Chronic administration of clozapine alleviates reversal-learning impairment in isolation-reared rats

Nanxin Li[a], Xihong Wu[a] and Liang Li[a,b]

Isolation rearing has been used for inducing schizophrenia-like symptoms in rats. Human schizophrenics have deficits in prefrontal-dysfunction-related cognitive/behavioral flexibility. Rats with lesions of the medial prefrontal cortex perform poorly in reversal learning. It is uncertain whether isolation rearing, however, causes reversal-learning impairment in adult rats. Using the rotating T maze, this study examined the effect of chronic administration of clozapine on visual discrimination learning and reversal learning in isolation-reared and socially reared adult rats. The results show that isolation-reared rats without clozapine injection performed significantly worse than socially reared rats in reversal learning but not in acquisition learning. Chronic injection of clozapine (5 or 10 mg/kg) in isolation-reared rats significantly improved reversal learning but had no effects on acquisition learning. Further data analyses show that in both the inhibition phase and the new-strategy-acquisition phase of reversal learning, isolation-reared rats needed significantly more correct-response trials to reach the criterion than socially reared rats, and clozapine significantly reduced the isolation-induced impairment of reversal learning only in the new-strategy-acquisition phase. In socially reared rats, clozapine had a dose-related interfering effect on reversal learning but not acquisition learning. This study supports the use of isolation rearing as a model for investigating the neurodevelopmental hypothesis of schizophrenia. ***Behavioural Pharmacology*** **18:135–145 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Increasing evidence has shown that schizophrenia is associated with certain disturbances that occur during individuals' early development (O'Connor and Rutter, 1996; Weinberger, 1996; Ellenbroek and Cools, 1998; Marenco and Weinberger, 2000; McGrath *et al.*, 2003; Tochigi *et al.*, 2004; Bembenek, 2005; Flagstad *et al.*, 2005; Rehn and Rees, 2005). The prefrontal cortex, which is typically implicated in schizophrenia, reaches its anatomical and functional maturity only in early adulthood. On the basis of the postulation by Weinberger (1987), if early neurological injuries in the prefrontal cortex occur before prefrontal maturity, the effects of the injuries may remain silent until the prefrontal cortex matures. This neurodevelopmental hypothesis of schizophrenia emphasizes that certain early-life environmental factors can have substantial influences upon processes of brain maturation. No single 'ideal' animal model can be expected to represent abnormalities in all human schizophrenia-relevant behaviors. With the advent of more new models, a single model, however, is likely to represent a subpopulation of schizophrenia or even a particular aspect or endophenotype of schizophrenia (Powell and Miyakawa, 2006). Thus, establishment of appropriate neurodevelopmental models in laboratory animals is critical for investigating the mechanisms underlying schizophrenia-related symptoms induced by early disturbances (Heidbreder *et al.*, 2000; Weiss and Feldon, 2001; Powell *et al.*, 2002, 2003; van den Buuse *et al.*, 2003; Powell and Miyakawa, 2006; Sullivan *et al.*, 2006). In rats, following isolation rearing, both substantial changes in central neurotransmission (Jones *et al.*, 1991, 1992; Whitaker-Azmitia *et al.*, 2000; Heidbreder *et al.*, 2001; Dalley *et al.*, 2002; Muchimapura *et al.*, 2003; Barr *et al.*, 2004; Harte *et al.*, 2004; Preece *et al.*, 2004; Meyera *et al.*, 2005) and remarkable behavioral abnormalities (Jones *et al.*, 1991; Geyer *et al.*, 1993; Wilkinson *et al.*, 1994; Reboucas and Schimdek, 1997; Paulus *et al.*, 1998; Varty and Geyer, 1998; Lapiz *et al.*, 2000; Varty *et al.*, 2000; Schrijver and Wurbel, 2001; Weiss *et al.*, 2001; Arakawa, 2005) can be observed.

Schizophrenia patients usually suffer from cognitive/behavioral perseveration (failure to switch from previously learned solution to a new solution), which is routinely estimated by the Wisconsin Card Sorting Test (WCST) (e.g. Milner, 1963; Kolb and Whishaw, 1983; Lanser *et al.*, 2002; Ritter *et al.*, 2004; Tanaka *et al.*, 2006), reversal learning, prompted discourse, and the generation of guessing sequences (for a review see Crider, 1997). For example, in the WCST, which is typically used to examine the prefrontal dysfunction (Janowsky *et al.*, 1989; Rogers

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et al., 2000; Demakis, 2003; Nagahama *et al.*, 2005), patients with schizophrenia continue to sort cards according to the same rule despite negative feedback. Bender *et al.* (2006) have recently reported that after 4–26 weeks of chronic clozapine (an atypical antipsychotic) treatment, performances in the WCST (measured by correct hits, perseverative errors, and total errors) improved significantly in the 54 patients with schizophrenia who were tested.

