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Early Adolescent Emergence of Reversal Learning Impairments in Isolation-Reared Rats

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Abstract

Cognitive impairments appear early in the progression of schizophrenia, often preceding the symptoms of psychosis. Thus, the systems subserving these functions may be more vulnerable to, and mechanistically linked with, the initial pathology. Understanding the trajectory of behavioral and anatomical abnormalities relevant to the schizophrenia prodrome and their sensitivity to interventions in relevant models will be critical to identifying early therapeutic strategies. Isolation rearing of rats is an environmental perturbation that deprives rodents of social contact from weaning through adulthood and produces behavioral and neuronal abnormalities that mirror some pathophysiology associated with schizophrenia, e.g. frontal cortex abnormalities and prepulse inhibition (PPI) of startle deficits. Previously, we showed that PPI deficits in isolation-reared rats emerge in mid-adolescence (4 weeks after weaning; approx. postnatal day 52) but are not present when tested at 2 weeks after weaning (approx. postnatal day 38). Because cognitive deficits are reported during early adolescence, are relevant to the prodrome, and are linked to functional outcome, we examined the putative time course of reversal learning deficits in isolation-reared rats. Separate groups of male Sprague Dawley rats were tested in a two-choice discrimination task at 2 and 8 weeks after weaning, on postnatal day 38 and 80, respectively. The isolation-reared rats displayed impaired reversal learning at both time points. Isolation rearing was also associated with deficits in PPI at 4 and 10 weeks after weaning. The reversal learning deficits in the isolated rats were accompanied by reductions in parvalbumin immunoreactivity, a marker for specific subpopulations of GABAergic neurons, in the hippocampus. Hence, isolation rearing of rats may offer a unique model to examine the ontogeny of behavioral and neurobiological alterations that may be relevant to preclinical models of prodromal psychosis.

Keywords

Isolation rearing; Reversal learning; Prepulse inhibition; Developmental model; GABA; Parvalbumin; Schizophrenia

Introduction

Growing evidence indicates that schizophrenia is an outcome of brain disturbances that occur during early development, including prenatal, early postnatal, and adolescent stages [1, 2]. As Insel [3] argues, a better understanding of the neurodevelopmental origins of schizophrenia and efforts at early detection and intervention (specifically targeting cognitive deficits) may be the most plausible path to developing effective treatments and improving the quality of life for patients. Cognitive deficits in schizophrenia occur early in the disease process, often precede psychosis manifestation, and are good predictors of conversion to psychosis [4–11]. Because cognitive deficits tend to manifest themselves during adolescence, the neuroanatomical systems subserving cognitive functions may be more vulnerable to, and mechanistically linked with, the initial pathology. Therefore, understanding the trajectory of behavioral and anatomical abnormalities relevant to the schizophrenia prodrome and their sensitivity to interventions in model organisms will be critical to identifying therapeutic strategies for schizophrenia early in the progression of the illness.

In animals and humans, social interaction is essential for pair bonding, parental care, and cooperation [12–14]. Failure to engage in appropriate social interactions at various stages of development can be detrimental to the organism. In humans, adolescent social isolation/withdrawal is associated with several neuropsychiatric conditions ranging from schizophrenia to anxiety and depression [15]. In schizophrenia, social withdrawal occurs early in the course of illness, prior to symptoms of psychosis, and predicts conversion to psychosis [16, 17]. Because of this observation, experimentally imposed social isolation rearing has been considered a relevant model of the symptomatically imposed social isolation in schizophrenia because both result in the loss of neurostimulatory social interaction, which has secondary biological consequences [18]. Social isolation of rodents from weaning produces several behavioral and neurochemical traits that relate to schizophrenia-like disorders [18, 19]. Isolation rearing-induced deficits in prepulse inhibition (PPI) begin during puberty and reach full expression only in the adult rat, a time course that appears to model the development of schizophrenia-specific symptoms in early adulthood in humans (e.g. psychosis [20]). A critical question is what biomarkers or pathologies *precede* these PPI deficits and thus are the most likely targets for therapeutic interventions to prevent the development of behavioral abnormalities in this developmental model. Determining the time course of cognitive deficits in isolation-reared rats will help identify specific brain regions involved in the initial effects of social isolation and determine therapeutic interventions that may thwart the effects of social isolation.

