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Post-weaning social isolation increases activity in a novel environment but decreases defensive burying and subchronic MK-801 enhances the activity but not the burying effect in rats

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ABSTRACT

Subchronic treatment with a non-competitive glutamate NMDA-receptor antagonist [e.g., MK-801 or phencyclidine] or social isolation (SI) from weaning (age 21 days) to adulthood (age 56 days) produce deficits similar to some of the positive and negative symptoms of schizophrenia. Few studies have evaluated the effects of these treatments on emotional behavior. We hypothesized that subchronic MK-801, post-weaning SI or the two in combination would alter activity in a novel environment, anxiety-like behaviors in the elevated plus-maze, coping responses in the defensive burying paradigm and social behavior. In experiment 1, SI rats ($n=17$) showed increased locomotor activity when exposed to a novel environment, no change in plus-maze behavior and decreased defensive burying when compared to group housed rats ($n=16$). Subchronic MK-801 enhanced the increase in activity but not the decrease in burying in SI rats. Experiment 2 evaluated the effects on social behavior of post-weaning SI. The locomotor and burying results of experiment 1 were replicated and SI rats ($n=9$) were found to decrease orientation towards a novel conspecific social target when compared to group housed rats ($n=8$). The behavioral abnormalities of SI rats may be a manifestation of GABAergic dysfunction that has recently become evident in schizophrenia.

1. Introduction

Schizophrenia affects about 1% of the general population. Although the etiology remains unknown, studies with animal models have begun to reveal possible mechanisms. It is difficult to produce animal models of hallucinations and delusions (Geyer and Moghaddam, 2002) but symptoms such as impairments in cognition, memory, emotion and social interaction, as well as impaired sensorimotor gating and hyperactivity in response to amphetamine can be investigated (Fone and Porkess, 2008).

Post-mortem studies of people with schizophrenia have revealed alterations in binding, transcription and subunit expression of glutamate N-methyl-D-aspartate (NMDA) receptors (Lewis and Moghaddam, 2006). People administered with NMDA receptor antagonists such as phencyclidine (PCP) or ketamine show schizophrenia-like symptoms (Geyer and Moghaddam, 2002) and animals treated subchronically (e.g., 2 injections a day for 7 days) with these drugs or with dizocilpine (MK-801) show deficits in pre-pulse inhibition, social interaction, locomotor activity and a set-shifting task (comparable to the Wisconsin card sorting task in which people with schizophrenia show impaired performance) (Morris et al., 2005 but see Marquis et al., 2007).

Post-weaning social isolation rearing (SI) has been proposed as a non-pharmacological animal model of schizophrenia-like symptoms. The model involves the social isolation of rats from weaning (postnatal day (P) 21) to sexual maturity (P56). SI is achieved by housing rats singly in clear plastic cages that allow them to see, hear and smell conspecifics but restrict physical contact. Although some deficits produced by SI are similar to schizophrenic symptoms but not unique to the disorder, behavioral changes across all three areas of impairment in schizophrenia (positive, negative and cognitive symptoms) have been produced with the SI model including impaired sensorimotor gating, social withdrawal and impaired cognitive flexibility (Powell and Miyakawa, 2006). The behavioral and cognitive effects of SI appear to be dependent upon social isolation during development, as similar isolation of adult rats fails to produce deficits (Geyer and Moghaddam, 2002). Further investigation of SI as a model of schizophrenia-like symptoms is needed to determine if the model can reliably produce behavioral effects characteristic of schizophrenia such as impaired social behaviors. It is important to characterize how SI affects social interaction because deficits in social behavior are critical negative symptoms associated with schizophrenia and one of the first symptoms to appear (Strous et al., 2004). In addition, we have begun an investigation to determine whether a combined, i.e., double

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hit, model utilizing SI plus subchronic MK-801 treatment will provide a more robust model of schizophrenia-like symptoms in a battery of behavioral tests.

The battery of behavioral tests that were chosen for this study probe multiple domains of cognitive and behavioral deficits that are associated with schizophrenia, and included: locomotor responses to the dopamine (DA) transport-reverser amphetamine, elevated plus-maze, defensive burying, and a social interaction test. Amphetamine produces elevated levels of DA release in people with schizophrenia (Balla et al., 2001). In rats, SI leads to increased DA release (Jones et al., 1992) and increased DA receptor sensitivity in the striatum and nucleus accumbens (Owen et al., 1978). Subchronic PCP or MK-801 leads to increased locomotor responses to amphetamine in rats (Balla et al., 2001; Beninger et al., 2009). Thus, we hypothesized that SI rats would show a greater increase in amphetamine-stimulated activity than group-housed rats and that subchronic MK-801 treatment would augment this effect.

