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Isolation rearing effects on probabilistic learning and cognitive flexibility in rats

Nurith Amitai · Jared W. Young · Kerin Higa · Richard F. Sharp · Mark A. Geyer · Susan B. Powell

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Abstract Isolation rearing is a neurodevelopmental manipulation that produces neurochemical, structural, and behavioral alterations in rodents that in many ways are consistent with schizophrenia. Symptoms induced by isolation rearing that mirror clinically relevant aspects of schizophrenia, such as cognitive deficits, open up the possibility of testing putative therapeutics in isolation-reared animals prior to clinical development. We investigated what effect isolation rearing would have on cognitive flexibility, a cognitive function characteristically disrupted in schizophrenia. For this purpose, we assessed cognitive flexibility using between- and within-session probabilistic reversal-learning tasks based on clinical tests. Isolation-reared rats required more sessions, though not more task trials, to acquire criterion performance in the reversal phase of the task, and were slower to adjust their task strategy after reward contingencies were switched. Isolation-reared rats also completed fewer trials and exhibited lower levels of overall activity in the probabilistic reversal-learning task than did the socially reared rats. This finding contrasted with the elevated levels of unconditioned investigatory activity and reduced levels of locomotor habituation that isolation-reared rats displayed in the behavioral pattern monitor. Finally, isolation-reared rats also exhibited sensorimotor gating deficits, reflected by decreased prepulse inhibition of the startle response, consistent with previous studies. We concluded that isolation rearing constitutes a valuable, noninvasive manipulation for modeling schizophrenia-like cognitive deficits and assessing putative therapeutics.

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Isolation rearing is a neurodevelopmental manipulation developed to mimic psychosocial deprivation. Social isolation of rodents after weaning produces permanent neurochemical, structural, and behavioral alterations (Bianchi et al., 2006; Fone & Porkess, 2008; C. A. Jones, Brown, Auer, & Fone, 2011; King, Seeman, Marsden, & Fone, 2009; Powell, 2010; Schubert, Porkess, Dashdorj, Fone, & Auer, 2009). At least some of these effects, such as disruptions in sensorimotor gating, are developmentally specific, in that they result only when animals are isolated as juveniles; social isolation of rodents after they have reached adulthood does not produce these alterations (Cilia, Reavill, Hagan, & Jones, 2001; Wilkinson et al., 1994).

Early-life stressors have been associated with neuropsychiatric vulnerability and an increased risk of several mental illnesses, including schizophrenia (Agid et al., 1999; Lim, Chong, & Keefe, 2009). Moreover, social isolation and impaired social functioning are nonspecific symptoms of schizophrenia that have been observed to predate the onset of psychotic symptoms in both retrospective and prospective studies (Addington, Penn, Woods, Addington, & Perkins, 2008; Häfner, Löffler, Maurer, Hambrecht, & an der Heiden, 1999; Møller & Husby, 2000) and that can predict conversion to psychosis in high-risk patients (Cannon et al., 2008). Notably, several of the abnormalities produced by isolation rearing strongly resemble findings in human schizophrenia (Fone & Porkess, 2008; Geyer, Wilkinson, Humby, & Robbins, 1993; Powell, 2010). For example, prepulse inhibition (PPI) of the startle response, an operational measure of sensorimotor gating, is robustly decreased in patients with schizophrenia (Braff, Geyer, & Swerdlow, 2001; Braff, Grillon, & Geyer, 1992; Braff et al., 1978), possibly reflecting

a pervasive information-processing deficit (Braff & Geyer, 1990). Isolation rearing reliably decreases PPI in rats (Cilia, Hatcher, Reavill, & Jones, 2005; Cilia et al., 2001; Geyer et al., 1993; Varty & Geyer, 1998; Varty & Higgins, 1995; Wilkinson et al., 1994). Strikingly, these PPI deficits are absent in juvenile isolation-reared rats but emerge after puberty (Bakshi & Geyer, 1999), a developmental pattern resembling the time course of schizophrenia (Weinberger, 1987). Impairments in cognitive performance have also been observed in isolation-reared animals (Bianchi et al., 2006; Gresack et al., 2010; Hellemans, Benge, & Olmstead, 2004; C. A. Jones et al., 2011; Valzelli, 1973), matching the cognitive deficits seen in schizophrenia (Elvevåg & Goldberg, 2000; Mortimer, 1997; Nelson et al., 1990; Nuechterlein et al., 2004; Sharma & Antonova, 2003; Tyson, Laws, Flowers, Tyson, & Mortimer, 2006). Several of the behavioral alterations induced by isolation rearing have been found to be reversible with antipsychotic treatment (Bakshi, Swerdlow, Braff, & Geyer, 1998; Cilia et al., 2001; Geyer et al., 1993; Li, Wu, & Li, 2007; Varty & Higgins, 1995; Wilkinson et al., 1994), further supporting the utility of this manipulation as an inducing condition for models of schizophrenia symptoms.

Schizophrenia patients frequently exhibit cognitive inflexibility (i.e., the inability to alter behavior in reaction to changing situational demands; Goldberg, Weinberger, Berman, Pliskin, & Podd, 1987; Leeson et al., 2009; Morice, 1990; Murray et al., 2008), a characteristic deficit of executive functioning that contributes to the difficulties with problem solving encountered by many schizophrenia sufferers (Hatashita-Wong, Smith, Silverstein, Hull, & Willson, 2002; Nuechterlein et al., 2004). A distinguishing symptom of this deficit is perseveration in outdated behavioral strategies that are no longer rewarded. Cognitive flexibility can be assayed in humans and experimental animals using reversal-learning tasks (Boulougouris, Dalley, & Robbins, 2007; Fellows & Farah, 2003). These tasks require the subject first to learn a reward contingency, and then to detect that the reward contingency has been switched to its exact opposite—that is, that the response previously associated with reward is now associated with nonreward, and vice versa. Several studies have reported impairments in reversal-learning tasks in isolation-reared animals (G. H. Jones, Marsden, & Robbins, 1991; Krech, Rosenzweig, & Bennett, 1962; Li et al., 2007; Schrijver, Pallier, Brown, & Wurbel, 2004; but see Wongwitdecha & Marsden, 1996).

Animal behavioral tasks frequently use reward contingencies that predict reward or nonreward with 100 % certainty. Such all-or-nothing contingencies may not suitably reflect real-life problem-solving situations, which usually involve maximizing the probability of reward under circumstances in which a given action does not guarantee a given outcome 100 % of the time. Probabilistic learning tasks, in contrast, require the subject to learn to choose the response that has the highest probability of resulting in a reward, on the basis of probabilistic contingencies that predict a certain outcome in some, but not all, cases. The subject's performance is therefore guided by punishing and rewarding feedback that is "degraded" (i.e., misleading on a subset of trials) and may more accurately represent real-life problem-solving situations. Schizophrenia patients exhibit significant impairments in a probabilistic reversal-learning task (Waltz & Gold, 2007), suggesting that deficits in feedback processing and reinforcement learning may contribute to the cognitive inflexibility observed in this population. The probabilistic reversal-learning paradigm was selected by Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS)—a translational research initiative sponsored by the National Institute of Mental Health—to assess reinforcement learning in schizophrenia (Ragland et al., 2009). Measuring such learning across species was supported by the development of a rat probabilistic reversal-learning task sensitive to serotonergic manipulation (Bari et al., 2010).

In the present study, we examined the performance of isolation-reared rats in two versions of a probabilistic reversal-learning task based on the task described by Bari et al. (2010). Rats were trained to respond preferentially in a location that offered a high probability of a food reward. On a minority of trials, however, responding in this "target" location resulted in a punitive timeout and no reward. Likewise, on a certain percentage of trials, responding in the "nontarget" location resulted in reward delivery, even though responding in this location resulted in a timeout and no reward during the majority of trials. These target–nontarget contingencies were then switched upon attainment of a set criterion; thus, probabilistic learning and reversal learning were measured in the same task.

In order to confirm the effectiveness of our isolation-rearing protocol, we also tested these rats in an acoustic startle/PPI paradigm. Additionally, rats were tested in the behavioral pattern monitor (BPM), a test used in rats (Geyer, Russo, & Masten, 1986), mice (Risbrough et al., 2006), and humans (Perry et al., 2009; Young, Minassian, Paulus, Geyer, & Perry, 2007) to profile the amount and pattern of unconditioned exploratory activity. Isolation rearing has been reported to decrease locomotor habituation and to increase rearing behavior and exploratory holepoking in the BPM or similar locomotor activity tests (Lapiz, Mateo, Parker, & Marsden, 2000; Paulus, Bakshi, & Geyer, 1998; Powell, Swerdlow, Pitcher, & Geyer, 2002; Sahakian, Robbins, Morgan, & Iversen, 1975; Varty, Paulus, Braff, & Geyer, 2000). Such reduced habituation is consistent with findings in patients with schizophrenia (Perry et al., 2009). A generalized increase in locomotion has also been found in some (Hall, Huang, Fong, Pert, & Linnoila, 1998; G. H. Jones, Robbins, & Marsden, 1989; Lapiz et al., 2000; Powell et al., 2002; Sahakian et al., 1975), but not all (Paulus et al., 1998; Varty et al., 2000), studies, and may be strain-dependent. Increases in unconditioned locomotor activity have been widely used as a model to develop antipsychotic

treatments for schizophrenia (van den Buuse, 2010). We hypothesized that isolation-reared rats would exhibit (1) impaired reversal learning in the probabilistic task; (2) impaired PPI; and (3) reduced locomotor habituation in a novel environment, thus mimicking the cognitive and behavioral changes observed in patients with schizophrenia.

Method

Animals

A group of 12 timed-pregnant Long Evans dams were shipped to our facility at gestation Day 18. On postnatal Day 3, litters were culled to ten with an equal number of males and females kept in each litter for a total of 118 rats (one litter had eight pups). Rats were weaned at 24 days postnatal and a total of 56 male Long-Evans rats (Charles River Laboratories, Wilmington, MA) were used in this study. Of the pups, 29 were single-housed (isolation-reared rats, or *isolates*), whereas the 27 remaining pups were housed in groups of three (socially reared rats, or *socials*). One of the socials died during the experiment, and its cage mates remained housed as a pair; as a result, 26 socials were tested in the study. Behavioral testing commenced at 8 weeks postweaning. Rats were allowed to reach a body weight of at least 300 g before the initiation of food restriction, which was calibrated to keep rats at 90 % of their free-feeding weights. Water was available ad libitum at all times except during testing. Rats were housed on a 12:12-h reversed light:dark cycle (lights off at 7:00 a.m.); all behavioral testing was conducted during the animals' dark cycle. Animals were treated in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and the National Research Council's Guide for Care and Use of Laboratory Animals. All experiments were approved by the Animal Care and Use Committee of the University of California San Diego.