Reversal learning is thought to be a suitable model of measuring cognitive flexibility in schizophrenia and other neuropsychiatric disorders [21, 22]. The effects of isolation rearing on reversal learning are not consistent, with some studies showing deficits in isolates [23, 24] and others showing no difference [25, 26], or even improvements, in isolates [27]. These differences may lie in the type of reversal task used, which can be either spontaneous, as used in these studies and consistent with human tests [28], or cued, with no change in isolates seen in the latter. Abnormalities of GABAergic interneurons have been reported in the frontal cortex and hippocampus of patients with schizophrenia [29] and in isolation-

reared rats [30, 31]. The extent to which these alterations in GABA interneuron function affect reversal learning in isolation-reared rats remains to be determined. In the present study, we examined the developmental trajectory of reversal learning deficits produced by social isolation and measured the integrity of parvalbumin (PV)-positive GABA interneurons in the cortex and hippocampus. Rats were tested in a two-choice discrimination learning task after either 2 or 8 weeks of social isolation, corresponding to postnatal day 38 (d38; birthdate = day 0) and d80, respectively. We chose the d38 time point because our hypothesis was that cognitive deficits may occur early in the course of isolation rearing, prior to the emergence of PPI deficits [20], during adolescence. In contrast, we also wanted to assess the cognitive effects of a longer period of isolation (8 weeks) in which we consistently observed PPI deficits [20, 32, 33] at an adult age (d80) where rats are sexually mature [34]. Rats were subsequently tested in an acoustic startle/PPI paradigm and evaluated for levels of PV+ interneurons in the hippocampus and cortex.

Materials and Methods

Animals

Twelve timed-pregnant Sprague Dawley dams (Harlan, San Diego, Calif., USA) were shipped to our facility on gestation day 18. On postnatal d3, the litters were culled to 8–10 pups with an equal number of males and females kept in each litter. At weaning (d24), rats from each litter were assigned to either social (n = 3 rats/cage) or isolation housing (n = 1 rat/cage) and housed in standard polycarbonate cages (25 × 48 × 20 cm) in a temperature- and humidity-controlled room. The rats were housed on a 12 h:12 h reversed light-dark cycle (lights off at 7:00 a.m.). Both socials (n = 27) and isolates (n = 28) were housed in the same room with minimal handling except for cage cleaning once every week, so that all animals had visual, olfactory, and auditory exposure to other rats but no physical interaction with them. Only male rats (n = 55) were used in these studies. Female rats were used for pharmacological experiments of PPI not reported here. Approximately 4 days prior to the reversal learning testing, the male rats were food restricted in order to attain an 85% free-feeding weight with water available ad libitum. On the 4th day of food restriction, approximately twenty 1/4 Honey Nut Cheerio® pieces were placed in the cage to familiarize the rats with the food reward used in the reversal learning test. At the end of the reversal learning testing, the rats were put back on free feeding for the remainder of the experiment. All experiments were conducted during the animals' dark cycle, and the animals were acclimatized to the behavioral suite at least 60 min prior to testing. The experiments were carried out in accordance with the University of California San Diego's Institutional Animal Care and Use Committee (IACUC) and the guidelines of the National Institute of Health. The animal facilities were approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Two-Choice Discrimination Learning Test

Digging-based, two-choice discriminative learning tasks have been established in rodents in which different odors, digging media, and bowl textures are used to assess reversal learning and set-shifting [35, 36]. The current test was conducted similarly to the attentional set-shifting tasks described in rats through the first reversal stage of the task [35]. The test

chamber was a modified polycarbonate home cage ($25 \times 48 \times 20$ cm) with plastic panels to separate half of the cage into 2 equal sections. Digging bowls (ceramic circular pots 4.5×2.5 cm) were placed in each quarter section, with access to these sections limited by removable dividers. The rats were given access to the digging bowls by lifting up the dividers; access was denied by closing the dividers after the rat had made a selection (fig. 1).