There is a relatively high rate of anxiety among those with schizophrenia (Cosoff and Hafner, 1998) and the elevated plus maze is a validated test of anxiety in rats (Lister, 1990; Pellow et al., 1985). We hypothesized that SI or MK-801-treated rats would show increased anxiety in the plus maze and that rats receiving SI plus subchronic MK-801 would show even greater anxiety.

Defensive burying is an innate, stereotypic response of rodents to noxious stimuli that pose a threat (De Boer and Koolhaas, 2003). Increased burying is indicative of anxiety (Treit et al., 1981; Rodgers, 1997). Decreased burying may reveal a lack of active coping skills (De Boer and Koolhaas, 2003). Individuals with schizophrenia often report difficulties coping with major and minor stresses (Mueser et al., 1997) and tend to avoid problems rather than attempting to solve them (Wilder-Willis et al., 2002). We hypothesized that SI or sub-chronic MK-801-treated rats would bury less and that rats receiving both treatments would bury the least. The decrease in burying may represent a lack of coping skills and may be analogous to a negative symptom of schizophrenia.

Social anxiety is commonly comorbid with schizophrenia but often goes unnoticed (Cosoff and Hafner, 1998) and a common characteristic of schizophrenia is social withdrawal (American Psychiatric Assoc., Diagnostic and statistical manual of mental disorders, 1994). The social interaction test quantifies social behavior between pairs of rats. Repeated treatment with PCP reduced social interaction between rats in a social interaction test (Sams-Dodd, 1996). The effects of SI on social interaction behaviors have not been well studied. Because we found that SI had a greater effect than subchronic MK-801, in experiment 2 we hypothesized that SI would lead to decreased interaction with a conspecific.

2. Method

2.1. Experiment 1

2.1.1. Subjects

Male Sprague Dawley rats ($n=41$; Charles River, St. Constant QC) were obtained at weaning (P21); 25 rats were randomly assigned to the SI condition and 16 rats were group housed (GH) with 4 rats per cage in clear Plexiglas cages, $45\times23\times20$ cm deep for SI rats and $47\times37\times20$ cm deep for GH rats. Immediately upon arrival all rats were socially isolated or group housed according to their randomly-assigned housing condition and remained in their assigned housing condition for the duration of the experiment. The floors were lined with bedding (Beta Chip, NEPCO, Warrensburg, NY) and the cages were located in a climate-controlled colony room (21 ± 1 °C; humidity 40–70%) on a reversed 12-h light/dark schedule (lights off at 07:00 h). Food (LabDiet rodent feed #5001, PMI Nutrition International, Brentwood, MO) and water were available ad libitum.

Treatment of the animals was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and pertinent University Policy, and the Queen's University Animal Care Committee approved the experimental protocol.

2.1.2. Apparatus

2.1.2.1. Locomotor activity. Six experimental chambers ($50\times40\times40$ cm high) were constructed from Plexiglas and housed in wooden, Styrofoam-insulated outer boxes. Each was illuminated with a 2.5 W incandescent bulb and ventilated by a small fan that also provided background noise. Seven pairs of photoemitters and detectors, 3 pairs equally spaced along the width and 4 along the length at a height of 5.0 cm above the metal-rod floor captured locomotor activity. For details of the apparatus see Beninger et al. (1985).

2.1.2.2. Elevated plus-maze. The plus-shaped apparatus made of urethane-sealed wood and elevated 50 cm above the floor consisted of two 50×10 cm open arms and two 50×10 cm arms enclosed by 40 cm high walls, all uncovered. The arms were connected by a 10×10 cm central square.

2.1.2.3. Shock-probe burying. A rectangular Plexiglas box ($30\times40\times40$ cm high) with no cover contained a clean, level, approximately 3 cm deep layer of wooden chip bedding (Beta Chip, NEPCO, Warrensburg, NY). The shock-probe consisted of two copper wires wrapped around a Plexiglas rod (0.75 cm diam × 7.0 cm long) that delivered an electric current from a 2000 V shock source with an intensity of 2.5 mA. The shock-probe could be inserted into the testing box through a small hole (2.5 cm diam) centered in one wall 10 cm from the floor of the box.