Study design

After 8 weeks of isolation or social rearing, rats were tested in an acoustic startle/PPI paradigm, followed by testing in the BPM. Food restriction was then initiated. After the rats' weights had stabilized at ~90 % of their free-feeding weight, they were trained to respond for a food reward in the operant testing chamber. Rats were first tested in a between-session probabilistic learning task. To further explore whether the performance differences observed in isolates in this task reflected a true learning deficit, we then tested the rats in a within-session version of the probabilistic learning task. To minimize overtraining on the probabilistic learning task and ensure a significant learning component for the within-session probabilistic learning task, rats were also trained and tested in several other reinforcement-motivated operant behavioral tasks for several months between the between-session and within-session probabilistic learning tasks. After completion of all other behavioral testing, 54 weeks postweaning, the rats were retested in the acoustic startle/PPI paradigm. See Fig. 1 for an overview of the study design.

Startle apparatus

Startle and PPI were assessed in startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA). Each chamber contained a Plexiglas cylinder (9 cm in diameter) into which the rat was placed. The jump or flinch responses of the rat were detected by a piezoelectric accelerometer mounted at the bottom of the cylinder. A loudspeaker (Radio Shack Supertweeter) located 24 cm above the cylinder provided the broadband background noise and acoustic stimuli. Each apparatus was housed within a ventilated, sound-attenuating startle chamber (39 × 38 × 58 cm). Presentations of the acoustic stimuli were controlled by the SR-LAB software and interface system, which also rectified, digitized [using a 12-bit (0-4095) digitizer], and recorded responses from the accelerometer. Calibrations were performed monthly to maintain accurate acoustic stimuli and mechanical vibration measures.

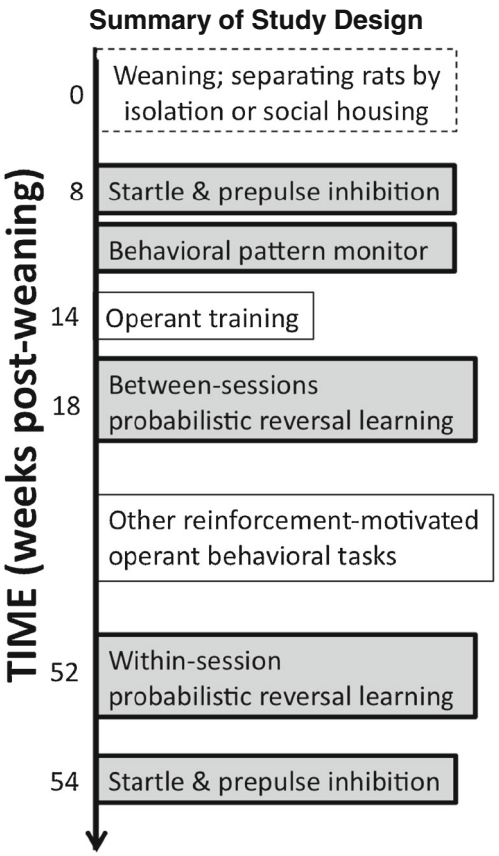

Fig. 1 Summary of the study design. Rats were initially weaned and either isolated or grouped into triads for the rest of the study. Tasks presented in this article are highlighted in gray

Startle/PPI procedures

The rats were allowed to acclimate to the testing room for 60 min before being placed in the startle chambers. The experimental session consisted of a 5-min acclimatization period to a 65-dB background noise (continuous throughout the session), followed by a 15-min PPI test session. Five trial types were presented: a 40-ms, 120-dB startle pulse (P120); three 20-ms prepulse + P120 pulse combinations with different prepulse intensities (3, 6, or 12 dB above background); and no-stimulus trials, which consisted of accelerometer recordings obtained in the absence of any stimulus and served to assess baseline motor activity. The interstimulus interval between prepulse and pulse was 100 ms. The first four trial types were presented in a pseudorandom order (12 presentations of the P120 trial, 12 presentations of each prepulse + pulse trial). The intertrial interval varied between 7 and 23 s, with an average duration of 15 s. No-stimulus trials were interspersed between each of these trials. In addition, six P120 trials were presented at both the beginning of the acoustic test session (Block 1: to assess startle reactivity before appreciable habituation) and its end (Block 2: to assess habituation across the session by comparison with Block 1). The mean startle magnitude for each trial type presentation was determined by averaging 100 one-millisecond readings taken from the onset of the P120 stimulus.

The following measures were calculated for the startle/PPI test:

- *Percentage of PPI*: Percentage decrease in startle magnitude in the prepulse + pulse trials, as compared to the P120 trials, calculated using the formula {[1 − (startle magnitude on prepulse + pulse trials/startle magnitude on P120 trials)] × 100}, such that a 0 % value indicated no difference between the responses to prepulse + pulse trials and to P120 trials (i.e., no PPI)
- *Startle reactivity*: Mean startle response to the P120 trials during the PPI test session
- *Startle habituation*: Percentage decrease in startle response to the P120 trials in Block 2 as compared to Block 1, calculated using the formula {[1 − (mean startle for Block 2 / mean startle for Block 1)] × 100}

Percentages of PPI were calculated separately for each prepulse intensity, along with the mean percentage of PPI across all prepulse intensities.

Behavioral pattern monitor (BPM) apparatus

Unconditioned exploratory locomotor activity was assessed in behavioral pattern monitors (BPM), 30.5 × 61.0-cm black Plexiglas chambers each enclosed within a ventilated sound-attenuated cabinet. Each chamber was outfitted with a 4 × 8 array of photobeams 2 cm above the floor used to detect the rat's position in an $x - y$ coordinate system with a resolution of 3.8 cm. Ten holes (2.5 cm in diameter; three in each long wall, one in the rear short wall and three along the center of the floor) containing photo beams were used to detect investigatory holepokes. Metal touchplates located along the walls 15.2 cm above the floor of the chamber were used to detect rearing. The status of all photo beams was monitored and recorded with 55-ms temporal resolution by an IBM PC-compatible computer. BPM chambers were lit by 7.5-W red lights.

BPM procedure

Rats were allowed to acclimate to the testing room for 60 min. They were then gently placed into the BPM chambers and allowed to explore the chambers for 60 min.

The following measures were calculated for the BPM test:

- *Crossings*: Number of transitions among eight equal 15.25 × 15.25 cm sectors, a measure of horizontal locomotion in the BPM
- *Holepokes*: Number of nosepokes into the holes in the BPM wall and floor
- *Rears*: Number of times that a rat reared up against the wall of the BPM

Crossings were assessed separately for the first and second 30-min periods of the session. In addition, *locomotor habituation* was assessed by calculating the percentage decrease in locomotion in the second 30-min period relative to the first, using the formula {[1 − (crossings for second period / crossings for first period)] × 100}.

Probabilistic learning apparatus

Training and testing were conducted in nine-hole operant testing chambers enclosed in ventilated sound-attenuating cabinets (Med Associates Inc., St. Albans, VT, and Lafayette Instrument Co., Lafayette, IN). Each testing chamber contained a curved rear wall with nine contiguous apertures. Metal inserts could be used to cover the apertures in the testing chamber. An infrared beam located at the entrance of each aperture detected nosepoke responses, and an LED stimulus light was located at the rear of each aperture. Liquid reinforcement, in the form of strawberry milkshake (Nesquik plus nonfat milk, 40 μl), could be delivered into a magazine located in the opposite wall via peristaltic pump; an infrared beam detected head entries into the magazine. A house light was located in the middle of the chamber ceiling. The control of stimuli and the recording of responses were managed by a SmartCtrl 8-In/16-Out Package with additional interfacing by MED-PC for Windows (Med Associates Inc., St. Albans, VT), using custom programming (written by R.F. Sharp & J.W. Young).

Operant response training

Rats were trained to retrieve the liquid reward from the magazine for two consecutive days in 10-min sessions, during which a 40-μl increment of strawberry milkshake was delivered noncontingently into the magazine every 15 s (Stage 1). Delivery of the reward was accompanied by illumination of the magazine light. Head entries into the magazine to consume the reward led to extinction of the magazine light until the delivery of the next reward.

Consequently, the rats were trained to nosepoke for the reward using a fixed ratio 1 schedule of reinforcement (Stage 2). Rats were trained in daily 30-min sessions. Metal inserts covered eight of the nine apertures in the testing chamber, leaving open only the central aperture (aperture 5). All trials were initiated by a head entry into the food magazine. An initial, noncontingent liquid reward was delivered into the magazine at the start of each session to facilitate the initiation of the first trial. Upon removal of the head from the magazine, a 4-s intertrial interval (ITI) began, after which the central aperture was illuminated. A nosepoke response into the aperture resulted in extinction of the aperture light and delivery of a reward into the food magazine.

Between-session probabilistic learning task

During the *initial learning phase*, rats were trained on the initial reward contingency using daily 30-minute sessions. Metal inserts covered seven of the nine apertures in the testing chamber, leaving open only apertures 3 and 7. Trials were initiated by a head entry into the food magazine. Upon removal of the head from the magazine, a 2-s ITI began, after which both of the uncovered apertures were illuminated. A nosepoke in the target aperture resulted in "reward" (i.e., extinction of the aperture light and delivery of a reward into the food magazine) 80 % of the time and in "punishment" (i.e., extinction of the aperture light, no reward delivery, and illumination of the house light for a 4-s timeout) 20 % of the time, following a randomized schedule. Nosepokes into the nontarget aperture resulted in reward 20 % of the time and punishment 80 % of the time. Target and nontarget locations were counterbalanced so that the target aperture was located on the right for half the animals and on the left for the other half. Nosepokes in either aperture made during the ITI, before illumination of the apertures ("premature responses"), were punished by a timeout and no reward. Continued nosepokes made into the same aperture after a rewarded response before retrieval of the reward ("reward-perseverative responses"), as well as continued responses after a punished response made into the same aperture ("punished-perseverative responses") or the other aperture ("timeout responses") were recorded, but had no programmed consequences. Animals were tested daily until they reached criterion performance—that is, until >90 % of their responses occurred in the target location on two consecutive days while performing at least 50 trials per session. This criterion performance was considered as reflective of having learned the target location.