The rats in each housing condition were subsequently assigned to be tested in the two-choice discrimination learning task at either 2 or 8 weeks after weaning, which corresponded to d38 and d80, respectively, with litters equally distributed across the 4 groups. Because of the time-restricted window of behavioral testing and the labor-intensive nature of the two-choice discrimination task, only a subset of rats was tested (d38 group: social = 11, isolate = 9; d80 group: social = 9, isolate = 11); however, all rats were food restricted. One day prior to the testing, animals were habituated to the testing room and chamber and trained to dig in unscented bedding for a food reward (1/4 Honey Nut Cheerio). Odors were derived from commercially available spices. On day 2, the rats were tested in the reversal learning procedure. Every trial was initiated by raising the dividers and allowing the rat to access the digging bowls. The rats were permitted to dig in both bowls for the first 4 trials, only 1 of which was baited, so if 1 bowl was investigated in error, the rat could move to the 2nd bowl. Trials were continued until the rat reached the criterion of 6 consecutive correct trials. Throughout the test session, the rats performed a series of discriminations where the choice was made depending upon a stimulus in a particular dimension, e.g. odor or digging medium. In the simple discrimination (SD), only 1 relevant dimension (either odor or digging medium) was used. Once the rats reached a criterion of 6 consecutive correct responses, a compound discrimination (CD) occurred in which the 2nd dimension, e.g. the digging medium, was introduced, but the relevant stimulus (odor) in the SD would still identify the correct bowl. The CD tests the distractibility of the rodent to novel stimuli [37]. Reversal learning (compound discrimination reversal; CDR) was then assessed by simply reversing the correct stimulus to the previously unrewarded stimulus within the same modality/dimension (e.g. from nutmeg to ginger). The correct and incorrect trials and their latencies were recorded for each stage of the task. The rats were counterbalanced so that the initial SD would either be odor or digging medium. Stimuli combinations and locations were selected in a varied order.

Acoustic Startle and PPI Testing

The acoustic startle testing was conducted using SR-LAB startle chambers (San Diego Instruments, San Diego, Calif., USA). Each chamber consisted of a clear Plexiglas cylinder (8.8×19.5 cm) mounted on a solid Perspex base situated inside the ventilated sound-attenuating and well-lit (15 W) chamber ($39 \times 38 \times 58$ cm). A high-frequency loudspeaker inside the chamber produced a background noise of 65 dB, acoustic stimuli of 120 dB, and 68-, 71- and 77-dB prepulse stimuli. The animals were presented with 5 different trial types: pulse-alone trials comprised of a 40-ms 120-dB pulse; prepulse + pulse trials in which the 120 dB pulse was preceded (100 ms) by 20-ms subthreshold 68-, 71- or 77-dB noises; and a no-stimulus trial that included only the 65-dB background noise. All trial types were presented in a pseudorandom order for a total of 120 trials (12 pulse-alone trials, 12 of each prepulse + pulse trials, and 60 hidden no-stimulus trials). Six pulse-alone trials were

presented at the beginning of the session, and another 6 pulse-alone trials were presented at the end of the session and used to assess startle habituation. The mean startle magnitude for each trial type presentation, the dependent measure, was determined by averaging one hundred 1-ms readings taken from the onset of the startle P120 stimulus. The amount of PPI was calculated as a percentage score for each prepulse + pulse trial type: $\%PPI = 100 - [([startle\ response\ for\ prepulse + pulse\ trial] / [startle\ response\ for\ pulse-alone\ trial]) \times 100]$. The rats tested in discrimination learning at 2 weeks after weaning were tested 4 and 10 weeks after weaning (d52 and d94, respectively) for startle and PPI. A total of 3 rats were removed from the startle/PPI analysis; 2 were removed (1 isolate, 1 social) due to the average PPI being <2.5 SD from the mean, and 1 isolate was removed due to startle being >2.5 SD from the mean (social = 11, isolate = 15). The rats tested in discrimination learning at 8 weeks after weaning were tested for startle and PPI 10 weeks after weaning (d94; social = 15, isolate = 11).