2.1.3. Drug treatment and housing groups

After 35 days (P56), subchronic (defined here as 7 days) treatments began; MK-801 (Sigma, Oakville ON) was dissolved in saline to a concentration of 0.5 mg/ml and injected (0.5 mg/kg i.p.) twice daily at approximately 08:30 and 20:30 h for 7 days. The dose of 0.5 mg/kg MK-801 has been used in previous studies (Beninger et al., 2009; Manalack et al., 1989; Miller and Abercromble, 1996). SI rats were randomly assigned to the following groups: SI ($n=8$), SI-Sal ($n=8$) or SI-MK ($n=9$), the SI group receiving no injections and the latter two groups receiving subchronic saline (1.0 ml/kg) or MK-801, respectively. The SI group that received no injections was included for comparison with the SI-Sal group to evaluate possible effects of handling on the effects of SI since it has been reported that handling can influence the effects of SI (Krebs-Thomson et al., 2001; Rosa et al., 2005). GH rats were randomly assigned to GH-Sal ($n=8$) or GH-MK ($n=8$) groups and received corresponding subchronic injections. Rats remained in their home cages for 7 days following subchronic injections (P64–P70) before behavioral testing began.

2.1.4. Behavioral testing

2.1.4.1. Locomotor activity. On P70, P71 or P72, activity was measured as the number of beam breaks over 3.5 h in 3 distinct phases with activity counts recorded in 5-min bins. The habituation phase lasted 60 min, after which the rats were removed from the chambers, injected with saline (1.0 ml/kg i.p.) and returned for another 60-min session. Following this, rats were again removed, injected with amphetamine (1.5 mg/kg i.p., dextro-amphetamine sulfate; Sigma, Oakville ON) dissolved in saline, and returned to the chambers for an additional 90-min session. This dose augments locomotor activity and has frequently been employed to assess possible increases in sensitivity to the locomotor stimulating actions of amphetamine (e.g., see Beninger et al., 2009; Lipska et al., 1993).

2.1.4.2. Elevated plus-maze. On P73, all testing took place in a room lit by red light. Animals were placed in the centre of the maze facing a

closed arm and their behaviors were recorded for 5-min. The experimenter remained in the room throughout testing, standing quietly at least one meter away from the maze. If an animal fell or jumped from the maze during testing the experimenter placed the animal back on the maze in the location from which they fell or jumped.

The experimenter recorded the total numbers of open and closed arm entries. An entry was recorded when an animal placed all four paws in a given arm of the maze. In addition, each animal's test was captured using a digital video camera. The videos were later scored, using Observer VideoPro (Noldus Technology, Wageningen, The Netherlands) for the following behaviors: (1) the duration of time spent in the closed arms, (2) the duration of time spent in the open arms, and (3) the duration of time spent in the 10×10 cm^2 in the centre of the maze. Open arm entries were analyzed as a percentage score by dividing the number of open arm entries by the total number of arm entries (open and closed). Time spent on the open arms of the maze was also analyzed as a percentage score by dividing the duration of time spent on an open arm by the total duration of time spent on any arm (open or closed).

2.1.4.3. Shock-probe burying. Beginning on P85 or P86, during the habituation phase, animals were placed in the testing apparatus for 15 min on each of the four consecutive days prior to testing. At this time, the hole in the testing chamber was left unplugged. During testing, the shock-probe was positioned in the hole and the animals were individually placed in the test chamber. Throughout testing the shock-probe was electrified. Test duration began following the first shock received by the rat in the testing apparatus and lasted for 15 min.

Each burying test was captured using a digital camera. The videos were later scored, using Observer VideoPro (Noldus Technology, Wageningen, The Netherlands). The frequency or total duration of time spent engaged in the following behaviors were coded: (1) burying (i.e., the duration of time moving bedding towards the probe using front limbs in a pushing motion), (2) rearing (i.e., the duration of time spent on hind legs with front limbs raised off the bedding), (3) number of shocks (i.e., the frequency of times the animal made contact with the probe and received a small electric shock).

The rats' reactivity to each shock was determined by assigning a shock reactivity score each time an animal received a contact-induced shock using a numeric scale: 1 = head flinch (no body movement), 2 = whole body flinch, 3 = whole body flinch followed by movement away from the probe, 4 = jump (all four feet in the air) following shock and rapid movement away from the probe. The mean shock reactivity score for each animal was determined by dividing the total shock reactivity score (sum of all reactivity scores to a shock) by the total number of shocks received.

2.1.5. Data analysis

After initial scoring of the behavioral videos, 20 elevated plus-maze videos (4 from each group) and 20 shock-probe videos (4 from each group) were randomly selected, the video file names were recorded by a member of the lab unfamiliar with the experiment and the videos were then rescored by the original experimenter. Videos were scored in 1-min intervals and rescored data for each interval were compared to the initial data. Total durations of the behaviors in each session were used for analyses.