Once a rat had reached criterion performance, it was switched to the *reversal-learning phase*. For each individual rat, this phase was started as soon as this rat had reached criterion performance, consistent with human reversal-learning tasks. Beginning with the next session after reaching criterion performance, reward contingencies were reversed, so that the previous location of the target aperture was now the nontarget aperture and vice versa. For example, if the left aperture had served as the target location in the initial learning phase and the right aperture as the nontarget location, the right aperture became the target location and the left aperture the nontarget location in the reversal-learning phase. Daily 30-min sessions continued with these reversed contingencies until the rat once again reached criterion performance (>90 % of responses into the new target location, ≥50 trials per session), which was considered indicative of successful reversal learning.

Once a rat reached criterion performance in the reversal phase, its reward contingencies were switched again on the next day. (In the above example, the target location, which had been on the left during the initial learning phase and on the right during the reversal phase, would now be again located on the left.) If the animal then reached criterion performance again under these re-reversed conditions, reward contingencies were again switched starting on the next day, and so on.

Rats were tested in the between-session probabilistic learning task for 15 days. During this time, reward contingencies were switched back and forth for each given animal as often as it managed to reach criterion performance. Unless otherwise stated, the analysis of results focuses on the animals' performance of the initial learning phase and the first reversal-learning phase that directly followed the initial learning phase. Not all rats managed to reach criterion performance in this first reversal phase, or even in the initial learning phase, during the 15 days of testing (see the Results).

Within-session probabilistic learning task

This task version was based on a similar task developed by Bari et al. (2010). Briefly, the task matched the between-session probabilistic learning task in terms of apertures uncovered, trial initiation, initial response contingencies, and parameters recorded. In contrast to the between-session task, however, sessions lasted 1 h, and criterion performance was now defined as the performance of eight consecutive nosepoke responses (rewarded or unrewarded) into the target location. As soon as this criterion was reached, the reward contingency

switched (former target location became nontarget location, and vice versa). This switch was not signaled to the rat in any way. If rats succeeded in again performing eight consecutive responses into the new target location, reward contingencies switched again, and continued to switch every time the rat reached criterion performance until the end of the session or completion of a maximal number of trials, whichever came first. The maximum trial number was set at 240 during the first session. For all subsequent sessions, it was increased to 600 to ensure that all rats performed the task for the full 1-h duration. Rats were tested in five sessions of this task on five separate days to assess improvements in reversal learning. Unlike the task employed by Bari et al. (but like the between-session task used earlier in this study), the illumination of the apertures did not end after a set time, but continued until the rat performed a nosepoke. This ensured that the focus of the task was strictly on the animal's ability to learn the target location, without any component of attention to a limited-duration stimulus.

Probabilistic learning measures

The following measures were calculated to assess task performance:

Measures assessed in the between-session task only

- *Sessions to initial acquisition*: Number of sessions required to reach criterion performance under the initial task contingencies
- *Sessions to reversal*: Number of sessions required to reach criterion performance under the reversed task contingencies.

Measures assessed in both versions of the probabilistic learning task

Overall performance

- *Number of reversals*: Number of times rat reached criterion performance and was switched to reversed task contingencies during the 15 days of the experiment (between-session task) or during the session (within-session task)
- *Trials per session*: Number of trials completed per session
- *Trials to initial acquisition*: Number of trials required to reach criterion performance under the initial task contingencies
- *Trials to first reversal*: Number of trials required to reach criterion performance after task contingencies were reversed for the first time

Strategy formation

- *Target win–stay ratio*: Proportion of target responses following a reward from responding in the target location (number of target responses after rewarded target response / number of total responses after rewarded target response)
- *Nontarget win–stay ratio*: Proportion of nontarget responses following a reward from responding in the nontarget location (number of nontarget responses after rewarded nontarget response / number of total responses after rewarded nontarget response)
- *Target lose–shift ratio*: Proportion of nontarget responses following a punished response into the target location (number of nontarget responses after punished target response / number of total responses after punished target response)
- *Nontarget lose–shift ratio*: Proportion of target responses following a punished response into the nontarget location (number of target responses after punished nontarget response / number of total responses after punished nontarget response)
- *Percentage of premature responses*: Number of premature responses per session divided by the number of trials per session.
- *Percentage of reward-perseverative responses*: Number of reward-perseverative responses per session divided by the number of trials per session.
- *Percentage of punished-perseverative responses*: Number of punished-perseverative responses per session divided by the number of trials per session.
- *Percentage of timeout responses*: Number of timeout responses per session divided by the number of timeout periods per session.

Statistical analyses

The data were analyzed using a mixed-factor two-way analysis of variance (ANOVA), with Rearing as the between-subjects factor and the following within-subjects factors, specific to the various tests:

- PPI task: Prepulse Intensity
- BPM (crossings): Time Block
- Between-session probabilistic learning task (sessions to initial acquisition vs. sessions to first reversal; trials to initial acquisition vs. trials to first reversal): Task Phase
- Between-session probabilistic learning task (win–stay ratios, lose–shift ratios): Day
- Within-session probabilistic learning task (all measures): Day

In addition, trials to initial acquisition versus trials to first reversal in the within-session probabilistic learning task were analyzed using a three-way ANOVA with the factors Rearing, Day, and Task Phase. When statistically significant effects were found in the ANOVA, post-hoc comparisons among means were conducted using Bonferroni tests. All remaining measures were analyzed using two-tailed *t* tests; Welch's correction was used when variances were significantly different among groups. The level of significance was set at .05. Statistical trends (.05 <

$p < .1$) are discussed in the Results. The data were analyzed using GraphPad Prism (GraphPad, San Diego, CA), BMDP (Statistical Solutions Inc., Saugus, MA) or SPSS Version 19 (IBM Corporation, Armonk, NY, USA).

Results

One isolate and one social were excluded from the probabilistic learning tasks because they never developed sufficient levels of operant responding. One additional isolate was excluded due to profoundly elevated levels of non-task-related nosepoking. Between the between-session and within-session probabilistic learning tasks, one isolate had to be euthanized due to suspicion of parvovirus infection. As a result, the final group sizes were 26 socials and 28 isolates (initial PPI and BPM tests), 25 socials and 27 isolates (between-session probabilistic learning task), 25 socials and 26 isolates (within-session probabilistic learning task), and 26 socials and 27 isolates (final PPI test).

PPI

The isolates exhibited consistently lower percentages of PPI than did socials (see Fig. 2), reflected by a significant main effect of rearing, irrespective of age [8 weeks postweaning, $F(1, 104) = 4.8$, $p < .05$; 54 weeks postweaning, $F(1, 102) = 4.9$, $p < .05$]. A significant main effect of prepulse intensity on percentages of PPI was also found [8 weeks postweaning, $F(2, 104) = 61.3$, $p < .0001$; 54 weeks postweaning, $F(2, 102) = 37.7$, $p < .0001$], with no Rearing × Prepulse Intensity interaction on percentages of PPI. Isolates and socials did not differ significantly in terms of absolute startle or startle habituation, although we did observe a trend toward less habituation in the isolates during the retest at 54 weeks postweaning [$t(51) = 1.8$, $p = .072$]. A similar reduction in habituation in isolates was also seen at 8 weeks postweaning, but it did not reach either significance or trend level [$t(40) = 1.7$ with Welch's correction, $p = .102$; see Table 1].

BPM

Crossings decreased in the second half of the session [$F(1, 52) = 282.5$, $p < .0001$], as would be expected given habituation to the BPM environment. No main effect of rearing on crossings was observed, but a Rearing × Time Block interaction [$F(1, 52) = 10.3$, $p < .01$; see Fig. 3a] was. Isolates exhibited less locomotor habituation in the BPM than did socials [$t(52) = 2.6$, $p < .05$; see Fig. 3b]. We found a trend toward more holepokes in isolates [$t(52) = 1.8$, $p = .073$; see Fig. 3c], and isolates performed more rears than did socials [$t(52) = 2.2$, $p < .05$; see Fig. 3d].

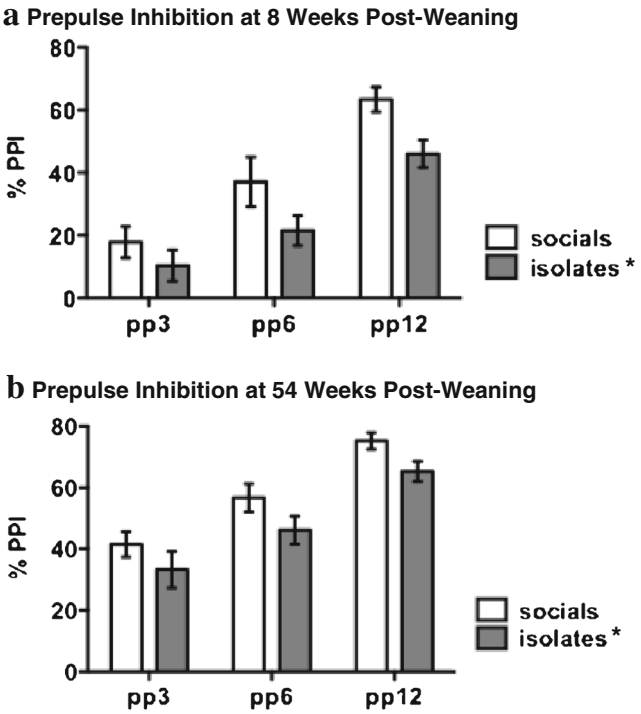

Fig. 2 Effects of isolation rearing on prepulse inhibition of the startle response. Isolation-reared rats (isolates) had decreased levels of prepulse inhibition (PPI) relative to socially reared rats (socials): PP3, PP6, and PP12 indicate prepulse intensities of 3, 6, and 12 dB over background. Values at 8 weeks (**a**) and 54 weeks (**b**) postweaning are expressed as means ± *SEM*s. Asterisks next to the group names reflect an overall main effect of rearing rather than a significant difference at any individual prepulse intensity

Between-session probabilistic learning task

Overall performance Rats required both more sessions [$F(1, 46) = 58.2$, $p < .0001$] and more trials [$F(1, 46) = 51.7$, $p < .0001$] to reach criterion performance after reward contingencies were reversed (see Fig. 4a and b). No rats reached the accuracy criterion for task performance (>90 % of responses to the target location) without also reaching the

Table 1 Effects of isolation rearing on acoustic startle

		Socially Reared	Isolation Reared
Startle	8 weeks postweaning	269.69 ± 41.58	313.71 ± 45.54
	54 weeks postweaning	364.31 ± 66.66	335.51 ± 42.85
Startle habituation	8 weeks postweaning	66.19 % ± 7.87 %	51.34 % ± 4.48 %
	54 weeks postweaning	68.13 % ± 5.58 %	53.87 % ± 5.59 %