Immunohistochemistry

At approximately 9 months of age, rats ($n = 5$ /housing condition) were deeply anesthetized using sodium pentobarbital. Once the anesthesia was deep enough that the foot pinch reflex was absent, the rats were perfused intracardially through the ascending aorta at 10 ml/min with approximately 50 ml of ice-cold saline followed by 200 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brains were then removed and postfixed in 4% paraformaldehyde in 0.1 M PBS at 4°C. After 48 h, the brains were transferred to 2% paraformaldehyde in 0.1 M PBS and stored at 4°C. Brain slices (50 μ m) obtained between bregma 2.0 and 1.3 mm for the prelimbic region and bregma -2.9 to -4.0 for the dorsal hippocampus were analyzed for PV expression using nickel-enhanced DAB staining (Vectastain, Vector). The brain slices were obtained consecutively, but 1 in every 4 slices was used for staining, resulting in 4 slices covering the prelimbic and orbitofrontal regions and 6 slices covering the hippocampus. The primary antibody used was a rabbit polyclonal (Swant) at a 1:5,000 dilution in PBS with the addition of 2% normal goat serum and 0.1% Triton X-100. The slices were incubated in primary antibody solution for 24 h. For the total counts, the images were analyzed as described [38]. Briefly, for the prelimbic region, a box of $800 \times 400 \mu$ m (on a 10 $\times$ microscope objective on a Nikon Diaphot) was positioned over the prelimbic region, and all positive cells within this box were counted. The dorsal hippocampal region was divided into 3 segments corresponding to CA1, CA2/3, and dentate gyrus. All positive cells in the pyramidal layers in CA1 and CA3, as well as in the dentate gyrus, were counted across 6 slices. The sum of cells across all slices in each region was calculated using the Abercrombie correction as described [39].

Statistical Analyses

All analyses were conducted using the SPSS version 21.0.

Two-Choice Discrimination Learning Task—To investigate the changes in outcome (trials to criterion) across stage, a linear mixed effects (LME) model was implemented using the SPSS Mixed procedure with a random intercept by subject. This procedure was chosen due to its ability to handle missing data (not all animals completed all the testing stages, see below) and to model time-varying covariates [40]. Housing and age were entered as fixed

effects, and stage was entered as a repeated fixed effect. All interactions were also modeled. The mean correct latency was entered into the model as a time-varying covariate to control for the speed-accuracy trade-off that can occur in rodent discrimination tasks [41, 42]. A variance components covariance structure was modeled for the random intercept, and a diagonal covariance structure was found to best fit the repeated measure. An initial evaluation of the stimulus dimension (e.g. odor vs. digging medium) revealed that the stimulus dimension did not affect the performance or interact with housing; thus, the starting dimension was not included in the final model. Post-hoc analyses were conducted on the estimated marginal means with Bonferroni correction.

PPI and Startle Data—The PPI data were initially analyzed with 2- or 3-factor analysis of variance (ANOVA) with housing and age as between-subjects factors and trial type (prepulse intensity) as a repeated measure (within-subjects) factor. The PPI data were then collapsed across prepulse intensity, and the average PPI was used as the main dependent measure because no significant interactions between housing and prepulse intensity were evident. The startle magnitude data were analyzed with 1- or 2-factor (housing and/or age) ANOVA.

PV Cell Counts—The mean numbers of PV+ cells for each brain region were compared using Student's t test. The α level for all analyses was set at 0.05. For post-hoc analyses, a Bonferroni correction was used, where $\alpha = 0.05/\text{number of comparisons}$.

Results

Two-Choice Discrimination Learning Task

The validity for the two-choice learning task arises from evidence of a greater difficulty for rats in terms of more trials to criterion during the CDR versus the SD stage [$F(2, 41) = 29.7$, $p < 0.001$]. When both stages were examined together, adult rats (d80, 8 weeks after weaning) performed slightly better than adolescent rats tested at 2 weeks after weaning (d38), the main effect of age being $F(1, 50) = 5.66$, with $p < 0.025$. Importantly, the isolation-reared rats showed deficits in reversal learning (CDR stage) at both d38 and d80 but not during the SD stage and only marginally during the CD stage, as evidenced by a housing $\times$ stage interaction [$F(2, 41) = 7.94$, $p < 0.001$] (fig. 2) and a main effect of housing [$F(1, 49) = 6.24$, $p < 0.025$]. A follow-up post-hoc analysis comparing the effects of housing at each stage of the task confirmed that the isolates required increased trials to criterion compared to the socially housed rats in the CDR stage ($p < 0.01$) and a marginal increase in the CD stage ($p < 0.05$). Two out of the 20 isolated rats failed to progress past the SD stage; however, the proportion of rats completing the task did not differ between housing conditions [completing task: socials = 20/20; isolates = 18/20; $\chi^2(1, n = 40) = 2.1$, not significant].