All analyses were performed using SPSS version 17.0. Three 2 (housing condition) × 2 (treatment) × 12 or 18 (bins) mixed-designed analyses of variance (ANOVA) were conducted to assess group (SI-Sal, SI-MK, GH-Sal, GH-MK) differences in locomotor activity over time in the habituation, saline and amphetamine phases. In addition 2 (housing condition) × 12 or 18 (bins) ANOVA, the SI and SI-Sal groups were compared. Separate 2 (housing condition) × 2 (treatment) ANOVA were conducted to assess group differences in the behaviors scored during the elevated plus maze and shock-probe burying tests; again the 4 main experimental groups were analyzed and then the SI and SI-Sal groups were compared. Bivariate Pearson correlations were conducted to compare initial and rescored behavioral data. Significance levels were set at $p<0.05$.

2.2. Experiment 2

2.2.1. Subjects

Male Sprague Dawley rats (Charles River, St. Constant Quebec), obtained at P21 and randomly assigned to SI-Sal ($n=9$) and GH-Sal ($n=8$) groups were housed as described in experiment 1. SI-MK, SI and GH-MK groups were not included because the SI-groups did not differ from SI-SAL and GH-MK did not differ from GH-SAL in experiment 1.

2.2.2. Apparatus

Locomotor activity and Shock-probe burying apparatus were the same as those described for experiment 1.

2.2.2.1. Social interaction. A square Plexiglas box ($101\times101\times50$ cm high) without a lid contained the social target cage, a white, rectangular, wire cage with a Plexiglas bottom ($36\times24\times30$ cm high) located in the middle of one side. Tape was used to section the floor into different zones to facilitate scoring. Two aversion corners (20×20 cm) were located opposite the social target cage, a 10-cm interaction zone surrounded the social target cage and a 10-cm wide zone was located along each wall of the Plexiglas box (Fig. 1).

2.2.3. Behavioral testing

From P57–P63 rats received twice-daily injections of saline (1 mg/kg, i.p.) for 7 days at approximately 08:30 and 20:30 h to make the protocols of this study and experiment 1 directly comparable. Rats remained in their home cages from P64 to P70.

Locomotor activity was tested on P70 or P71 and Shock-probe burying tests began on P84 or P85 according to the protocol described for experiment 1.

2.2.3.1. Social interaction test. On P77 or P78 animals were individually habituated to the apparatus for 10 min in the absence of the social target (one of 2 novel, male, Sprague Dawley rats of approximate age P84), removed and then immediately placed back into the apparatus for 10 min with a social target in the social target cage. The 2 social targets were presented equally to each housing group. Each social interaction test was captured using a digital camera and the video was later scored, using Noldus software (Noldus Technology, Wageningen, The Netherlands). The total duration of time spent in the following areas or engaged in the following behaviors were scored: (1) in the

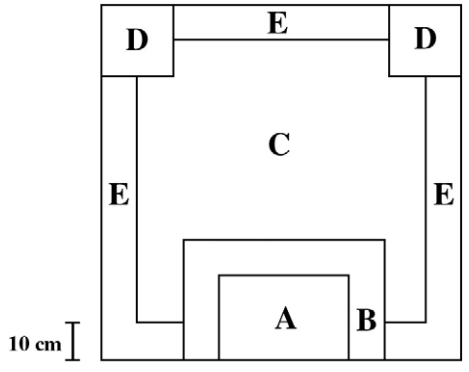

Fig. 1. The social interaction testing apparatus. The social target cage (A) was surrounded by the interaction zone (B) and located opposite to two aversion zones (D). Wall zones (E) were located along each wall of the apparatus and the remaining area was referred to as the central area (C).

interaction zone, (2) in the interaction zone oriented towards the social target, (3) in an aversion zone, (4) in the central area, and (5) in a wall zone. Orientation towards the social target was defined as the amount of time a rat spent with its nose oriented within 45° of the social target or time spent actively moving to remain close to the social target.

2.2.4. Data analysis

After initial scoring of the behavioral videos, 6 social interaction videos (3 SI and 3 GH rats) and 6 shock-probe videos (3 SI and 3 GH rats) were randomly selected, the video file names were recorded by a member of the lab unfamiliar with the experiment and the videos were then rescored by the original experimenter. Videos were scored in 1-min intervals and rescored data for each interval were compared to the initial scores. Total durations of the behaviors in each session were used for analyses.