Absolute startle and startle habituation did not differ significantly between isolates and socials. However, we did observe a trend toward less habituation in isolates than in socials at 54 weeks postweaning ($p = .072$). Values are expressed as means ± *SEM*s

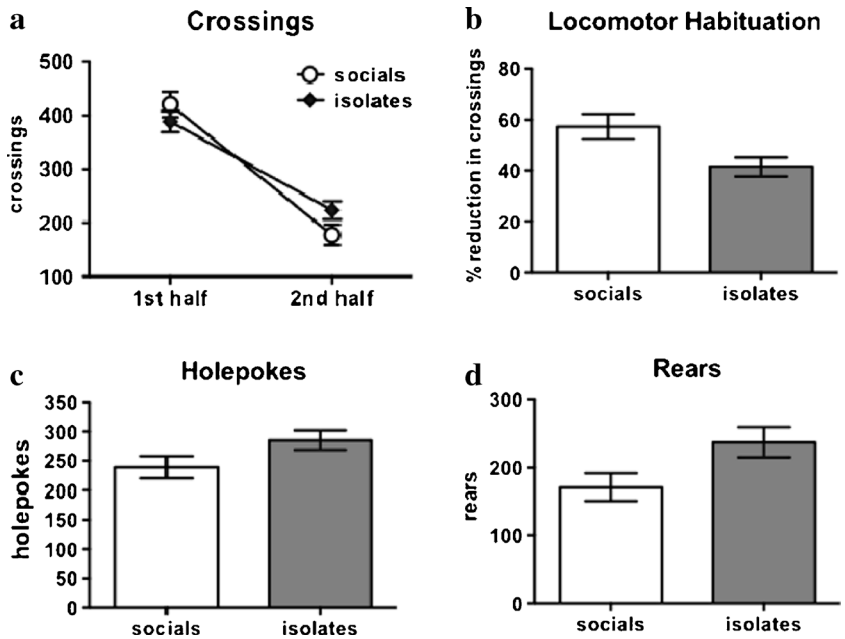

Fig. 3 Effects of isolation rearing on locomotion and exploratory behavior in the behavioral pattern monitor. Crossings (**a**) decreased from the first to the second half of the test in both isolates and socials. However, a significant Rearing × Session Period interaction reflected the fact that crossings decreased less in isolates over time than in socials. This finding was confirmed by the fact that locomotor habituation (**b**) was significantly lower in isolates than in socials. We observed a trend ($p = .073$) toward more holepokes (**c**) in isolates, and isolates also performed more rears (**d**) than socials. Values are expressed as means ± *SEM*s. *Significant differences relative to socials ($p < .05$)

trial criterion (≥50 trials per session). Failures to reach criterion were therefore driven predominantly by a lack of selectivity for the target location, not by low levels of responding.

No main effect of rearing and no Rearing × Task Phase interaction were found for trials to initial acquisition/first reversal. However, a significant main effect of rearing did emerge on the number of sessions required to reach initial acquisition/first reversal [$F(1, 46) = 5.6$, $p < .05$]. Although no Rearing × Task Phase interaction occurred on sessions to initial acquisition/first reversal, post hoc testing detected significant differences between the isolates and socials only during the reversal phase ($p < .01$), with no significant differences during the initial learning phase.

It should be noted, however, that not all rats reached criterion performance after the first reversal of reward contingencies. The comparisons of initial versus reversal acquisition therefore did not include the initial-learning-phase performance of rats that did not reach criterion during the reversal phase. In order to compare the performance of *all* rats during the initial learning phase, we also performed separate *t* tests comparing performance during either the initial learning phase or the reversal-learning phase alone. The following analyses, depicted in Fig. 5, include the rats that did not reach criterion during the reversal-learning phase.

Isolates strongly tended to take longer to attain initial criterion performance than did socials [$t(37) = 2.0$ with Welch's correction,

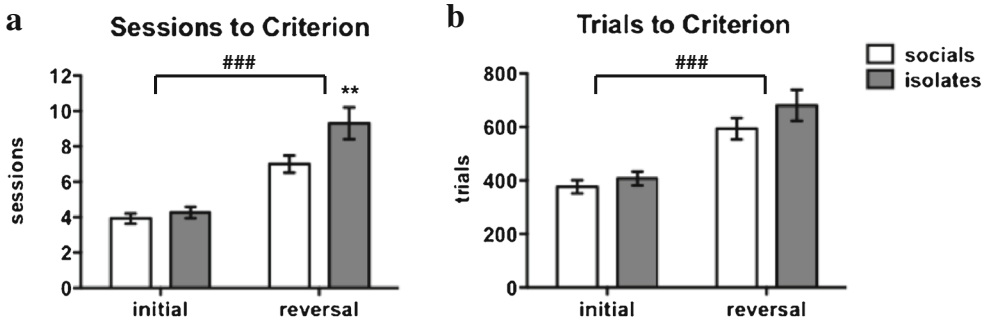

Fig. 4 Effects of task phase on performance in the between-session probabilistic learning task. Rats required more sessions (**a**) and more trials (**b**) to reach criterion performance in the reversal phase than in the initial learning phase. Rats that did not reach criterion during the reversal-learning phase were excluded from the analysis, due to ANOVA requirements. Values are expressed as means ± *SEM*s. Pound signs ($^{\#\#\#\#}p < .0001$) denote significant differences between the initial learning phase and reversal phase; asterisks ($^{**}p < .01$) denote a significant difference relative to socials. initial, initial learning phase; reversal, reversal-learning phase

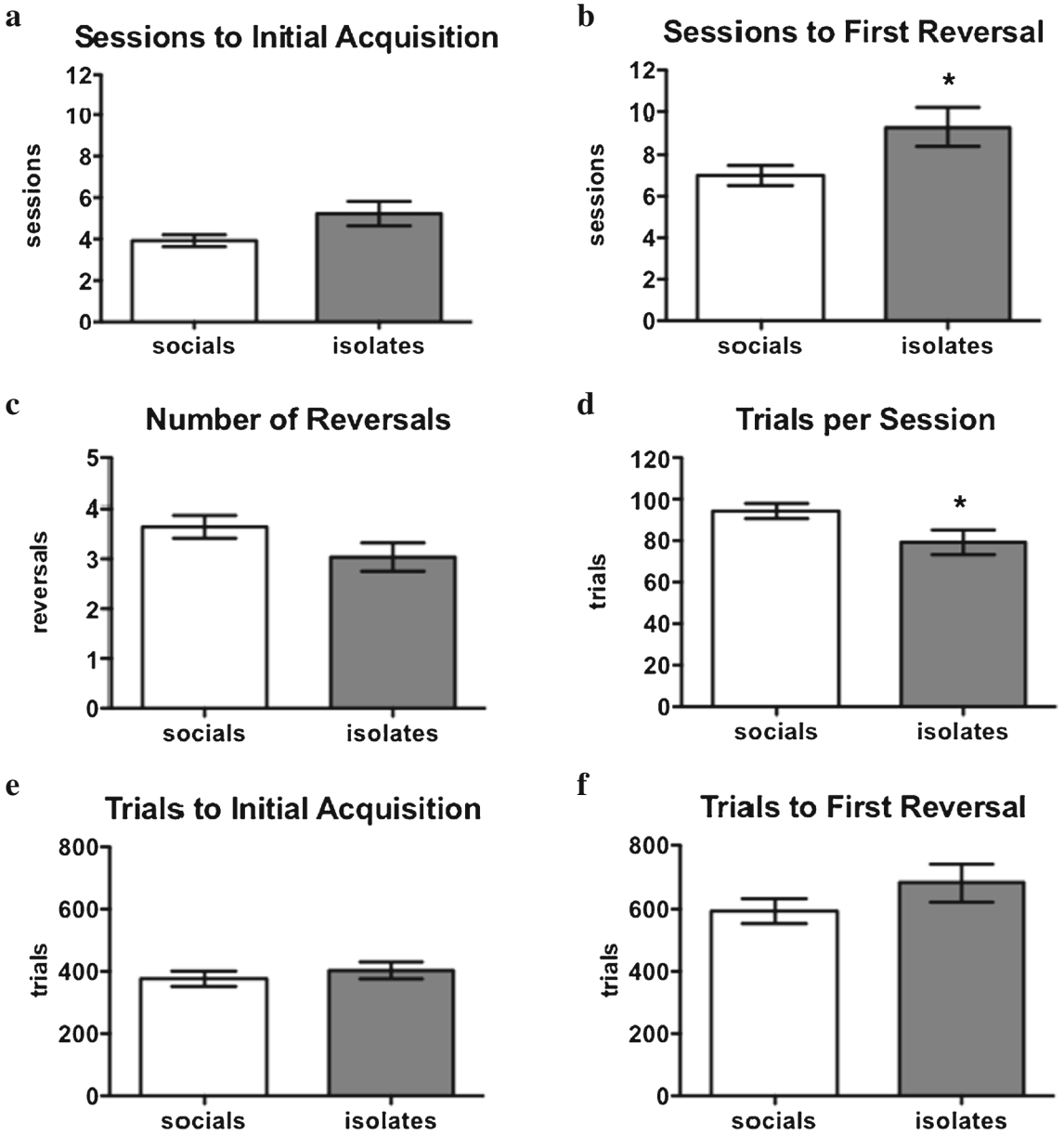

Fig. 5 Effects of isolation rearing on performance in the between-session probabilistic learning task. We observed a strong trend (p = .054) for isolates to require more sessions to reach criterion performance in the initial learning phase (**a**), and isolates required significantly more sessions to reach criterion in the reversal phase (**b**). Isolates did not differ significantly from socials in terms of reversals completed during the duration of the experiment (**c**), but they performed fewer trials per session (**d**) than did socials. No significant differences emerged regarding trials to initial acquisition (**e**) and trials to first reversal (**f**) between isolates and socials. Values are expressed as means ± *SEM*s. The depicted analyses include rats that did not reach criterion during the reversal-learning phase. *Significant differences relative to socials ($p < .05$)

p = .054; see Fig. 5a]. Isolates also required significantly more sessions to reach criterion performance under the reversed task conditions [$t(34) = 2.3$ with Welch's correction, $p < .05$; see Fig. 5b]. Indeed, four isolates never reached criterion performance under the reversed task conditions during the 15 days of the experiment, whereas all socials did. Inspection of the data also indicated that isolates on average tended to complete slightly fewer reversals during the 15 days of the experiment (see Fig. 5c), although the difference between the groups did not reach statistical significance [$t(50) = 1.6$, p = .109].