In animals, reversal learning, which requires animals to both inhibit the previously reinforced behavioral strategy and acquire the previously declined behavioral strategy, is often used for testing cognitive/behavioral flexibility (Sperling, 1965; Mackintosh and Holgate, 1969; Jones *et al.*, 1991; Hartmann and Gunturkun, 1998; Li and Shao, 1998; Abdul-Monim *et al.*, 2003, 2006; Russig *et al.*, 2003; van der Meulen *et al.*, 2003; Clarke *et al.*, 2004, 2005; Idris *et al.*, 2005). In rats, successful reversal-learning performance largely depends on the ventral medial prefrontal cortex (mPFC) and the orbital prefrontal cortex (oPFC). Damage to the infralimbic area or the prelimbic area of rats' ventral mPFC (which is possibly homologous to the dorsolateral prefrontal cortex in primates) impairs reversal learning (spatial or nonspatial) but not acquisition learning (Li and Shao, 1998; Chudasama and Robbins, 2003; Salazara *et al.*, 2004). Damage to the oPFC also impairs reversal learning but not acquisition learning (Schoenbaum *et al.*, 2002; Chudasama and Robbins, 2003; McAlonan and Brown, 2003). Although mPFC-lesion rats and oPFC-lesion rats commit more errors in reversal than controls, oPFC-lesion rats commit more errors in suppressing the previously rewarded stimulus–reward association and mPFC-lesion rats commit more errors in learning the new stimulus–reward association (Chudasama and Robbins, 2003). Injection of phencyclidine (PCP), D-amphetamine, or MK801, which affects prefrontal functions, or depletion of serotonin in the mPFC, significantly impairs rats' reversal-learning performance (Jentsch and Taylor, 2001; Abdul-Monim *et al.*, 2003, 2006; van der Meulen *et al.*, 2003; Idris *et al.*, 2005; Robbins, 2005). PCP-induced impairment of reversal learning can be significantly attenuated by injection of atypical antipsychotics, such as clozapine, ziprasidone, or olanzapine (Abdul-Monim *et al.*, 2003, 2006; Idris *et al.*, 2005).

In the study by Joel *et al.* (1997), one task consisted of both a delayed nonmatch-to-sample training and a reversal from the nonmatch-to-sample training to the match-to-sample training. The results show that rats with mPFC lesions did not exhibit working memory impairment but were slower in reversal from the nonmatch-to-sample performance to the match-to-sample performance. Joel *et al.* (1997) suggested that the performance impairments in mPFC-lesion rats are due to a difficulty in changing behavioral strategy, therefore the reversal component of the task is an analog of the WCST.

It has not been clear, however, whether isolation rearing impairs, has no effect on, or even facilitates reversal learning in adult rats (Krech *et al.*, 1962; Jones *et al.*, 1991; Wongwitdecha and Marsden, 1996; Schrijver and Würbel, 2001; Abdul-Monim *et al.*, 2003; Schrijver *et al.*, 2004). For example, some studies reported that isolation rearing impaired reversal learning but did not affect the original learning of visual discrimination (Krech *et al.*, 1962; Jones *et al.*, 1991; Schrijver *et al.*, 2004). In the studies by Schrijver and Würbel (2001) and Abdul-Monim *et al.* (2003), however, neither acquisition learning nor reversal learning was affected by isolation rearing. Moreover, in the study by Wongwitdecha and Marsden (1996), both acquisition learning and reversal learning in a Morris water maze were improved in isolation-reared rats compared with socially reared controls. Thus, it is very necessary to carefully reevaluate the effects of isolation rearing on reversal learning using more precisely controlled testing paradigms. As in rats isolation rearing alters dopamine activity in the mPFC (Jones *et al.*, 1991, 1992) and lesions of the mPFC during either the neonatal age or the adulthood impair reversal learning (Li and Shao, 1998; Chudasama and Robbins, 2003; Salazara *et al.*, 2004; Schwabe *et al.*, 2004), this study used a behavioral procedure that can efficiently reveal the impairing effect of local lesions of the subregions of the ventral mPFC on reversal learning (Li and Shao, 1998).

As an efficient atypical antipsychotic drug, clozapine has been widely used to alleviate both schizophrenics' cognitive symptoms (Meltzer, 1989; Deutch *et al.*, 1991; Mcelroy *et al.*, 1991; Pickar *et al.*, 1992; Robertson and Fibiger, 1992; Meltzer and McGurk, 1999; Sharma *et al.*, 2003; Galletly *et al.*, 2005) and rats' schizophrenia-like behavioral impairments (Swerdlow and Geyer, 1993; Varty and Higgins, 1995; Hitchcock *et al.*, 1997; Cilia *et al.*, 2001, 2005; Bardgett *et al.*, 2006; Levin and Christopher, 2006; Ortega-Alvaro *et al.*, 2006). The affinities of clozapine at various receptors play an important role in ameliorating schizophrenia-related neurochemical abnormalities and cognitive/behavioral deficiencies (Sebban *et al.*, 2002; Robbins, 2005). It is not clear, however, whether isolation-rearing-induced impairments of reversal learning (Krech *et al.*, 1962; Jones *et al.*, 1991; Schrijver *et al.*, 2004) can be ameliorated following treatment with clozapine. According to the study by Abdul-Monim *et al.* (2003), PCP impaired reversal learning in both isolation-reared and socially reared rats, but the atypical antipsychotic ziprasidone (2.5 mg/kg, intraperitoneal route) significantly reversed the PCP-induced impairment only in socially reared rats but not in isolation-reared rats.

The aim of this study was to examine the effects of chronic administration of clozapine on original visual discrimination learning (acquisition learning) and reversal learning in adult rats with or without isolation-rearing

experience. Both acquisition learning and reversal learning were tested in a rotating T maze (Li and Shao, 1998).