PPI and Startle Magnitude

The rats tested in the reversal learning task at 2 weeks after weaning (d38) were tested in the PPI/startle paradigm 4 and 10 weeks after weaning, corresponding to d52 and d94 (fig. 3). Overall, the isolation-reared rats showed a decreased PPI at both d52 and d94 compared

with socially reared rats [housing: $F(1, 24) = 4.92$, $p < 0.05$] with no week $\times$ housing interaction ($F < 1$; fig. 3a). There was, however, a main effect of week [$F(1, 24) = 21.44$, $p < 0.001$] indicating a higher PPI at 10 weeks after weaning. The isolation-reared rats tested in discrimination learning at d38 (2-week postweaning group) also had a higher startle magnitude [housing: $F(1, 24) = 6.25$, $p < 0.05$] which did not interact with week (fig. 3e). The rats in the d80 discrimination group (8 weeks after weaning) were also tested for startle and PPI at 10 weeks after weaning (d94) and showed a decreased PPI compared to socially reared rats [housing: $F(1, 24) = 9.23$, $p < 0.01$] (fig. 3b) and a trend toward an increased startle magnitude [housing: $F(1, 24) = 2.08$, $p = 0.16$] (fig. 3f). To assess the impact of the startle magnitude on PPI in the group tested in the d38 discrimination group, a linear mixed-effects model was used to compare the housing effects of PPI, while covarying the startle magnitude by week. When the startle magnitude was used as a covariate, the main effect of housing on the PPI remained [$F(1, 24) = 4.85$, $p < 0.05$]. A similar analysis covarying for the startle magnitude was done in the d80 discrimination group, and the housing effect on PPI remained significant [$F(1, 23) = 8.87$, $p < 0.01$]. Additionally, in both the d38 and d80 discrimination groups when rats were ‘matched’ for the startle magnitude (i.e. extreme values removed), the isolation rearing-induced PPI deficits remained.

PV Immunohistochemistry

The immunohistochemical analysis of PV+ cells in the brains of the social and the isolate rats revealed a decreased number of PV+ cells in the CA1 [$t(8) = -2.74$, $p < 0.05$] and CA2/3 [$t(8) = -2.59$, $p < 0.05$] hippocampal regions in isolation-reared compared to socially reared rats, but no differences in the dentate gyrus, prelimbic cortex, or orbitofrontal cortex (both medial and ventral orbitofrontal cortex regions; fig. 4).

Discussion

The aims of the present study were to (1) evaluate the effects of isolation rearing on reversal learning in rats; (2) determine when reversal learning deficits emerged in isolated rats, and (3) further assess PV+ interneuron function in the isolation-rearing model. The experiments showed that isolation rearing disrupted reversal learning in a two-choice discrimination task and that these effects were present as early as d38, 2 weeks after weaning. These data indicate an adolescent emergence of isolation-induced cognitive deficits in this model. Reversal learning deficits were also present at d80, 8 weeks after weaning. In addition to deficient reversal learning, impairments in PPI and decreased PV-expressing neurons in the CA1 and CA2/3 of the hippocampus were observed in the isolation-reared compared to the socially housed rats.

In reversal learning tasks, subjects are required to suppress a previously learned response and acquire a new behavioral strategy [43]. In the current study, the rats readily learned to discriminate bowls on the basis of odor and digging medium. At the reversal learning stage, however, all rats took more trials to reach criterion compared to the previous 2 stages, with the isolation-reared rats showing a significant impairment at this stage. In the first experiment, the rats tested at 8 weeks after weaning (~ 80 days old; adults) performed better on the task overall compared to rats tested 2 weeks after weaning (~ 38 days old;

adolescents), suggesting an improved performance with age/adulthood. The increased difficulty performing the task in the d38 group, particularly at the reversal stage, is consistent with previous studies showing that adolescent rats have more difficulty learning both reversals and extradimensional shifts in the Attentional Set Shifting Task (ASST) relative to adult rats [44]. Thus, the current data corroborate previous studies indicating a deficient cognitive flexibility during the adolescent stage.