Isolates also performed significantly fewer trials per session than did socials [$t(43) = 2.2$, $p < .05$; see Fig. 5d]. Therefore, when summing trials across days, isolates did not require more trials than did socials to attain either initial learning [$t(50) = 0.7$, $p > .1$; see Fig. 5e] or reversal [$t(46) = 1.3$, $p > .1$; see Fig. 5f].

Strategy formation We analyzed win–stay and lose–shift ratios for the first three days of both the initial learning phase and the reversal-learning phase. We focused on these initial sessions because significant numbers of rats reached criterion

performance within these three sessions. During the initial learning phase, rats increased their target win–stay ratio [$F(2, 50) = 7.8$, $p < .001$] and decreased their target lose–shift ratio [$F(2, 50) = 4.1$, $p < .05$] across days, indicating that they became more likely over time to continue responding at the target location after receiving a reward from that location, and less likely to shift away from the target location after a punishment (see Fig. 6a and b). Nontarget shifting or staying did not change over days (see Table 2). Rearing did not affect shifting and staying for either the target or the nontarget location during the initial learning phase, and we found no Rearing × Day interaction.

After reward contingencies had been reversed, rats increased their tendency to continue responding at the new target location after receiving a reward from that location [$F(2, 49) = 17.8$, $p < .0001$] and decreased their tendency to shift away from this new target location after a punishment [$F(2, 48) = 11.1$, $p < .0001$] across days (see Fig. 6c and d). Additionally, over time they decreased their tendency to continue responding at the new nontarget location after receiving a reward from that location [$F(2, 49) = 22.8$, $p < .0001$] and increased their tendency to shift away from this new nontarget location after a punishment [$F(2, 49) = 28.7$, $p < .0001$] (see Table 2). Rearing interacted significantly with day on both target win–stay ratio [$F(2, 49) = 4.9$, $p < .01$] and nontarget win–stay ratio [$F(2, 49) = 6.5$, $p < .01$] during the first reversal phase. Post-hoc testing revealed that isolates exhibited lower target win–stay ratios ($p < .05$) and higher nontarget win–stay ratios ($p < .01$) than socials on Day 3 of the reversal-learning phase. In other words, when reward contingencies were reversed, isolates were less successful in increasing their tendency to continue responding in the new target location, after rewarded target responses (see Fig. 6c), and in decreasing their tendency to continue responding in the new nontarget location, after rewarded nontarget responses (see Table 2).

The groups did not differ significantly regarding their mean target latencies; although target latencies were shorter in socials, this difference did not reach either significance or trend level [$t(50) = 1.4$, $p = .159$]. We observed a trend toward longer nontarget latencies in isolates [$t(32) = 1.7$ with Welch's

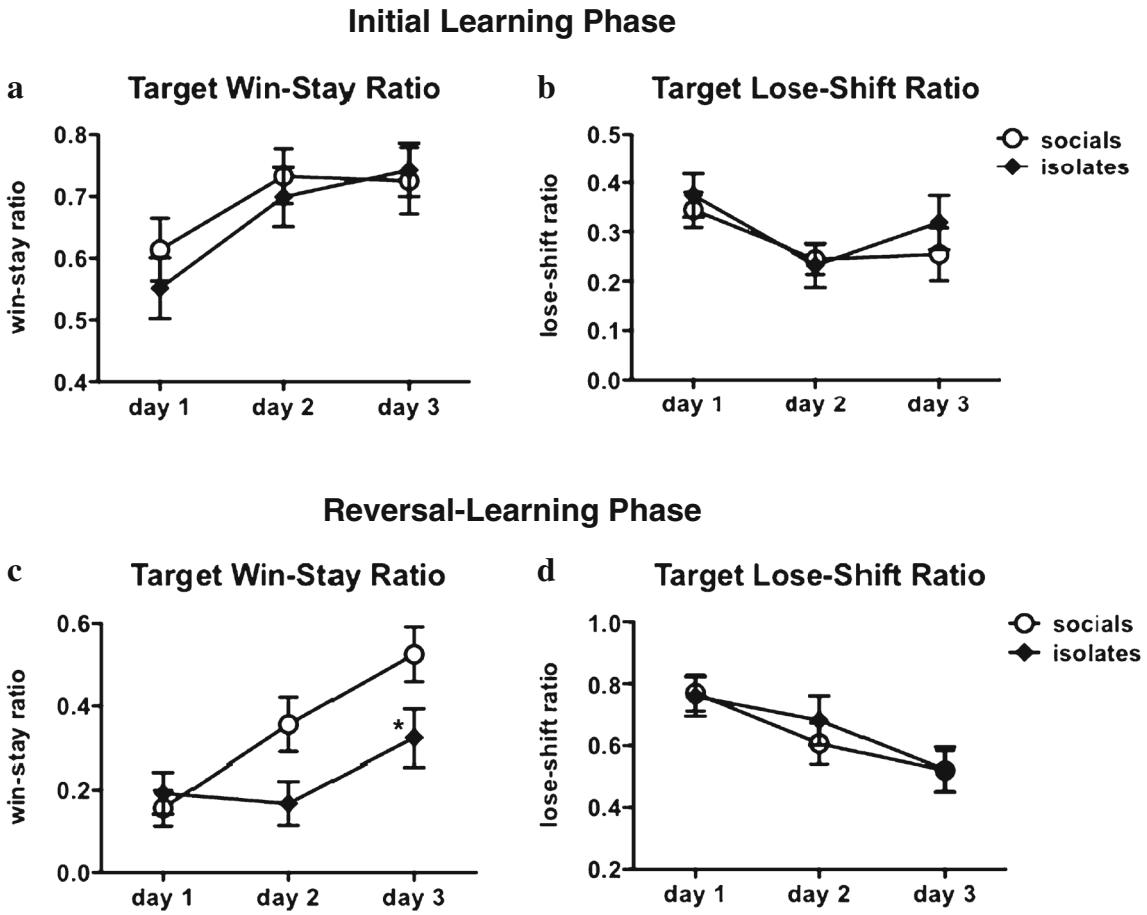

Fig. 6 Effects of isolation rearing on target win–stay and lose–shift ratios in the between-session probabilistic learning task. Rats increased their target win–stay ratios and decreased their target lose–shift ratios during the first three days of the initial learning phase (**a**, **b**), and again during the first three days of the reversal phase (**c**, **d**). Isolates exhibited less of an increase in their target win–stay ratio and less of a decrease in their nontarget win–stay ratio than did socials (see Table 2). Values are expressed as means ± *SEM*s. *Significant difference relative to socials ($p < .05$)

Table 2 Effects of isolation rearing on nontarget win–stay and lose–shift ratios in the between-session probabilistic learning task

		Socially Reared	Isolation Reared
Initial Learning Phase			
Nontarget win–stay ratio	Day 1	.33 ± .06	.33 ± .05
	Day 2	.20 ± .04	.33 ± .07
	Day 3	.31 ± .07	.27 ± .06
Nontarget lose–shift ratio	Day 1	.61 ± .04	.59 ± .04
	Day 2	.67 ± .04	.64 ± .06
	Day 3	.68 ± .05	.63 ± .04
Reversal-Learning Phase			
Nontarget win–stay ratio	Day 1	.88 ± .03	.83 ± .04
	Day 2	.68 ± .06	.77 ± .05
	Day 3	.47 ± .07	.70 ± .06**
Nontarget lose–shift ratio	Day 1	.20 ± .03	.17 ± .03
	Day 2	.28 ± .04	.21 ± .03
	Day 3	.43 ± .05	.32 ± .05

During the first three days of the initial learning phase, no significant differences were apparent across days or between isolates and socials regarding nontarget win–stay and lose–shift ratios. In contrast, during the first three days of the reversal-learning phase, rats decreased their nontarget win–stay and increased their nontarget lose–shift ratios over time, and isolates exhibited less of a decrease in their nontarget win–stay ratios than did socials. Values are expressed as means ± *SEM*s. ** Significant difference relative to socials ($p < .01$)

correction, $p = .098$]. Isolates exhibited a lower percentage of premature responses than did socials [$t(50) = 2.4$, $p < .05$], but the groups did not differ in terms of percentages of reward-perseverative responses, percentages of punished-perseverative responses, or percentages of timeout responses (see Table 3).

Table 3 Effects of isolation rearing on activity in the between-session probabilistic reversal-learning task

	Socially Reared	Isolation Reared
Target latency (s)	6.49 ± 2.14	11.18 ± 2.53
Nontarget latency (s)	7.00 ± 2.18	17.69 ± 6.00
Reward latency (s)	1.17 ± 0.06	1.43 ± 0.27
Percentage of premature responses	5.90 % ± 0.82 %	3.40 % ± 0.72 %*
Percentage of reward-perseverative responses	2.07 % ± 0.20 %	1.76 % ± 0.19 %
Percentage of punished-perseverative responses	12.11 % ± 1.06 %	11.32 % ± 1.07 %
Percentage of timeout responses	22.60 % ± 1.86 %	20.94 % ± 2.34 %

Isolates and socials did not differ significantly in terms of mean target latencies, but a trend toward longer nontarget latencies did emerge in isolates ($p = .098$). Premature responding rates were lower in isolates than in socials, but we found no differences in the percentages of reward-perseverative responses, punished-perseverative responses, or timeout responses. Values are expressed as means ± *SEM*s. * Significant difference relative to socials ($p < .05$)

Within-session probabilistic learning task

Overall performance Isolates completed fewer reversals per session [$F(1, 196) = 8.1$, $p < .01$], as well as fewer trials per session [$F(1, 196) = 8.1$, $p < .01$], than did socials (see Fig. 7a and b). Post-hoc testing showed that the difference in reversals per session reached significance on Day 3 ($p < .01$) and Day 5 ($p < .05$), though a similar pattern could be observed on the other days of the task (see Fig. 7a). For trials per session, post-hoc testing showed significant differences between isolate and socials on Days 2–5 ($p < .05$; see Fig. 7b).

The numbers of reversals per session [$F(4, 196) = 19.0$, $p < .0001$] and trials per session [$F(4, 196) = 38.6$, $p < .0001$] increased robustly over time. The large increase in trials per session after Day 1 was likely due to the change in task conditions, which increased the maximum number of trials that rats could complete per session. The main effect of day on trials per session remained, however, even when analyzing only Days 2–4 [$F(3, 147) = 6.3$, $p < .001$]. We found no Rearing × Day interaction on either reversals per session or trials per session.