Methods

Participants

Forty-eight male Sprague-Dawley rats aged 21 days (the age of weaning) were purchased from the Beijing Vital River Experimental Animals Technology Ltd. (Beijing, China). They were randomly assigned into two main groups: the isolation-reared group (24 rats) and the socially reared group (24 rats). Each of the two main groups was further divided randomly into three subgroups injected with different doses of clozapine (see below).

For isolation-reared rats, each individual was housed in a single transparent plastic cage ($48 \times 30 \times 18$ cm). For socially reared rats, three individuals were housed in a cage. As both isolated and socially reared rats were kept in the same room, isolated rats were able to hear, smell and see other rats. All rats had free access to food (Beijing Vital River Experimental Animals Technology Ltd., Beijing, China) and water. They were maintained under the condition of a constant temperature of 24°C (± 2°C), humidity of 40–50%, and a 12 h light/dark cycle (lights on 07.00 h). Six weeks after weaning (2 weeks before testing), rats were gradually food deprived to approximately 85% the free-feeding body weight. The reduced body weight was kept by restricting the amount of food, and it reached the range between 240 and 260 g at the time of testing.

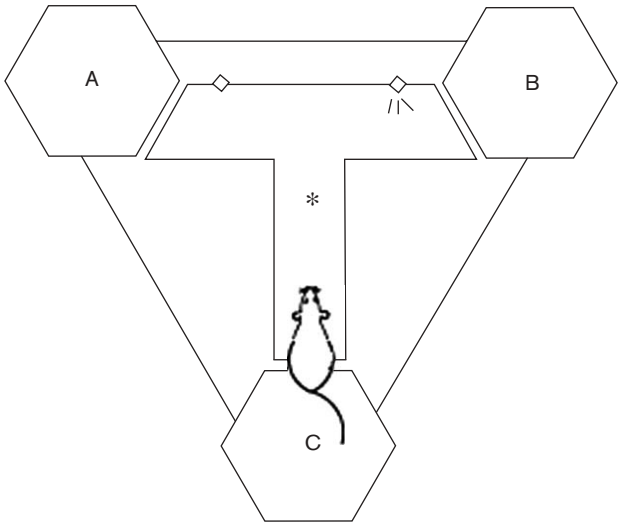
All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experiments were carried out according to the guidelines of the Beijing Laboratory Animal Center, the guidelines of the Canadian Council of Animal Care, and the Policies on the Use of Animals and Humans in Neuroscience Research revised and approved by the Society for Neuroscience in January 1995.

Rotating T maze

Figure 1 shows the diagram of the rotating T maze used in this study. The rotating T maze has also been described in detail elsewhere (Li and Shao, 1998). Briefly, the maze had three hexagonal boxes and one T tunnel. The maze floor was made of stainless-steel wire meshes. Uncolored transparent Plexiglas lids were on the three hexagonal boxes and the T tunnel. The three hexagonal boxes were made of dark Plexiglas and were referred to as box A, box B, and box C, respectively. The edge length of each hexagonal box was 9.0 cm. Each box had a door that could be opened to the T tunnel.

The T tunnel was also made of dark Plexiglas. The length of each arm was 13.5 cm at its longest edge. The stem had the width of 7.3 cm and the length of 22.0 cm. As the T tunnel could be rotated around its axis (whose position is shown as an asterisk in Fig. 1), the entrance to the T-tunnel stem or either of the two exits from the T-tunnel arms could connect to any hexagonal box of the three. On the frontal wall (25.5 cm in length) of the T tunnel, there were two holes (1 cm in diameter, 8.5 cm apart from each other). A light emitting diode (LED) was installed inside each of the holes, providing visual cues for food reward.

Fig. 1



Overhead schematic view of the rotating T maze used for visual discrimination tests. The maze has (1) three hexagonal boxes (box A, box B, and box C), and (2) a T tunnel. In this figure, box C represents the start box, and the entrance of the T tunnel is connected to box C. A light spot is on the right side of the front wall of the T tunnel. The position of the axis of the T tunnel is indicated by the asterisk.

Visual discrimination learning and reversal learning

In the eighth week after weaning (8 weeks of social isolation for isolation-reared rats), all rats started their training in a dark room in which the only light source was the LED(s) in the T maze. All tests were conducted during the light phase.

During the first 3 days of training, each rat was put into the maze for 30 s in a single day, when all the doors in the maze were open, the two LEDs were on, and food pellets were placed in each of the three boxes.

On the fourth day, rats were trained to respond for food by learning the correct strategies. In each trial, the light stimulus was presented only on one side of the two arms of the T tunnel. For half of the rats, the box on the light side was the target box that was baited, and for the other half of rats, the box on the side without light was the target box. After the door of the start box was opened, the rat stepped out of the box and the door of the box was

then closed to prevent the rat from turning back. If the rat stepped into the target box (a 'correct response'), it was rewarded with a food pellet (50 mg, Beijing Vital River Experimental Animals Technology Ltd., Beijing, China). If the rat entered the box opposite to the target box (an 'error response'), it received no food. The door of the terminal box containing the rat was then closed, and the T tunnel was rotated with the entrance connecting to this box, which then became the start box in the next trial. Thirty seconds later, a new trial started. Thus, a noncorrection-trial testing procedure was used.