The deficits in reversal learning shown in the current studies corroborate previous studies showing reversal learning deficits in isolation-reared rats using a very similar digging-based two-choice discrimination task [24]. Several other studies have shown that isolation rearing impaired reversal learning in different behavioral tasks such as spatial learning in the Morris water maze [23, 45] and two-choice discrimination learning similar to the one used in the current studies [24]. Conversely, several other studies have failed to show isolation rearing-induced reversal learning deficits or have shown increases. These discrepancies may be based on the type of reversal learning task and the sex of the rats. For example, isolation-reared female rats showed no reversal learning deficits in an operant task relying on cued associations [25] and in digging-based two-choice discrimination tasks [26]. In contrast to studies reporting isolation rearing-induced reversal learning deficits after longer periods of isolation, well into adulthood [23, 24], the current data indicate that these deficits emerge early in the course of isolation (d38) and remain consistent during prolonged periods of isolation (d80). These findings mirror observations in patients with schizophrenia wherein cognitive deficits are observed during adolescence in subjects with a high risk of schizophrenia prior to an initial psychotic episode [4, 10]. In contrast, studies assessing the age of emergence of PPI deficits in isolation-reared rats showed that the PPI deficits were not present at 2 weeks after weaning but were apparent at 4 weeks after weaning [20]. Similarly, in the current study, the isolation-reared rats showed PPI deficits at 4 weeks after weaning (d52) and remained decreased after 10 weeks of isolation (d94). Thus, disruptions in reversal learning in isolated rats appear to emerge prior to deficits in PPI. Additionally, the PPI deficits in the isolation-reared rats tested in the d38 discrimination learning task were associated with elevations in startle magnitude, particularly at week 10 of isolation (fig. 3 e), indicating that startle increases in isolation-reared rats became more pronounced with repeated testing. The PPI deficits in isolation-reared rats tested in the 8-week discrimination learning task were accompanied by moderate but nonsignificant increases in startle magnitude (fig. 3 f). This pattern of startle and PPI changes suggests that the PPI deficits in isolation-reared rats are not a confound of increased startle because isolation rearing-induced PPI deficits remained when the groups were ‘matched’ for the startle magnitude and when the startle magnitude was used as a covariate in the PPI analysis.

GABAergic interneurons are dysfunctional in a number of brain regions in schizophrenia [46, 47], including the frontal cortex [48, 49] and hippocampus [50]. Similar decreases are observed in several neurodevelopmental models of schizophrenia [51]. The isolation-reared rats displayed decreased PV+ cell counts in the CA1 and CA2/3 regions of the hippocampus (fig. 4) consistent with what we have reported previously [30]. These findings demonstrate selective abnormalities of subpopulations of GABAergic interneurons in the hippocampus of the isolation-reared rats, which resemble the neuronal deficits seen in this region in schizophrenia. We do not know, however, whether the reduction in PV appears prior to or

following the emergence of reversal learning and PPI deficits. Thus, we cannot conclude the specific role of PV loss in the adolescent emergence of behavioral abnormalities in the isolation-rearing model. Previous studies have also reported decreases in PV+ immunoreactive cells in the frontal cortex of isolation-reared rats [31]. In the current set of studies, however, no differences in PV immunoreactivity were observed in the prefrontal cortex (PFC) or OFC (fig. 4). There are several differences between our studies and the study by Schiavone et al. [31], including the strain of rat (Sprague Dawley vs. Wistar, respectively) and the age/length of isolation at the time of euthanasia across the two studies (9 months vs. 7 weeks, respectively). Additionally, the trend for a decrease in PV immunoreactivity in the PrL mPFC and the OFC (fig. 4) might have reached statistical significance if the sample size was increased.

Disruptions in inhibitory signaling in the hippocampus may contribute to the reversal learning and PPI deficits observed in isolation-reared rats. The CA1-subicular-PFC projection relays spatial information from the hippocampus to the PFC and is modulated by local GABA inhibitory neurons in CA1 [52–54]. Thus, disruption of this circuit due to deficits in inhibitory interneurons in CA1 in isolated rats may contribute to a disinhibition of hippocampal-PFC projections and deficits in reversal learning. Rats with hippocampal lesions show impaired place reversals in the water maze [55], and hippocampal long-term depression is required for spatial reversal learning in the water maze [56]. Additionally, patients with bilateral hippocampal damage due to hypoxia were not impaired in the initial acquisition of stimulus-response learning, but were impaired in reversals [57]. We have shown previously that deficits in PV+ interneurons in the hippocampus are correlated with PPI deficits in isolation-reared rats [30], suggesting that the loss of PV in hippocampus contributes to the behavioral deficits in isolates. The small sample size for PV immunohistochemistry in the current study precludes any correlational analyses between PV loss and behavior.