Comparing across days, rats required fewer trials to reach initial learning over time [$F(1, 196) = 5.8$, $p < .001$], but the number of trials to first reversal did not change significantly. Rearing did not affect trials to initial learning or first reversal, nor did it interact with day on either measure (see Fig. 7c and d).

A three-way ANOVA comparing trials to criterion in both task phases (i.e., both the initial learning and reversal-learning phases) for both groups revealed that rats required more trials to reach criterion in the first reversal phase than in the initial learning phase of the task [$F(1, 26) = 15.8$, $p < .001$]. A three-way ANOVA also confirmed a main effect of day on trials to criterion [$F(4, 104) = 4.3$, $p < .01$], likely driven by the decrease in trials to initial learning across days.

Strategy formation Target win–stay ratios increased steadily across days [$F(4, 196) = 2.6$, $p < .05$; see Fig. 8a]. Although a main effect of day emerged on nontarget win–stay ratios [$F(4, 196) = 3.8$, $p < .01$], no clear pattern explained this finding (see Table 4). Whereas on Days 1–3 rats appeared to more efficiently disregard rewards from nontarget locations, this effect was apparently reversed on Days 4 and 5. Target and nontarget lose–shift ratios were unaffected by day (Fig. 8b, Table 4). Rearing did not affect staying or shifting during the initial learning phase, and no Rearing × Day interactions were observed. We did observe a trend toward a main effect of rearing on nontarget lose–shift ratios [$F(1, 196) = 3.0$, $p = .091$], but inspection of the data revealed no clear pattern of difference between isolates and socials on this measure (see Table 4).

During the first reversal-learning phase, isolates tended to have lower target win–stay ratios [$F(1, 184) = 3.01$, $p = .089$; see Fig. 8c]. Target lose–shift ratios decreased slightly across days [$F(4, 184) = 4.8$, $p < .01$; see Fig. 8d]. No other main effects of

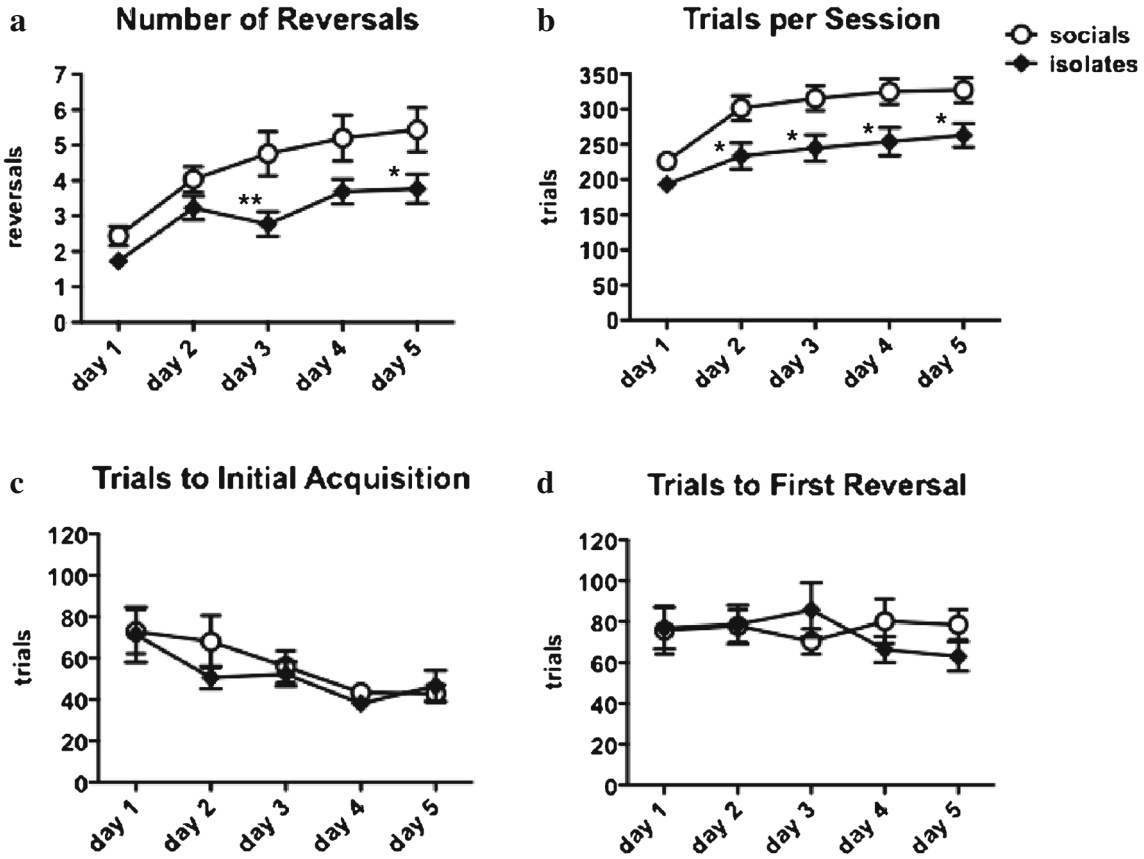

Fig. 7 Effects of isolation rearing on performance in the within-session probabilistic learning task. Isolates completed fewer reversals per session (**a**) and performed fewer trials per session (**b**) than did socials. Over time, all rats completed more reversals per session and performed more trials per session. Rearing groups did not differ on trials to initial acquisition (**c**) or to first reversal (**d**). Values are expressed as means ± *SEM*s. Asterisks denote significant differences relative to socials ($^{*}p < .05$, $^{**}p < .01$)

day and rearing on staying and shifting, and no Rearing × Day interactions, were found (see Fig. 8c and d, Table 4).

Isolates had both longer target latencies [$F(1, 196) = 4.38$, $p < .05$] and longer nontarget latencies [$F(1, 196) = 6.29$, $p < .05$] than did socials. Post-hoc testing detected a significant difference in nontarget latencies on Day 4. No main effects of day and no Rearing × Day interaction on target and nontarget latencies were seen. Reward latencies decreased over days [$F(4, 196) = 30.02$, $p < .0001$], and no main effect of rearing and no Rearing × Day interaction emerged for reward latencies (see Table 5).

Isolates exhibited lower percentages of premature responses than did socials [$F(1, 196) = 9.8$, $p < .01$]. Whereas percentages of premature responses increased over time [$F(4, 196) = 4.3$, $p < .01$], we observed a strong trend toward a Day × Rearing interaction [$F(4, 196) = 2.4$, $p = .055$]. Post-hoc testing confirmed that the levels of premature responding were lower in isolates than in socials, a difference that reached significance on Days 4 and 5 ($p < .01$). Reward-perseverative responses [$F(4, 196) = 3.4$, $p < .05$] and punished-perseverative responses [$F(4, 196) = 5.43$, $p < .001$] decreased slightly over time; no main effect of rearing and no Rearing × Day interaction were found on these measures. Finally, isolates tended to exhibit lower percentages of timeout responses than did socials [$F(1, 196) = 3.32$, $p = .074$]. No main effect of day and no Rearing × Day interaction on timeout responding were observed (see Table 5).

Discussion

Consistent with previous studies (Cilia et al., 2005; Cilia et al., 2001; Geyer et al., 1993; Varty & Geyer, 1998; Varty & Higgins, 1995; Wilkinson et al., 1994), isolates had decreased PPI as compared to social controls (see Fig. 2). These PPI deficits were detected robustly at both the beginning and the end of our study, confirming that the behavioral effects of isolation rearing persisted throughout the duration of the study. Isolates also exhibited increased investigatory behavior, as measured by increased holepokes and rears in the BPM (see Fig. 3c and d). Importantly, whereas the overall locomotor

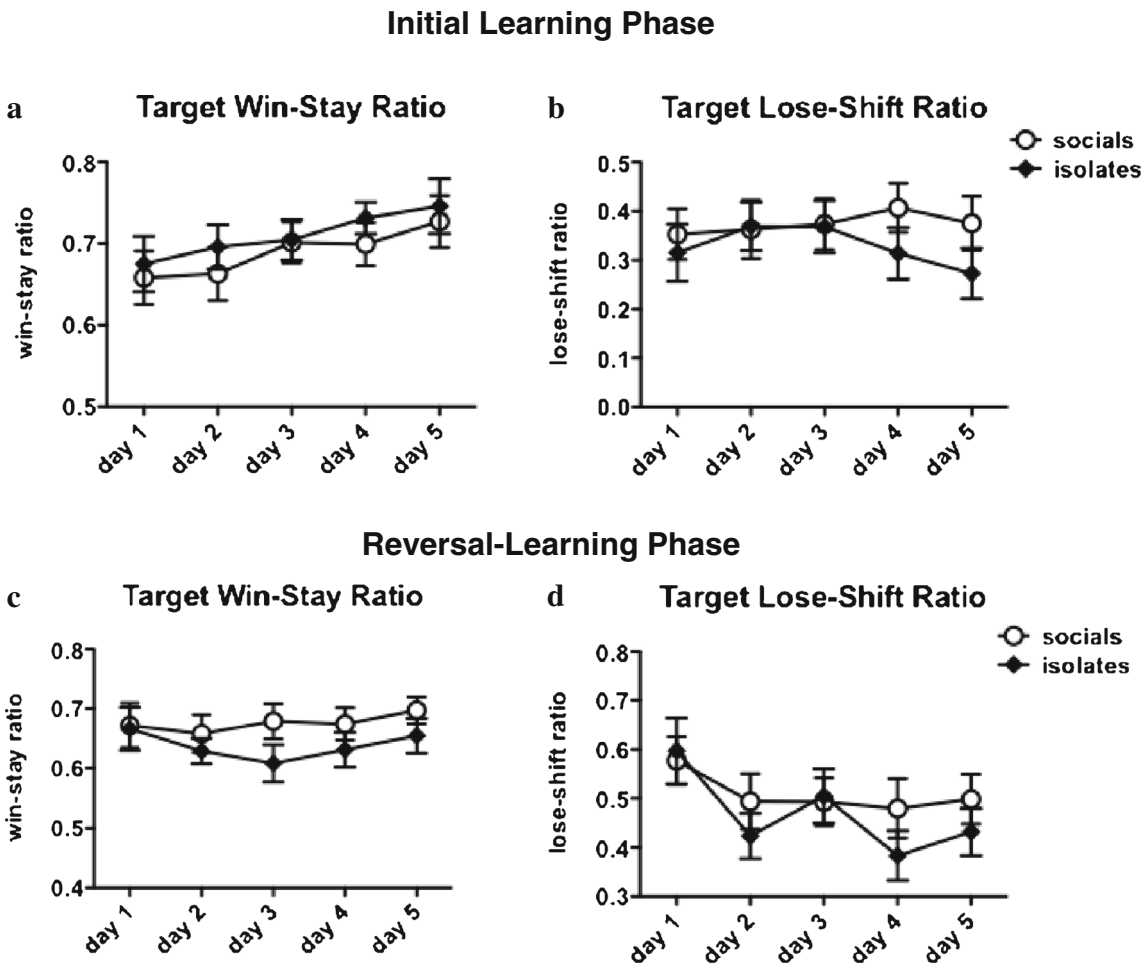

Fig. 8 Effects of isolation rearing on target win–stay and lose–shift ratios in the within-session probabilistic learning task. During the initial learning phase, rats increased their target win–stay ratios over time (**a**); no systematic changes emerged over time in target lose–shift ratios (**b**). During the reversal-learning phase, we observed a trend ($p = .089$) toward lower target win–stay ratios (**c**) in isolates than in socials. All rats decreased their target lose–shift ratios (**d**) over time during the reversal-learning phase. Values are expressed as means ± *SEM*s