All analyses were performed using SPSS version 17.0. Two-way group × bin repeated measures ANOVA assessed group differences in activity over time in the habituation, saline and amphetamine phases. One-way ANOVA assessed group differences in the behaviors scored during the social interaction and shock-probe burying tests. Bivariate Pearson correlations were conducted to compare initial and rescored behavioral data. Significance levels were set at $p<0.05$.

3. Results

3.1. Experiment 1

3.1.1. Locomotor activity

Note that the two housing conditions (SI and GH) and the two drug conditions (MK and Sal) were analyzed first in a 2 × 2 design followed by separate comparisons of the SI and SI-Sal groups. The SI-Sal and SI-MK rats appeared to be more active during the habituation and saline phases, with SI-MK rats appearing most active. All groups were similar following amphetamine (Fig. 2A). For the habituation phase, ANOVA revealed significant main effects of time (5-min bins), $F(11, 319) = 80.99$, $p<0.001$, and housing, $F(1, 29) = 29.51$, $p<0.001$, accounted for by greater activity of SI-Sal and SI-MK groups combined. The significant interaction of housing x time, $F(11, 319) = 5.85$, $p<0.001$, occurred because housing conditions differed significantly from bins 5–12, $Fs(1, 31) \geq 5.63$, $ps \leq 0.02$, but not during bins 1–4. The housing x drug interaction, $F(1, 29) = 5.77$, $p = 0.023$, occurred when bins were combined. Test of simple effects of drug condition for each housing group revealed that the mean ± SEM for the SI-MK (99.6 ± 4.3) group was higher than that (81.51 ± 4.6) for the SI-Sal group, $F(1, 15) = 8.03$, $p = 0.013$, whereas the GH-MK (64.7 ± 4.4) and GH-Sal groups (68.0 ± 4.4) did not differ significantly.

