b: example trace). EPSP amplitude in animals socially isolated between P21 and 42 (ISO; c; $n = 17$ cells from eight animals; d: example trace). A summary of the EPSP amplitude following application of SKF38393 and quinpirole is shown in e. Location of pyramidal cells, mostly in infralimbic mPFC layer V, recorded from is shown in f (white circles are cells from SOC animals, gray circles are cells from adult animals socially isolated from PND 21–42; ISO). Asterisk indicates $P < 0.05$ compared with SOC group. Data are expressed as mean ± SEM.

contribute to the development of social and cognitive functions, and in particular to acquire the ability to flexibly use these capacities under changeable circumstances (Pellis and Pellis, 2009; Špinka *et al*, 2001; Vanderschuren *et al*, 1997; Trezza *et al*, 2010). Previous work has shown that deprivation of social contact in post-weaning rats results in behavioral deficits within the social domain (Lukkes *et al*, 2009; Van den Berg *et al*, 1999). These changes lasted into adulthood, even when the isolation period was followed by re-socialization. The present observations extend these findings, as social isolation during the juvenile and early adolescent stages resulted in cognitive control deficits in adulthood. Impulsive action, measured as premature responses in the 5-CSRTT, was enhanced in ISO rats when test conditions were unexpectedly made more demanding (ie, long ITI and short stimulus duration). Moreover, early social isolation resulted in impaired decision making in a rodent variant of the Iowa gambling task. Both humans and SOC rats develop a preference for the advantageous options within the first session of the IGT or rGT, respectively (Baarendse *et al*, 2013; Bechara *et al*, 1994), but this acquisition curve was absent in ISO rats. Importantly, these impairments in cognitive control arose mainly under challenging (long ITI and short stimulus duration in the 5-CSRTT) or novel (first session in the rGT) circumstances. This is consistent with the hypothesis that the expression of social play behavior contributes to the ability to act flexibly in a changeable, dynamic environment (Špinka *et al*, 2001).

Previously, we showed that during long ITI conditions in the 5-CSRTT, amphetamine and the DA reuptake blocker GBR12909 enhanced, and the NA reuptake blocker atomoxetine and 5-HT reuptake blocker citalopram reduced impulsive action (Baarendse and Vanderschuren, 2012). Here, we found that early social isolation resulted in impaired impulse control and a reduced sensitivity to amphetamine and GBR12909, but not atomoxetine or citalopram, in the 5-CSRTT under long ITI conditions. These effects were selective for impulsive action, as neither attentional nor motivational processes in the 5-CSRTT, nor impulsive choice in the DRT were affected by early adolescent social isolation. In addition, the sensitivity to amphetamine and GBR12909 was not changed under baseline conditions in the 5-CSRTT, which underscores the notion that the consequences of early social isolation become especially apparent under challenging conditions. This suggests that dopamine function required for coping with sudden changes in task requirements (perhaps at the level of the mPFC) is compromised as a result of social isolation. The blunted response to amphetamine in the 5-CSRTT is reminiscent of earlier findings in isolation-reared rats (Dalley *et al*, 2002a). However, unlike the present data, this latter study found no behavioral differences in the 5-CSRTT under baseline and challenging conditions between isolation-reared and control rats. Together, these findings therefore suggest that post-weaning social isolation can induce long-lasting changes in impulse control, but that the exact pattern of behavioral changes depends on the precise period of social isolation. Thus, social isolation during a period in life when social play behavior is highly abundant causes persistent alterations in the neural circuits underlying the control of impulsive actions, in particular its dopaminergic mechanisms (Eagle and Baunez, 2010; Pattij and Vanderschuren, 2008).