activity of isolates in the BPM did not differ from that of socials, isolates exhibited less locomotor habituation (see Fig. 3a and b), consistent with findings in patients with schizophrenia (Perry et al., 2009). These BPM results were also consistent with other studies of isolates (Lapiz et al., 2000; Paulus et al., 1998; Powell et al., 2002; Sahakian et al., 1975; Varty et al., 2000).

The present data extend the behavioral phenotype of isolates to deficits in cognitive flexibility, using a cross-species-relevant test of reinforcement learning (Ragland et al., 2009). Conceptual validation of the cognitive flexibility required for this probabilistic reversal-learning task is supported by the finding that all rats required more trials to reach criterion during the reversal phase than during the initial learning phase in both versions of the task. Likewise, in the between-session version of the task, *sessions* to criterion were also higher in the reversal-learning phase (see Fig. 4). This pattern reflects the increased difficulty of task performance after the reversal of reward contingencies, because in addition to learning the new reward contingency, the animal must also inhibit the previously learned strategy and suppress the prepotent previously rewarded responses, a process that is likely to engage executive processes (Gilmour et al., in press).

In the between-session probabilistic learning task, isolates required more sessions to reach criterion performance than did socials (see Fig. 5a and b). This difference reached significance only during the reversal-learning phase of the task, possibly highlighting a selective effect of isolation rearing on reversal learning versus simple acquisition, although we also observed a strong trend toward a group difference during the initial learning phase, with no Rearing × Task Phase interaction. Isolates also exhibited a nonsignificant tendency toward completing fewer reversals over the duration of the experiment than did socials (see Fig. 5c). Finally, four isolates never reached criterion performance under the reversal-phase conditions

Table 4 Effects of isolation rearing on nontarget win–stay and lose–shift ratios in the within-session probabilistic learning task

		Socially Reared	Isolation Reared
Initial Learning Phase			
Nontarget win–stay ratio	Day 1	.42 ± .05	.32 ± .03
	Day 2	.35 ± .05	.38 ± .06
	Day 3	.24 ± .06	.18 ± .04
	Day 4	.40 ± .06	.39 ± .07
	Day 5	.41 ± .07	.36 ± .05
Nontarget lose–shift ratio	Day 1	.59 ± .04	.64 ± .03
	Day 2	.65 ± .03	.64 ± .04
	Day 3	.62 ± .04	.68 ± .03
	Day 4	.59 ± .03	.69 ± .04
	Day 5	.68 ± .04	.69 ± .04
Reversal-Learning Phase			
Nontarget win–stay ratio	Day 1	.62 ± .05	.52 ± .04
	Day 2	.60 ± .06	.65 ± .05
	Day 3	.67 ± .05	.58 ± .05
	Day 4	.48 ± .05	.56 ± .05
	Day 5	.51 ± .07	.62 ± .06
Nontarget lose–shift ratio	Day 1	.48 ± .03	.51 ± .03
	Day 2	.52 ± .04	.60 ± .04
	Day 3	.52 ± .04	.59 ± .04
	Day 4	.54 ± .03	.53 ± .03
	Day 5	.55 ± .03	.52 ± .03

During the initial learning phase, we found a significant main effect of day on nontarget win–stay ratios and a trend (p = .091) toward a main effect of rearing on nontarget lose–shift ratios, but no clear patterns explaining these findings could be identified. No other effects on nontarget win–stay and lose–shift ratios were seen during the initial learning phase. Likewise, no effects of day or rearing on nontarget win–stay and lose–shift ratios and no interactions were found during the reversal-learning phase. Values are expressed as means ± *SEM*s

Table 5 Effects of isolation rearing on activity in the within-session probabilistic reversal-learning task

		Socially Reared	Isolation Reared
Target latency[#] (s)	Day 1	2.53 ± 0.36	3.77 ± 0.55
	Day 2	2.58 ± 0.54	4.51 ± 1.18
	Day 3	2.63 ± 0.70	4.25 ± 0.71
	Day 4	2.07 ± 0.38	3.83 ± 0.77
	Day 5	2.03 ± 0.36	3.15 ± 0.38
Nontarget latency[#] (s)	Day 1	2.93 ± 0.41	5.45 ± 0.99
	Day 2	2.72 ± 0.50	5.50 ± 1.18
	Day 3	2.68 ± 0.63	4.83 ± 0.89
	Day 4	2.39 ± 0.47	5.74 ± 1.63
	Day 5	2.91 ± 0.69	4.09 ± 0.72[*]
Reward latency (s)	Day 1	1.16 ± 0.04	1.15 ± 0.05
	Day 2	0.93 ± 0.06	1.02 ± 0.05
	Day 3	0.90 ± 0.06	1.01 ± 0.07
	Day 4	0.86 ± 0.06	0.91 ± 0.04
	Day 5	0.86 ± 0.06	0.92 ± 0.05
Percentage of premature responses[#]	Day 1	0.28 % ± 0.13 %	0.02 % ± 0.02 %
	Day 2	0.25 % ± 0.10 %	0.03 % ± 0.03 %
	Day 3	0.50 % ± 0.17 %	0.07 % ± 0.05 %
	Day 4	0.97 % ± 0.33 %	0.12 % ± 0.07 %
	Day 5	0.98 % ± 0.38 %	0.15 % ± 0.06 %[**]
Percentage of reward-perseverative responses	Day 1	2.39 % ± 0.55 %	1.24 % ± 0.32 %[**]
	Day 2	1.63 % ± 0.45 %	1.79 % ± 0.40 %
	Day 3	1.56 % ± 0.35 %	1.67 % ± 0.34 %
	Day 4	1.47 % ± 0.37 %	0.97 % ± 0.22 %
	Day 5	1.11 % ± 0.19 %	1.03 % ± 0.19 %
Percentage of punished-perseverative responses	Day 1	5.12 % ± 0.81 %	5.54 % ± 0.75 %
	Day 2	3.37 % ± 0.49 %	4.02 % ± 0.64 %
	Day 3	3.71 % ± 0.52 %	4.87 % ± 0.59 %
	Day 4	4.13 % ± 0.53 %	4.72 % ± 0.58 %
	Day 5	3.59 % ± 0.48 %	4.16 % ± 0.62 %
Percentage of timeout responses	Day 1	2.86 % ± 0.55 %	1.47 % ± 0.32 %
	Day 2	1.74 % ± 0.42 %	1.28 % ± 0.38 %
	Day 3	2.75 % ± 0.46 %	1.85 % ± 0.39 %
	Day 4	2.17 % ± 0.54 %	1.43 % ± 0.32 %
	Day 5	1.94 % ± 0.57 %	1.47 % ± 0.43 %

Isolates had longer target and nontarget latencies than did socials. Reward latencies decreased across days, but did not differ between isolates and socials. Premature, reward-perseverative, and punished-perseverative responses, but not timeout responses, changed significantly over time. Isolates exhibited lower rates of premature responding and a trend (p = .074) toward lower timeout responding, as compared to socials. Values are expressed as means ± *SEM*s. Pound signs ($^{\#}p < .05$) denote a significant main effect of rearing; asterisks ($^{*}p < .05$, $^{**}p < .01$) denote significant differences relative to socials

within the 15 days of testing, whereas all socials completed at least the first reversal phase during this period.

Although the findings above support deficient learning in isolates under probabilistic learning conditions, these animals also completed significantly fewer trials per session (see Fig. 5d). No difference between isolates and socials was observed in the total numbers of trials required to reach criterion in either phase (see Fig. 5e and f). Given that isolates reached criterion performance within the same number of trials as socials, the differences in sessions to acquisition and first reversal in the between-session probabilistic learning task may not reflect a true learning deficit, but may instead be driven simply by a lower trial completion rate of isolates in the probabilistic reversal-learning task.

To clarify the role of trials per session in the effects of isolation rearing on probabilistic reversal-learning performance, we tested the same group of rats in a version of the probabilistic reversal-learning task that assessed reversal learning within a single testing session. Isolates reached criterion less often and completed significantly fewer reversals per session than did socials (see Fig. 7a). Again, however, this difference could be explained by fewer trials completed per

session by isolates (see Fig. 7b). Isolates did not differ from socials in terms of the numbers of trials to initial acquisition or reversal (see Fig. 7c and d).

To further explore the effect of isolation rearing on rule acquisition and reversal learning under probabilistic conditions, we analyzed the response strategies of our rats in the task by calculating their win–shift and lose–stay ratios after target versus nontarget responses. This analysis allowed for the assessment of whether subjects used model-free or model-based choice strategies to perform a task. Model-free strategies would entail a nonselective bias toward choosing previously rewarded locations or shifting away from punished locations. In the present task, a model-free strategy would result in a preference for responding in a previously rewarded location (i.e., making a win–stay choice) or shifting from a previously punished location (i.e., making a lose–shift choice), regardless of whether this location constituted the target or nontarget site. In contrast, model-based strategies would reflect the subject forming a model of which location represents the higher probability of reward and selectively favoring positive feedback and discounting negative feedback from this target location (Otto, Gershman, Markman, & Daw, 2013; Wunderlich, Smittenaar, & Dolan, 2012).