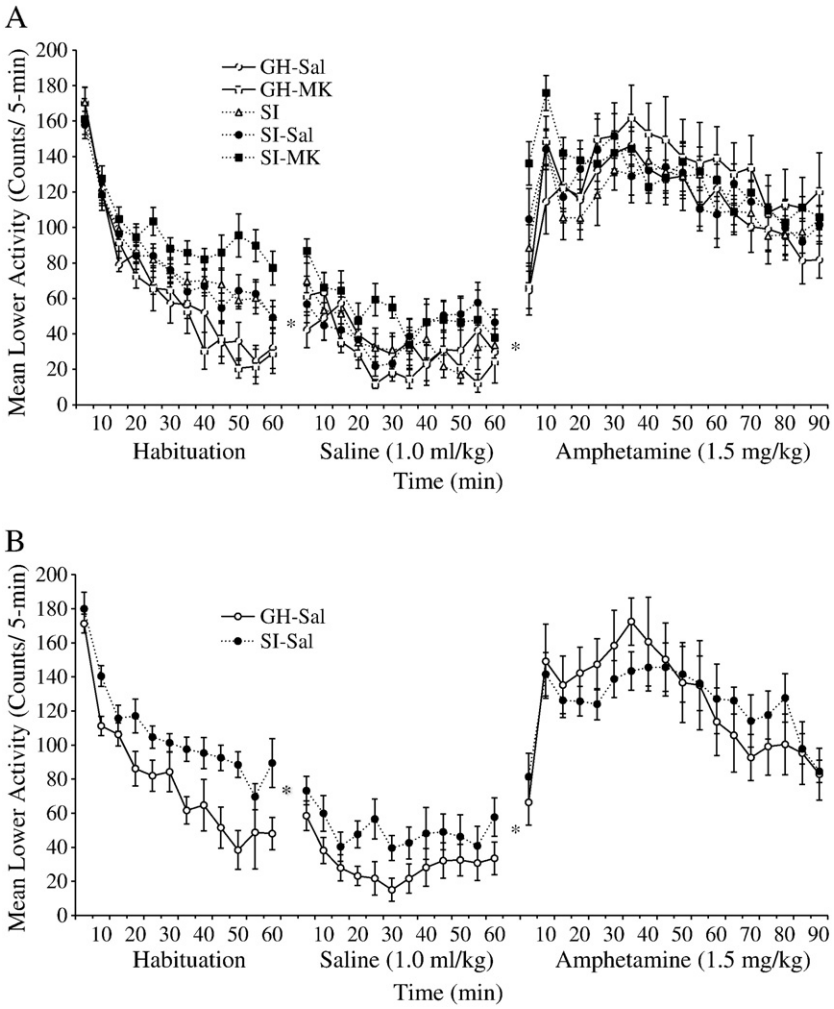

Fig. 2. A: Mean (± SEM) beam breaks per 5-min for habituation, saline, and amphetamine phases for groups that were socially isolated and received no injections (SI; $n = 8$), saline (SI-Sal; 1.0 ml/kg twice daily for 7 days; $n = 8$) or MK-801 (SI-MK; 0.5 mg/kg twice daily for 7 days; $n = 9$) injections, or were group housed and similarly received saline (GH-Sal; $n = 8$) or MK-801 (GH-MK; $n = 8$) injections in experiment 1. *Significant main effect of housing (SI-Sal plus SI-MK combined compared to GH-Sal plus GH-MK combined) by analysis of variance (ANOVA). B: Like A, SI-Sal ($n = 9$) and GH-Sal ($n = 8$) groups from experiment 2. *Significant main effect of housing by ANOVA.

For the saline phase, ANOVA revealed a significant main effect of time, $F(11, 319) = 4.78$, $p < 0.001$, and housing, $F(1, 29) = 8.99$, $p = 0.006$, accounted for by greater activity of the combined SI-MK and SI-Sal groups compared to the combined GH-MK and GH-Sal groups.

For the amphetamine phase, ANOVA revealed a significant main effect of time, $F(17, 493) = 13.11$, $p < 0.001$, and a significant interaction of housing and time, $F(17, 493) = 3.22$, $p = 0.004$. The interaction occurred because the SI rats (SI-MK and SI-Sal) were more active than the GH rats (GH-MK and GH-Sal) on bins 1 and 2, $Fs(1, 31) \geq 4.15$, $ps \leq 0.050$.

The SI and SI-Sal groups did not differ significantly in activity during the habituation, saline or amphetamine phases.

3.1.2. Elevated plus-maze

ANOVA revealed no significant effects of housing or drug treatment, nor any interaction between these dependent measures (see Table 1). The bivariate Pearson correlation of the 1-min rescored video intervals and the corresponding intervals of original data revealed a strong positive relationship for the percentage of open arm entries and percentage of open arm time, $r(99) = 0.915$, $p < 0.01$ and $r(99) = 0.901$, $p < 0.01$, respectively.

3.1.3. Shock-probe burying

The SI-MK and SI-Sal rats spent less time burying (Fig. 3 upper panel, left) and more time rearing (Fig. 3 lower panel, left) revealed by significant effects of housing in separate 2-way ANOVA, $F(1, 27) = 5.36$, $p = 0.028$ and $F(1, 27) = 20.18$, $p < 0.001$, respectively. All rats received a shock within the first 8 s of being placed in the testing apparatus. There was a main effect of housing on the number of shocks received, $F(1, 27) = 5.37$, $p = 0.029$, accounted for by GH-MK and GH-Sal rats receiving more mean (±SEM) shocks per session than the SI-MK and SI-Sal rats (Table 1). There was a significant main effect of drug on mean shock reactivity, $F(1, 27) = 6.16$, $p = 0.020$, accounted for by higher scores in GH-MK and SI-MK groups in comparison to GH-Sal and SI-Sal rats (Table 1). ANOVA comparing the SI and SI-Sal conditions revealed no significant effects.

The bivariate Pearson correlation of the 1-min rescored video intervals and the corresponding intervals of original data revealed a strong positive relationship for the amount of time spent burying and rearing and mean shock reactivity, $r(300) = 0.92$, $p < 0.01$, $r(300) = 0.94$, $p < 0.01$, and $r(20) = 0.86$, $p < 0.01$, respectively.

3.2. Experiment 2

3.2.1. Locomotor activity

The SI-Sal group showed greater activity than the GH-Sal group during the habituation and saline phases of testing. During the amphetamine testing phase the SI-Sal group was lower than the GH-Sal group early in testing but higher later on (Fig. 2B). Separate ANOVA for habituation and saline showed significant or near significant main effects of time (5-min bins), $Fs(11, 165) = 32.43$, $p < 0.01$ and $= 2.24$, $p = 0.06$, respectively, and main effects of housing, $Fs(1, 15) = 10.58$ and 10.65, $ps < 0.01$, respectively, confirming that the SI-Sal group was more active. For the amphetamine phase, ANOVA revealed no significant effects.

3.2.2. Social interaction

The average time spent in the interaction zone oriented towards the social target was lower for the SI-Sal group than the GH-Sal group (Table 1) and ANOVA revealed a significant difference, $F(1, 15) = 5.98$, $p = 0.03$. The amount of time spent in the interaction zone for the SI and GH groups was not significantly different, $F(1, 15) = 0.04$, $p = 0.84$. No effects were seen for time in the aversion zones, central area or wall zones (Table 1).