A total of 12 trials were carried out per day for each rat. This discrimination training lasted approximately 2 weeks until rats reached the acquisition-learning criterion, that is over 11 correct trials out of 12 trials for two consecutive days.

When a rat reached the criterion of acquisition learning, it entered the stage of reversal learning on the next day. In reversal learning, the procedure and the criterion remained the same as those in acquisition learning, except for that the stimulus–reward contingency was reversed, that is previous 'light–food' ('dark–no food') association became 'dark–food' ('light–no food') association, or vice versa.

According to the previous studies (e.g. Mackintosh and Holgate, 1969; Jones *et al.*, 1991; Ragozzino *et al.*, 2002; Chudasama and Robbins, 2003), to reach the criterion of reversal learning, rats must first inhibit the previously rewarded strategy and then acquire the new strategy. In this study, it was defined that if a rat reached more than six correct trials (over 50% correct) on each of three successive days, the previously learned behavioral strategies (obtained during acquisition learning) was inhibited. Thus, the rat's performance in reversal learning was divided into two phases: (i) the inhibition phase and (ii) the new-strategy-acquisition phase.

Clozapine administration

Clozapine (Sigma-Aldrich Corporate, St Louis, USA) was dissolved in acetic acid saline solution, whose pH was adjusted to neutral with 10 mol/l NaOH. Chronic administration of clozapine was applied during the whole course of testing for each rat (6–8 weeks, depending on performance). On the basis of the clozapine doses used in previous studies (e.g. Swerdlow and Geyer, 1993; Idris *et al.*, 2005; Abdul-Monim *et al.*, 2006), in this study the dose for an individual rat was 0, 5 or 10 mg/kg (with the constant injection volume of 0.1 ml). Thus, isolation-reared rats and socially reared rats were further grouped according to the dose of clozapine. For a rat, the injection was administered once a day via the intraperitoneal route 30 min before putting the rat into the maze.

Statistics

A complete 2 (rearing type: socially rearing, isolation rearing) by 2 (visual cue pattern across acquisition learning and reversal learning: light/dark, dark/light) by 3 (clozapine dose: 0, 5, 10 mg/kg) between-groups design was used in this study. Group mean differences were tested using three-way analyses of variance (ANOVAs). The analyses were performed using SPSS 11.5 software (Chicago, Illinois, USA). Significant level was set at 0.05. As Scheffé's test is used to make unplanned comparisons among the means and is one of the most stringent post-hoc tests, in this study Scheffé's test was used for further localizing the sources of significant mean differences.

Results

Original visual discrimination learning (acquisition learning)

All the rats used in this study reached the criterion of visual discrimination learning, even though there were fluctuations of the total number of testing sessions to the criterion across individual rats. Figure 2 shows the numbers of correct responses to the criterion (panel a) and the numbers of errors to the criterion (panel b).

For both the number of correct-response trials and the number of error-response trials to the criterion, ANOVAs showed that none of the main effects or interactions among the three factors was significant ($P > 0.05$).

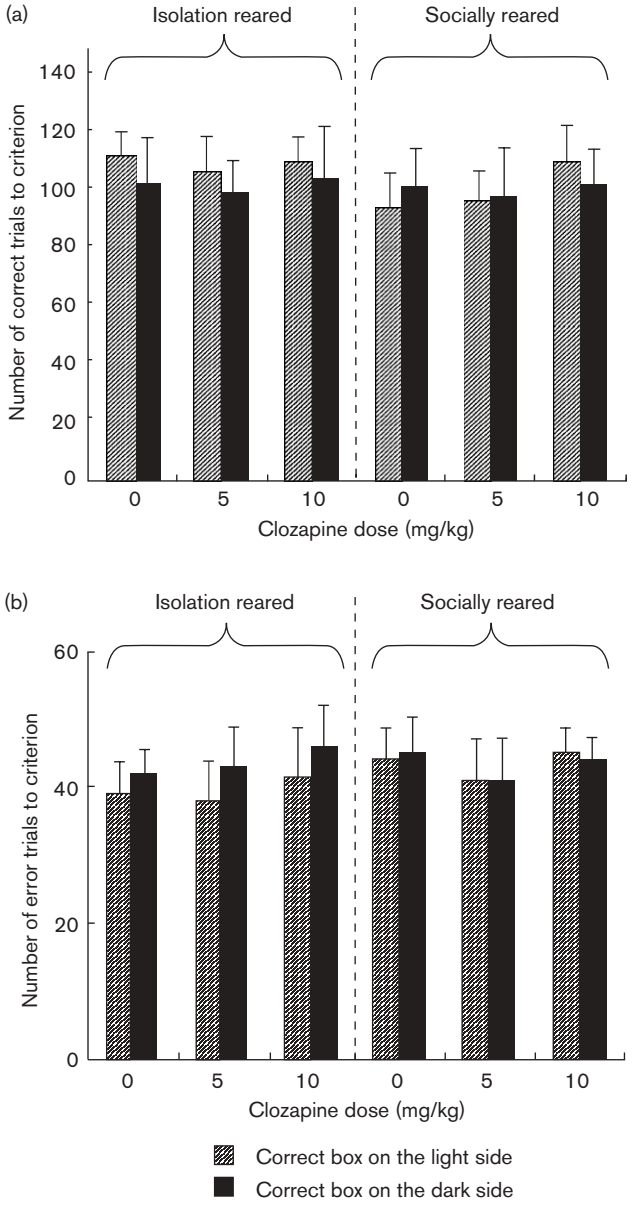
Reversal learning

All the rats eventually reached the criterion of reversal learning, even though there was great variation in the number of testing sessions across individual rats. Figure 3 shows the numbers of correct-response trials to reach the criterion (panel a) and the numbers of error-response trials to reach the criterion (panel b). Obviously, rats needed more trials to reach the performance criterion in reversal learning than in previous acquisition learning. In addition, isolation-reared rats treated with saline injection needed more trials to reach the learning criterion than any other subgroups.