Over the course of the first three sessions of the initial learning phase of the between-session probabilistic learning task, rats selectively increased their win–stay and decreased their lose–shift ratios after responses in the target location only (see Fig. 6a and b), indicating the development of a model-based strategy. Moreover, in the first days after reward contingencies were switched, the rats selectively increased their target win–stay and nontarget lose–shift ratios, while decreasing their nontarget win–stay and target lose–shift ratios (see Fig. 6c and d, Table 2).

Repeated challenge with the within-session probabilistic learning task led to further selective increases in the target win–stay ratio (during the initial learning phase of each session) and decreases in the target lose–shift ratio (during the first reversal phase of each session) across days (see Fig. 8). Hence, with increased repetitions of the task, rats learned to more quickly favor positive feedback and discount negative feedback associated with an apparent target location. With increased repetitions of the task, rats also managed to complete more reversals per session—that is, to reach criterion performance more often during the time allotted (see Fig. 7a)—consistent with earlier reports (Bari et al., 2010), supporting the idea that they were acquiring a higher-order strategy optimizing their performance in the task. The development of such higher-order strategies in response to "serial reversal learning"—that is, exposure to multiple contingency reversals—has been postulated in standard (nonprobabilistic) reversal-learning tasks (Rygula, Walker, Clarke, Robbins, & Roberts, 2010; Warren, 1966).

Isolates did not differ from socials in terms of win–stay and lose–shift ratios during the initial learning phase of the between-session probabilistic reversal-learning task (see Fig. 6a and b). Once reward contingencies were switched, however, isolates were significantly slower than socials to increase their win–stay ratios after responses in the new target location (see Fig. 6c). At the same time, isolates were slower to decrease their win–stay ratios after responses in the new nontarget location (see Table 2). A similar pattern was seen in the within-session probabilistic reversal-learning task, with no differences between isolates and socials during the initial learning phase (see Fig. 8a and b), but a trend toward slower increases in target win–stay ratios during the reversal-learning phase (see Fig. 8c). In other words, isolates were less able to shift their focus toward newly significant positive feedback and discount formerly significant positive feedback that had become nonsignificant. This inability, in combination with the normal win–stay and lose–shift ratios seen in isolates during the initial acquisition phase, suggests that isolates were able to develop an intact model-based choice strategy, but unable to readily update this model to respond to changing environmental demands.

Thus, despite possible interpretative difficulties of the overall performance due to a reduced number of trials being completed, isolates did exhibit decision-making deficiencies in the probabilistic reversal-learning tasks in response to changing contingencies. Such a deficit reflects a specific impairment in cognitive flexibility, a cognitive modality that is characteristically impaired in schizophrenia (Goldberg et al., 1987; Leeson et al., 2009; Morice, 1990; Murray et al., 2008). This finding is consistent with the results seen in other reversal-learning tasks, in which isolates showed no deficit in the initial acquisition of a rotating T-maze task (Li et al., 2007), a simple visual discrimination task (G. H. Jones et al., 1991; Krech et al., 1962), or an odor/digging medium discrimination task (Schrijver et al., 2004), but exhibited impairment in the reversal-learning phase of these tasks. Selective deficits in cognitive flexibility in isolates were also detected using a related assay, the attentional set-shifting task (McLean et al., 2010; Schrijver & Wurbel, 2001). Wongwitdecha and Marsden (1996), however, reported improved reversal learning of isolates in a water maze task. Task differences such as using rewarding versus aversive motivators (see below) and/or differences in handling and housing during the isolation procedure may explain these divergent findings. Similarly, subtle differences in task requirements between the between-session and the within-session probabilistic learning tasks may explain why, in our study, the difference between isolates and socials in reversal-phase target win–stay ratios reached significance in the former, but not the latter task. A possible explanation is the more stringent performance criterion in the between-session task, which required >90 % of responses to the target location, out of at least 50 trials per session in two consecutive sessions (i.e., at least 45 accurate target responses on two consecutive days), versus only eight consecutive target responses in the within-session probabilistic learning task. Moreover, in the between-session design, 24 h

passed between consecutive assessments of criterion performance, which added a long-term memory component that was largely absent from the within-session design. In previous studies, isolates had exhibited memory deficits in the novel object recognition task that were apparent after long (3.5–24 h) but not after shorter delays (Bianchi et al., 2006; Lukasz et al., 2013; McLean et al., 2010). The memory demands of the between-session probabilistic learning tasks may therefore make this task particularly challenging for isolates, contributing to more robust deficits.

Notably, in our study, the lose–shift ratios of isolates did not differ from those of socials during the reversal phase. In other words, isolates were just as successful as socials in decreasing their lose–shift ratios after responses to the new target location (see Figs. 6d and 8d) and increasing their lose–shift ratios after responses to the new nontarget location (see Table 2) during the reversal phase. The reversal-learning deficit in isolates was therefore driven predominantly by impaired processing of positive feedback, whereas negative feedback processing appeared largely unaltered. Such a dissociation of positive and negative reward feedback learning may explain why isolates were not impaired in the aversely motivated water maze task (Wongwitdecha & Marsden, 1996) but were impaired in these reward-based tasks. Importantly, studies of reinforcement learning in schizophrenia patients have found the same pattern of deficient positive feedback processing, but normal processing of negative feedback (Waltz, Frank, Robinson, & Gold, 2007). This is particularly relevant, because social isolation also plays a role in psychiatric illnesses other than schizophrenia, such as major depression (Barnett & Gotlib, 1988; Becker & Kleinman, 1991). Likewise, probabilistic reversal learning is disrupted in a number of different psychiatric disorders, such as depression (Murphy, Michael, Robbins, & Sahakian, 2003) and bipolar disorder (Roiser et al., 2009). In these other psychiatric conditions, however, poor reinforcement learning is mediated by differing aspects, such as hypersensitivity to reward in patients with bipolar disorder (Meyer, Johnson, & Winters, 2001) or hypersensitivity to punishment in depressed subjects (Santesso et al., 2008; Taylor Tavares et al., 2008). Isolation rearing may therefore most closely mirror the deficient reinforcement learning of people with schizophrenia, rather than that seen in other disorders.

It is not clear why isolates completed fewer trials per session in the probabilistic reversal-learning tasks. Although we saw no significant difference in target and nontarget latencies between isolates and socials in the between-session version of the task, isolates tended to have longer response latencies (see Table 3). Such long latencies of isolates were significant in the within-session version of the task (see Table 5). Longer response latencies may have prevented isolates from completing as many trials during the session as socials. Moreover, although the reward latencies of isolates did not differ from those of socials in either version of the task (see Tables 3 and 5), new trials were only initiated upon removal of the head from the magazine. Thus, isolates may have taken more time to consume the reward, delaying their initiation of the next trial. In addition, isolates may have taken longer than socials to perform head entries into the magazine after the end of the timeout after a punished response. Given the lack of an effect of isolation rearing on overall locomotor activity in the BPM (see Fig. 3a) and on reward latencies (see Tables 3 and 5), the lower trial completion rate in isolates was unlikely to be attributable to locomotor impairment, sedation, or decreased motivation, a conclusion that is further corroborated by their normal break points when responding in a progressive ratio schedule.

The lower response rates of isolates in the probabilistic reversal-learning task were also observed as reduced levels of premature responding in both versions of the task (see Tables 3 and 5). In the within-session task, isolates also tended to perform fewer timeout responses (see Table 5). These decreased rates of behavior across various types of responding—target, nontarget, premature, and timeout responses—in isolates are in striking contrast to the increased levels of unconditioned locomotor activity in the BPM, which include increased holepokes and rears, along with less locomotor habituation to a novel environment (see Fig. 3). Isolation rearing may therefore produce a pattern of elevated unconditioned activity, but decreased activity in a goal-oriented task. This notion is supported by findings of increased locomotion, holepokes, and rearing in the BPM or open-field tests in isolation-reared rats (Hall et al., 1998; G. H. Jones et al., 1989; Lapiz et al., 2000; Paulus et al., 1998; Powell et al., 2002; Sahakian et al., 1975; Varty et al., 2000), along with fewer total trials and premature responses with longer choice latencies in a gambling task (Zeeb, Wong, & Winstanley, 2013) and longer response latencies in an ethanol self-administration task (McCool & Chappell, 2009) in these animals. Isolates also tended to make fewer premature responses during ITI manipulations in the five-choice serial reaction time task (Dalley, Theobald, Pereira, Li, & Robbins, 2002), although increased perseverative responses during presentation of an auditory distractor were also observed.

These contrasting effects of isolation rearing may be due to differential changes in neurotransmission in the cortical versus subcortical brain structures of isolates. Although the effects of isolation rearing on neurotransmitter levels and function remains to be fully elucidated, some studies have reported increased basal dopamine levels (Hall, 1998; Robbins, Jones, & Wilkinson, 1996), increased dopamine turnover (Blanc et al., 1980; Heidbreder et al., 2000), and/or psychostimulant-induced (Howes, Dalley, Morrison, Robbins, & Everitt, 2000; G. H. Jones, Hernandez, Kendall, Marsden, & Robbins, 1992) or footshock-induced (Fulford & Marsden, 1998) dopamine hyperresponsivity in the nucleus accumbens, which plays an essential role in exploratory locomotion (Mogenson & Nielsen, 1984; Mogenson & Wu, 1991; Svensson & Ahlenius, 1983).

On the other hand, dopamine turnover in the prefrontal cortex was found to be decreased (Hall, 1998; Heidbreder et al., 2000; Robbins et al., 1996). These findings are not surprising, since cortical and subcortical dopamine projections often show reciprocal changes in activity. Given the critical involvement of the prefrontal cortex in higher-level cognition and executive function, this pattern of neurochemical changes may explain why unconditioned exploratory activity is increased in isolates, whereas responding in cognitive tasks, such as the probabilistic reversal-learning task, is reduced. Indeed, an increase in non-goal-specific behaviors such as exploration of the testing chamber may have contributed to less efficient performance of the task (leading to, e.g., longer pauses before initiation of a new trial after the timeout following a punished response), and thus fewer trials completed per session.

In summary, isolation rearing produced robust sensorimotor gating deficits and changes in exploratory locomotor behavior. The cognitive deficits in isolation-reared rats were more subtle, and were driven by poor processing of positive feedback reinforcement learning. Additionally, isolates exhibited a general response suppression in the cognitive tasks that contrasted with the elevated levels of unconditioned exploratory locomotion seen in these animals. The isolation-rearing model recreates many of the behavioral and cognitive deficits characteristic of schizophrenia, and thus may prove suitable for testing putative therapeutics for treating dysfunction in schizophrenia.

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