Although several studies have shown altered dopaminergic function in the PFC and striatum by chronic social

isolation (Fone and Porkess, 2008), evidence about the contribution of social behavior during the specific time period when social play behavior is highly abundant is scarce. Here, we showed that the behavioral deficits induced by social isolation during PND 21–42 were accompanied by a loss of sensitivity to dopamine in the mPFC. Thus, a combination of dopamine D1 and D2 receptor agonists reduced EPSP amplitude in mPFC pyramidal neurons from SOC animals, but not from ISO rats. Dopamine is a critical modulator of the efficacy of both excitatory and inhibitory synaptic activity in the PFC (Seamans and Yang, 2004). Fibers from dopamine neurons originating in the ventral tegmental area increasingly innervate the PFC through adolescence (Kalsbeek *et al*, 1988), and the modulation of PFC circuits by dopamine D1 and D2 receptors responsible for the excitation–inhibition balance changes considerably during post-weaning development (Brenhouse *et al*, 2008; Tseng and O'Donnell, 2007). The changes in dopamine modulation of synaptic responses that we observed following early social isolation are reminiscent of the adolescent phenotype where D2 receptor stimulation has no effect on excitatory synaptic transmission, which is dependent on the maturation of D2 receptors on interneurons (Tseng and O'Donnell, 2007). As activity-dependent processes of receptor and synaptic pruning during development fine-tune neural circuitry in an input-dependent manner (Lichtman and Colman, 2000), this suggests that the lack of social contact during early post-weaning development interferes with PFC maturation (see also Leussis *et al*, 2008; Makinodan *et al*, 2012). Whether the changed sensitivity for dopamine in the mPFC in the present study is dependent on the maturation of interneurons and causally related to the observed deficits in impulse control and decision making remains to be established. As yet, there is only scarce information about the role of dopamine in the PFC in the regulation of impulsive action and decision making in the 5-CSRTT and rGT, respectively (Dalley *et al*, 2002b; Economidou *et al*, 2012). Interestingly, nucleus accumbens dopamine release has been shown to be potentiated when mPFC dopamine activity is reduced (Louilot *et al*, 1989; Mitchell and Gratton, 1992), suggesting an inverse relationship between dopamine activity in the mPFC and nucleus accumbens. Therefore, it could be that the behavioral deficits by early social isolation are due to altered dopamine activity in the nucleus accumbens as a result of disrupted PFC input into the mesoaccumbens dopamine system, as dopaminergic activity in the nucleus accumbens has been implicated in impulsive action in the 5-CSRTT (Cole and Robbins, 1989; Pattij *et al*, 2007).

Several limitations of the present study should be acknowledged. First, different strains of rats were used in the behavioral (Lister Hooded) and electrophysiological (Long Evans) experiments. Although it is reasonable to assume that the behavioral and electrophysiological changes induced by early social isolation are related (see above), future studies should pertinently address this issue, within a single strain of rats. Second, because of logistic reasons, the animals were socially isolated the day after arrival in the laboratory. Shipping stress may therefore have interfered with the effects of early social isolation. Interestingly, a recent study has shown that corticosterone treatment during PND 30–50 has long-lasting effects on impulsive behavior in the 5-CSRTT and DRT (Torregrossa *et al*, 2012), but the pattern of effects was different from that seen in the present study. This suggests that the effects of social isolation in the present study are not merely the result of stress during the juvenile and early adolescent stages, although further work is necessary to investigate this in more detail.

In conclusion, the present findings indicate that the lack of proper social experience during the juvenile and early adolescent stages of life has long-lasting effects on the function of PFC circuits underlying cognitive control. This impaired cognitive control may account for an increased vulnerability for psychiatric disorders as a result of early social insults (Cacioppo and Hawkley, 2009; Paus *et al*, 2008). Indeed, impulsivity and decision-making deficits have been associated with a variety of psychiatric disorders, including attention-deficit/hyperactivity disorder and drug addiction (Chamberlain and Sahakian, 2007; Moeller *et al*, 2001). Our results underscore that experimental approaches which investigate the link between social experiences and neurobehavioral outcomes can extend our understanding of the pathways leading to increased risk or resilience to psychiatric illness.

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DISCLOSURE

The authors declare no conflict of intertest.

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