For the number of correct-response trials to the criterion, the interaction between rearing type and clozapine dose was significant, [$F(2,36) = 89.72$, $P < 0.001$], as were the main effect of rearing type [$F(1,36) = 78.46$, $P < 0.01$], and the main effect of clozapine dose [$F(2,36) = 46.62$, $P < 0.001$]. Thus, both rearing type and clozapine dose affected reversal learning.

The main effect of visual cue and all of the interactions involving this factor were nonsignificant ($P > 0.05$). Therefore, further statistical analyses were focused only on the factors of rearing type and clozapine dose and their interactions. Thus, data based on the two visual cue patterns were combined for each subgroup of rats.

Fig. 2



Group means of total numbers of correct-response trials (panel a) and error-response trials (panel b) to the acquisition learning criterion for the six subgroups. In each subgroup, the hexagonal box on the light side was the target box (that was baited) for half of the rats (light bars), and the hexagonal box on the side without light was the target box for the other half of the rats (dark bars). The error bar in this and the following figures indicates the standard error of the mean.

As the interaction between rearing type and clozapine dose was significant, further separate one-way ANOVAs were performed. These showed that there was a significant difference between isolation-reared rats and socially reared rats injected with saline [$F(1,14) = 246.73$, $P < 0.001$], indicating that isolation-reared rats without clozapine injection needed significantly more correct trials than their socially reared control group to reach the criterion. Surprisingly, isolation-reared rats injected with clozapine at a dose of 5 mg/kg needed significantly fewer correct trials to reach the learning criterion than socially reared rats injected with clozapine (5 mg/kg) [$F(1,14) = 12.08$, $P < 0.001$]. The difference between isolation-reared rats and socially reared rats injected with clozapine at a dose of 10 mg/kg, however, was not significant, $P > 0.05$.

Separate ANOVAs also showed that for isolation-reared rats, there was a significant effect of clozapine dose [$F(2,21) = 123.14$, $P < 0.001$]. Scheffé's post-hoc tests showed that all the three groups were significantly different from each other. The dose of 5 mg/kg produced a greater impairment-reducing effect than the dose of 10 mg/kg in isolation-reared rats (Fig. 3, panel a). Interestingly, for socially reared rats, there was also a significant effect of clozapine dose, $F(2,21) = 8.71$, $P < 0.01$. Scheffé's post-hoc tests showed that only the 0 mg/kg group and the 10 mg/kg group were significantly different. The 10 mg/kg group needed the highest number of correct-response trials to the criterion among the three socially reared groups (Fig. 3, panel a).

For the numbers of error-response trials to the criterion, a three-way between-group ANOVA showed that there were no significant main effects or interactions ($P > 0.05$).

Inhibition of previous strategies in reversal learning

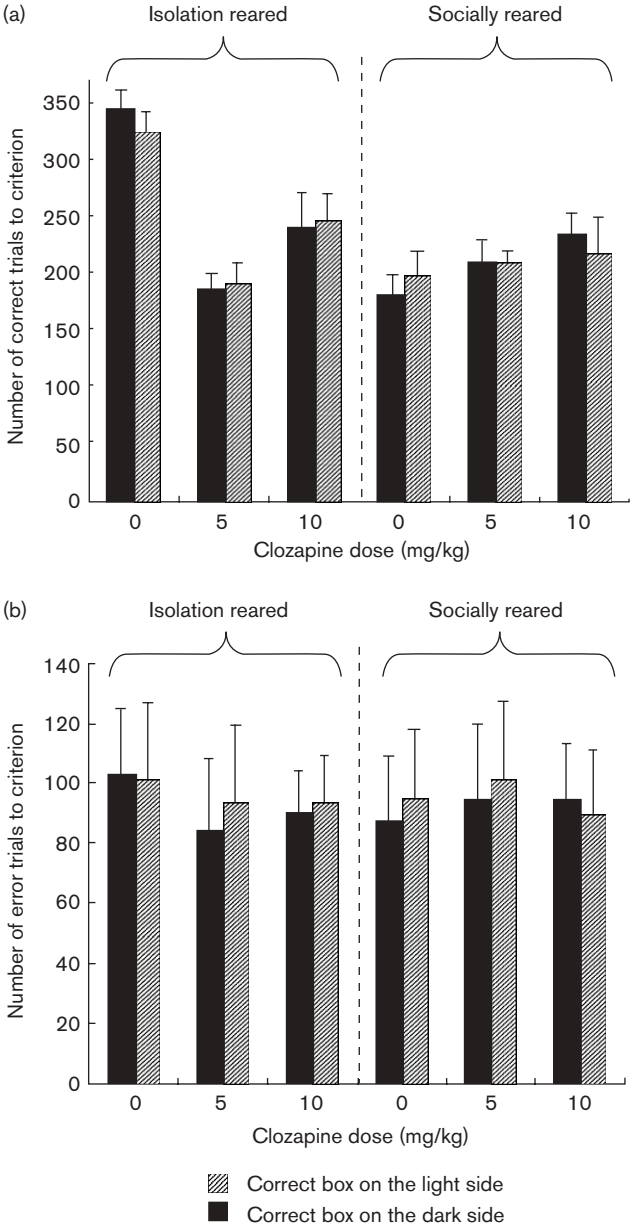
As errors committed during reversal learning did not differ significantly between subgroups, only the numbers of correct responses were analyzed for each of these two phases. In addition, the effect of visual cue pattern was not analyzed.