A bivariate Pearson correlation of the 1-min rescored video intervals and the corresponding intervals of original data revealed a strong positive relationship for the amount of time oriented towards the social target while in the interaction zone, $r(60) = 0.89$, $p = 0.01$.

3.2.3. Shock-probe burying

The SI-Sal group spent less time burying (Fig. 3, upper panel, right), $F(1, 15) = 11.02$, $p = 0.02$, and a significantly longer duration of rearing than the GH-Sal group, $F(1, 15) = 7.272$, $p = 0.017$ (Fig. 3, lower panel, right). All rats received a shock within the first 5 s of being placed in the testing apparatus. There was no significant difference in the number of shocks or mean shock reactivity between groups (Table 1). A bivariate Pearson correlation of the 1-min rescored video intervals and the corresponding intervals of the original data revealed a strong positive relationship for the amount of time spent burying, $r(90) = 0.94$, $p = 0.01$.

4. Discussion

We demonstrated that SI rats showed greater locomotor activity in response to a novel environment and following saline injections in

Table 1
Mean (±SEM) for each group in plus-maze and shock-probe burying tests from experiment 1 and in social interaction and shock-probe burying tests from experiment 2.

	GH-Sal	GH-MK	SI-Sal	SI-MK	SI
Plus-maze (exp 1)					
Open arm entries	3.13 ± 0.79	4.25 ± 0.98	1.88 ± 0.95	4.00 ± 1.11	3.25 ± 1.08
Closed arm entries	9.75 ± 0.84	9.75 ± 0.94	10.5 ± 1.15	11.33 ± 1.27	9.25 ± 0.84
Percentage open arm entries	22.1 ± 4.67	27.4 ± 5.05	14.24 ± 6.72	25.9 ± 6.18	24.74 ± 7.66
Time (s) in open arms	30.0 ± 6.49	46.0 ± 13.0	28.5 ± 13.7	46.7 ± 13.0	42.2 ± 14.8
Time (s) in closed arms	114.7 ± 15.4	110.3 ± 9.76	115.3 ± 17.5	122.1 ± 10.8	119.8 ± 18.1
Percentage of open arm time	18.2 ± 6.09	24.3 ± 4.54	16.38 ± 8.13	25.8 ± 6.67	25.11 ± 9.22
Time (s) spent in the centre square	148.7 ± 11.0	140.5 ± 5.70	154.3 ± 8.00	139.8 ± 9.01	142.1 ± 7.98
Shock-probe burying (exp 1)					
Number of shocks	3.00 ± 0.46	3.71 ± 0.52	2.25 ± 0.49*	2.38 ± 0.32*	2.38 ± 0.38
Shock reactivity	1.86 ± 0.20	2.34 ± 0.20†	2.00 ± 0.14	2.42 ± 0.18†	1.97 ± 0.33
Social inter-action (exp 2)					
Time (s) in interaction zone (B)	434.0 ± 18.6		429.1 ± 11.2		
Time (s) in B oriented to target	337.6 ± 19.4		286.1 ± 9.86Δ		
Time (s) in central zone (C)	12.86 ± 4.13		18.90 ± 4.79		
Time (s) in aversion zone (D)	34.18 ± 14.9		31.59 ± 3.43		
Time (s) in wall zone (E)	119.9 ± 10.6		118.6 ± 8.80		
Shock-probe burying (exp 2)					
Number of shocks	4.12 ± 0.66		2.78 ± 0.70		
Shock reactivity	2.24 ± 0.06		1.83 ± 0.21		

*Significant main effect of housing (GH vs. SI); †Significant main effect of treatment (MK vs. Sal); ΔSignificant effect of housing.