The performance of rodents in spontaneous reversal learning tasks depends on the functioning of the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), striatum, and other forebrain structures [58–60]. In particular, lesion studies have demonstrated the importance of the frontal cortex, particularly OFC, in reversal learning [61–63]. For example, OFC lesions disrupted reversal learning in the CDR stage of the attentional set-shifting task [64], similarly to what is observed here in the isolation-reared rats. In the current studies, however, no differences in PV+ cells were observed in the OFC. However, this lack of PV+ effect does not preclude the role of the OFC in mediating the effects of isolation rearing on impaired reversal learning. Previous studies have reported decreases in [³H] dopamine uptake [65] and decreased dendritic complexity [66] in the OFC of isolated rats, each of which may underlie the reversal learning deficits reported here.

One goal of these studies is to develop a neurodevelopmental model that demonstrates a progression of behavioral abnormalities that may aid in our understanding of the early prodromal stages of psychotic illness. In studies examining neuroanatomical changes in prodromal schizophrenia, young high-risk individuals show smaller volumes in the hippocampal-amygdala complex and thalamus [67–70] and alterations in dorsolateral PFC activation during working memory tasks when compared to controls [71, 72]. These

neuroimaging studies have also been shown to increase the prediction of conversion to psychosis [73]. Similarly, cognitive deficits in schizophrenia occur early in the disease process, often precede psychosis manifestation, and are good predictors of conversion to psychosis [4–10]. Our findings indicate that isolation rearing follows a similar trajectory with cognitive deficits preceding deficits in behaviors predictive of psychosis in humans (e.g. PPI) and thus provide a model to test hypotheses regarding the progression of neuropathology and early interventions in the prodromal stage of illness.

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Fig. 1. Schematic of the two-choice discrimination task apparatus and example of the testing stages and possible stimulus combinations.

Fig. 2.

Isolation rearing disrupted reversal learning in the two-choice discrimination test (a) and showed impaired reversal learning at both d38 (social = 11, isolate = 9) and d80 (social = 9, isolate = 11) (b). * $p < 0.05$, ** $p < 0.01$, versus the respective social group.

Fig. 3.

a Isolation-reared rats tested in the 2-week discrimination learning task (d38) exhibit PPI deficits at 4 and 10 weeks after weaning, d52 and d94, respectively (social = 11, isolate = 15). **b** Isolation-reared rats tested in the 8-week discrimination task (d80) exhibit PPI deficits at 10 weeks after weaning (d94) (social = 15, isolate = 11). PPI deficits in isolation-reared rats were apparent across prepulse intensities in both the 2-week (**c**) and the 8-week (**d**) discrimination learning groups. Startle was significantly increased in the isolation-reared rats from the 2-week group (**e**), whereas the rats from the 8-week group tested for the first time in startle/PPI at 10 weeks showed only moderate, nonsignificant increases in the startle magnitude (**f**). Data are means $\pm$ SEM. * $p < 0.05$ versus the respective social group.

Fig. 4.

a PV immunohistochemistry in social and isolation-reared rats in the CA1 and CA2/3 hippocampus, dentate gyrus (DG), prelimbic (PrL) mPFC, medial orbitofrontal (MO) cortex, and ventral orbitofrontal (VO) cortex. Data are presented as mean cell density (cells/mm³) $\pm$ SEM. * $p < 0.05$ versus the social group. **b** Schema defining areas of the frontal cortex for cell counting taken from [74]. Image at Bregma 5.16 mm [from 74]. aci = Anterior commissure, intralobular; AOD = anterior olfactory nu, dorsal; AOL = anterior olfactory nu, lateral; AOM = anterior olfactory nu, medial; AOVP = anterior olfactory nu, anteropost; DLO = dorsolateral orbital cortex; E/OV = ependymal and sube layer OV; GrO = granular cell layer olf bulb; LO = lateral orbital cortex; lo = lateral olfactory tract; M2 = secondary motor cortex; MO = medial orbital cortex; PrL = prelimbic cortex; rf = rhinal fissure; ri = rhinal incisure; VO = ventral orbital cortex. The definitions of the hippocampal areas for cell counting were conducted according to our previously published methods [38].