For the number of correct-response trials to reach the inhibition criterion (Fig. 4, panel a), a 2 (rearing type) by 3 (clozapine dose) two-way between-group ANOVA showed that neither the interaction between the two factors nor the main effect of clozapine dose was significant ($P > 0.05$). The main effect of rearing type was significant, however [$F(1,42) = 7.79$, $P < 0.05$] indicating that isolation-reared rats needed significantly more correct trials to inhibit the previously rewarded strategy than socially reared rats. Interestingly, although clozapine injection had no significant effect in this phase, it appeared to make performance of socially reared rats slightly worse.

Acquisition of the new strategy in reversal learning

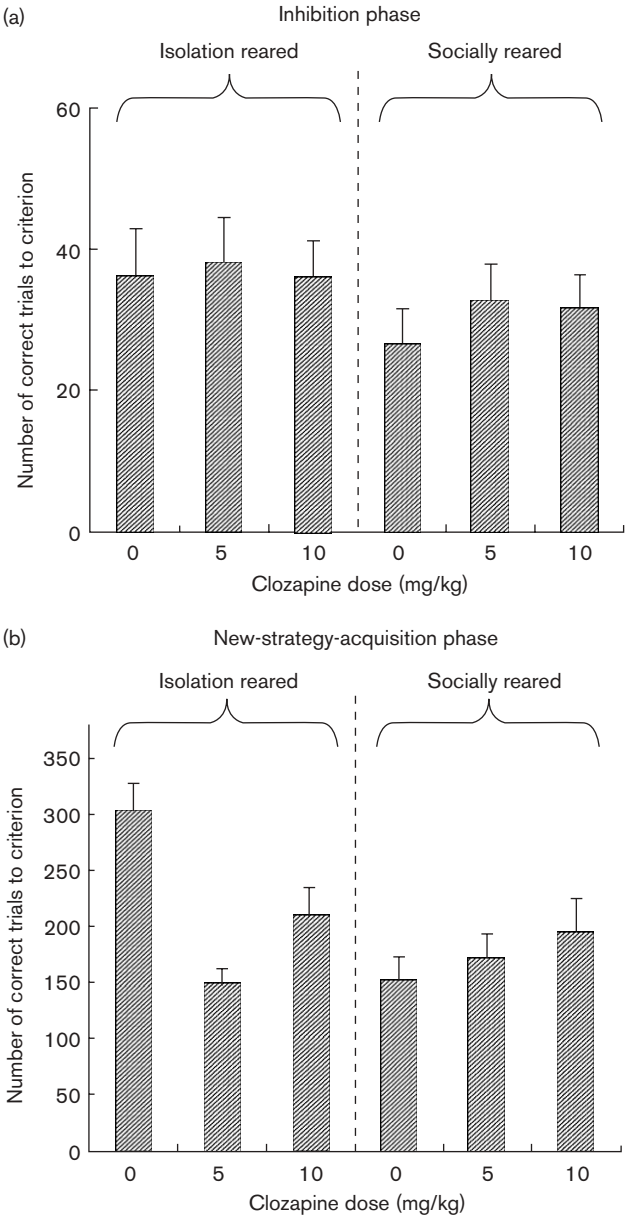
For the performance in the new-strategy-acquisition phase of reversal learning, isolation-reared rats without receiving clozapine injection needed more correct-response trials to reach the learning criterion than both

Fig. 3



Group means of total numbers of correct-response trials (panel a) and error-response trials (panel b) to the reversal-learning criterion for the six subgroups. In each subgroup, the hexagonal box on the side without light was the target box for half of the rats (dark bars), and the hexagonal box on the light side was the target box for the other half of the rats (light bars).

Fig. 4



Comparisons of group means of total numbers of correct-response trials during the inhibition phase (panel a) and the new-strategy-acquisition phase (panel b) of reversal learning.

isolation-reared rats receiving clozapine injection and socially-reared rats (Fig. 4, panel b). A 2 (rearing type) by 3 (clozapine dose) ANOVA showed that the interaction between the two factors was significant [$F(2,42) = 83.85$, $P < 0.001$] as were the main effect of rearing type [$F(1,42) = 56.34$, $P < 0.001$] and the main effect of clozapine dose [$F(2,42) = 42.96$, $P < 0.001$].

For isolation-reared rats, Scheffé's post-hoc tests showed that all the three subgroups were significantly different from each other, showing the clozapine dose effect. For socially reared rats, Scheffé post-hoc tests showed that only the 0 mg/kg subgroup and the 10 mg/kg subgroup were significantly different. The 10 mg/kg group needed highest number of correct-response trials to reach the reversal-learning criterion among the three socially reared subgroups.

Discussion

As mentioned in Introduction, previous studies have not reached an agreement about the effect of isolation rearing on reversal learning (Krech *et al.*, 1962; Jones *et al.*, 1991; Wongwitdecha and Marsden, 1996; Schrijver and Würbel, 2001; Abdul-Monim *et al.*, 2003; Schrijver *et al.*, 2004), implying that the effect of isolation rearing on reversal learning largely depends on the nature of the test. Here, some important features of the rotating T maze used in this study should be emphasized because these features are critically associated with the efficiency of the testing paradigm for revealing the impairing effect of isolation rearing on reversal learning.

First, as mentioned in Methods, the LED cue was the only light source in the testing room during a testing session. In such an environment, rats were able to concentrate only on the visual cue indicating the 'correct' target box, without receiving irrelevant visual disruptions. In addition, as tests were carried out in the rotating maze, there were no interactions, such as physical contacts, between tested rats and experimenters during a testing session. Moreover, in a testing session, because each hexagonal box was used as the start box, correct target box, and wrong target box, no preference for a specific box (location) was reinforced. Finally, because the rotating T tunnel had three different positions relative to an individual hexagonal box, no specific maze-pathway preference was reinforced. Therefore, using the rotating T maze, any possible disruptive effects of redundant cues that occur in some other testing apparatus, such as those in Skinner box, Morris water maze, and traditional T (or Y) maze, were substantially reduced.

The results of this study show that although neither isolation rearing nor clozapine injection had significant effects on acquisition of the simple visual discrimination, isolation-reared rats, if they were not injected with clozapine, needed significantly more correct-response trials than socially reared rats to reach the performance criterion in reversal learning. These findings support the previous reports by Krech *et al.* (1962), Jones *et al.* (1991), and Schrijver *et al.* (2004), indicating the impairing effect of isolation rearing on reversal learning.

In this study, however, when the rat's discrimination behavior in the maze was measured with the total number of error-response trials, the effect of isolation rearing on reversal learning was not significant. Thus, the measurement of maze-performance using the total number of correct-response trials is more sensitive to reversal-learning deficits than measurement with the total number of error-response trials. It should be noted that the sensitivity of maze-performance measurement using the number of error-response trials is also influenced by the strictness of the learning criterion. When the learning criterion was stringent (as the criterion used in this study), large numbers of sessions were needed to reach the criterion across individual rats, and relative differences in the total numbers of error-response trials across rats were reduced.

To perform effectively in a reversal-learning task, animals must first suppress previously acquired behavioral strategies and then establish new strategies (Mackintosh and Holgate, 1969; Jones *et al.*, 1991; Ragozzino *et al.*, 2002; Chudasama and Robbins, 2003). The results of this study indicate that isolation-reared rats showed a small but significant impairment in the initial phase (inhibition phase) of reversal learning, characterized by the need of more correct-response trials to inhibit the previously reinforced behavioral strategy. Chronic administration of clozapine, however, did not alter the isolation-rearing-induced impairment in the inhibition phase of reversal learning. In the new-strategy-acquisition phase of reversal learning, isolation-reared rats without clozapine injection needed significantly more correct-response trials to reach the learning criterion than both isolation-reared rats with clozapine injection and socially reared rats. Thus, isolation rearing leads to impairment of reversal learning in both the inhibition phase and the new-strategy-acquisition phase of reversal learning, but clozapine reduced the impairment only in the new-strategy-acquisition phase.

Selective lesions of the prelimbic area or the infralimbic area of the mPFC lead to similar impairment of reversal learning in rats tested in the same T maze (Li and Shao, 1998). Moreover, in the study by Chudasama and Robbins (2003), selective lesions of the infralimbic area of the mPFC specifically impaired rats' performance in the new-strategy-acquisition phase of reversal learning, suggesting that the infralimbic area of the mPFC is more involved in learning the previously unrewarded stimulus–reward association. Selective lesions of the oPFC specifically impaired rats' performance in the inhibition phase of reversal learning, suggesting that the oPFC is more involved in suppressing the previously rewarded stimulus–reward association. The reversal learning deficits observed in this study might be associated with dysfunctions in both the mPFC and the oPFC. Particularly, the results showing clozapine reduced reversal-learning deficits only in the new-strategy-acquisition (late) phase suggest that certain isolation-rearing-induced neurotransmission changes, which were responsible for the behavioral impairment in the late phase of reversal learning, were associated with the mPFC.

It has been well known that isolation rearing causes various damages to the mPFC, including volume loss (Day-Wilson *et al.*, 2006), increase of acetylcholine and serotonin fiber densities (Lehmann *et al.*, 2003, 2004),

reduction of 5-HT release (Crespi *et al.*, 1992; Bickerdike *et al.*, 1993; Dalley *et al.*, 2002), reduction of 5-HT level (Miura *et al.*, 2002), reduction of dopamine level (Jones *et al.*, 1991; Miura *et al.*, 2002; Eells *et al.*, 2006), increase of 5-$HT2_A$ receptor binding-site densities and decrease of 5-$HT1_A$ receptor binding-site densities (Preece *et al.*, 2004), and abnormal responses to stimulation of the ventral tegmental area (Peters and O'Donnell, 2005). The affinities of clozapine at various receptors may play an important role in alleviating isolation-rearing-induced prefrontal abnormalities and the behavioral impairment observed in the late phase of reversal learning.

Growing evidence has shown that the central serotonin system plays a critical role in the syndrome of behavioral and cognitive abnormalities observed in animals deprived of social contact from an early age (e.g. Bickerdike *et al.*, 1993; Fone *et al.*, 1996; Whitaker-Azmitia *et al.*, 2000; Dalley *et al.*, 2002; Robbins, 2005). Clozapine has a high affinity at both 5-HT_{1A} and 5-HT_{2A} receptors. 5-HT_{2A} receptors selectively regulate mesocortical dopamine projections (Meltzer, 1989; Ichikawa *et al.*, 2001; Bortolozzi *et al.*, 2004; Pehek *et al.*, 2006). Therefore, the ability of clozapine to block 5-HT_{2A} receptors in the mPFC may lead to an increase in dopamine transmission and alleviate some aspects of cognitive dysfunction associated with dopamine deficits. A high 5-HT_{2A}/dopamine D_2 receptor affinity ratio has been correlated with successful treatment of negative symptoms of schizophrenia (Altar *et al.*, 1986).

In addition to neurotransmission in the mPFC, isolation rearing also changes neurotransmission in other forebrain structures, including the nucleus accumbens, striatum, hippocampus, and amygdala (e.g. Jones *et al.*, 1992; Heidbreder *et al.*, 2001; Dalley *et al.*, 2002; Muchimapura *et al.*, 2003; Barr *et al.*, 2004; Harte *et al.*, 2004; Preece *et al.*, 2004; Meyera *et al.*, 2005). The study by Jones *et al.* (1991) showed that alterations in dopaminergic, serotoninergic, and cholinergic markers occurred in brains of isolation-reared rats. In addition, the study by Hall *et al.* (2002) showed that isolation rearing decreased the level of the *N*-methyl-D-aspartate-receptor subunit ($NMDAR_{1A}$) in the striatum and prefrontal cortex, but increased the subunit level in the hippocampus. Obviously, the alleviating effect of clozapine on isolation-rearing-induced reversal-learning/neurotransmission deficits is a critical research issue for understanding the mechanisms underlying symptoms of schizophrenia associated with disturbances occurring during individuals' early development.

Isolation-reared adult rats also show significant prepulse-inhibition (PPI) deficiency (Cilia *et al.*, 2001, 2005; Geyer *et al.*, 2001; Swerdlow *et al.*, 2001; Weiss and Feldon, 2001; van den Buuse *et al.*, 2005), which can be compensated by either antipsychotic treatments or postweaning manipulations (Cilia *et al.*, 2001, 2005; Geyer *et al.*, 2001; Powell *et al.*, 2002). PPI is the reduction of a startle reflex when the intense startling stimulus is preceded by a weaker stimulus and considered as a model of sensorimotor gating (for a review see Li and Yue, 2002). Some studies have reported that PPI is impaired in patients with schizophrenia (for reviews see Braff *et al.*, 2001; Geyer *et al.*, 2001; Swerdlow *et al.*, 2001; Weiss and Feldon, 2001; van den Buuse *et al.*, 2005) and atypical antipsychotics, especially clozapine, can normalize PPI deficits in these patients (Kumari *et al.*, 1999, 2000, 2002; Leumann *et al.*, 2002; Oranje *et al.*, 2002). Therefore, the common mechanisms underlying isolation-rearing-induced sensorimotor gating impairment and cognitive/behavioral flexibility impairment are also an important topic for future studies.

In this study, for socially reared rats, the high dose of clozapine (10 mg/kg) significantly interfered performance in the new-strategy-acquisition phase but not in the inhibition phase of reversal learning. These data suggest a complex bidirectional effect of clozapine on the late phase of reversal learning. The study by Heidbreder *et al.* (2001) showed that in socially reared rats, clozapine (5, 10 mg/kg, subcutaneously) produced a significantly dose-dependent increase in dopamine outflow in the mPFC. Thus, the increased release of dopamine in the mPFC might be able to cause certain disruptive influence to the performance during the new-strategy-acquisition phase of reversal learning in socially reared rats. Moreover, whether there is a link between the modulating effect of chronic administration of clozapine on neural responses of the mPFC to glutamate excitatory inputs (Jardemark *et al.*, 2000) and the new-strategy-acquisition phase of reversal learning is another important research issue in the future.

In summary, the results of this study indicate that isolation rearing leads to impairment of reversal learning, but not of acquisition, of simple visual discrimination in adult rats. In isolation-reared rats, the impairment that occurs during the new-strategy-acquisition phase, but not the inhibition phase, of reversal learning can be alleviated by chronic administration of clozapine, even though chronic administration of high-dose clozapine disrupts reversal learning in the new-strategy-acquisition phase in socially reared rats. Thus, this study has advanced the animal model used for both studying the effect of isolation rearing on cognitive/behavioral flexibility and estimating the efficiency of new antipsychotics.

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