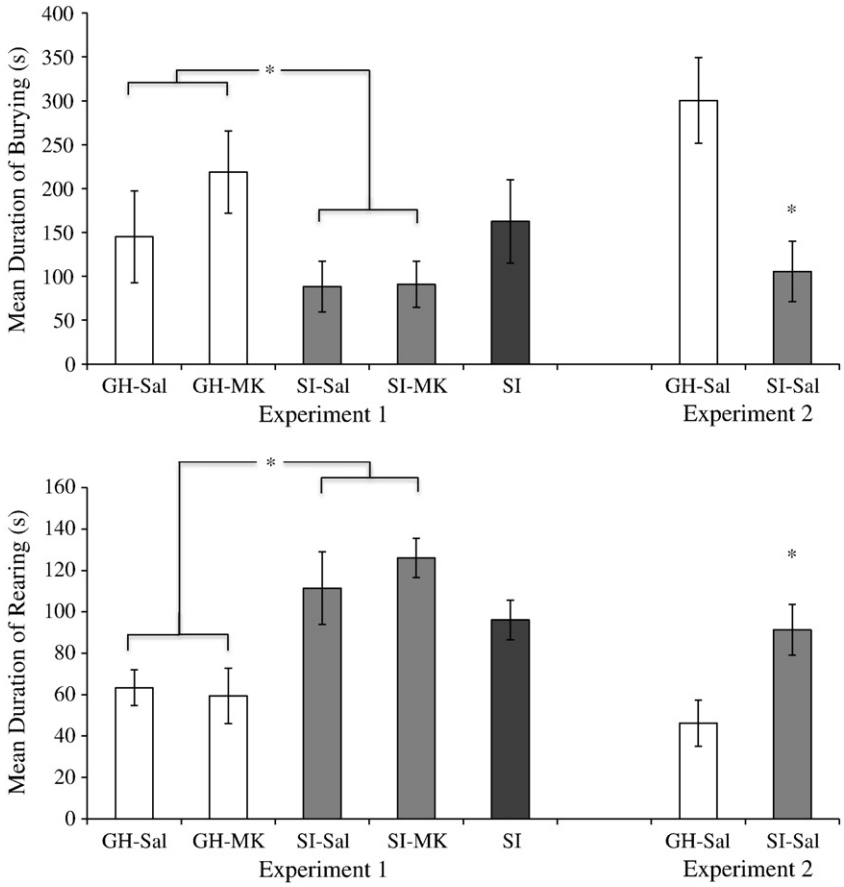

Fig. 3. Mean (±SEM) duration (s) of burying (upper panel) and rearing (lower panel) for groups that were socially isolated and received no injections (SI; $n=8$), saline (SI-Sal; 1.0 ml/kg twice daily for 7 days; $n=8$) or MK-801 (SI-MK; 0.5 mg/kg twice daily for 7 days; $n=8$) injections, or were group housed and similarly received saline (GH-Sal; $n=8$) or MK-801 (GH-MK; $n=8$) injections in experiment 1 (left) and corresponding groups from experiment 2 (right). *Significant main effect of housing (SI-Sal plus SI-MK combined compared to GH-Sal plus GH-MK combined in experiment 1) by analysis of variance.

comparison to GH rats. SI-MK rats showed even greater activity than SI-Sal rats in response to a novel environment. No group differences were observed in the plus-maze. SI rats showed decreased levels of defensive burying in the shock-probe test and decreased orientation towards a novel social target.

The data from the initial scoring of the elevated plus maze, shock-probe and social interaction test videos were highly correlated with the rescored data. The videos were rescored without knowing the treatment condition of the animal. The strong correlation eliminates the concern of possible experimenter bias. When the SI group was compared to the SI-Sal group for each behavioral test, no significant differences were found. This result shows that the handling associated with twice-daily drug injections did not alter the effects of social isolation.

The present observation, replicated in experiments 1 and 2, that SI rats showed increased locomotor activity in comparison to GH rats during the habituation phase of activity testing when the environment was novel agrees with the results of several previous studies (Bakshi and Geyer, 1999; Geyer et al., 1993; Hall et al., 1998; Phillips et al., 1994; Smith et al., 1997). Some investigators have failed to observe a difference between SI and GH animals (Jones et al., 1992; Varty et al., 2000), but variables such as illumination (Hall et al., 1998), length of SI (Bakshi and Geyer, 1999) or strain (Geyer et al., 1993) have been shown to interact with the SI effect and may account for the discrepant findings. We also found in experiments 1 and 2 that SI animals were more active following saline injection. Two previous studies have reported no effect of SI on locomotor activity (Jones et al., 1992; Phillips et al., 1994) but in these studies the rats had extensive exposure to the apparatus before saline testing occurred. By comparison, our saline test occurred after the rats had only 1 hr exposure to the apparatus so the environment was still relatively novel during the saline phase of testing. Thus, SI may lead to an enhanced locomotor response to novelty but has no significant effect on locomotor activity in a familiar environment.

We observed no significant main effect of subchronic treatment with MK-801 on locomotor activity during habituation or following saline, in agreement with previous findings (Beninger et al., 2009; Dall'Olio et al., 1992; Mandillo et al., 2003; Sams-Dodd, 2004). A novel finding from the present study was the observation that SI-MK rats showed increased activity in a novel environment when compared to SI-Sal rats. This effect was no longer significant during the second hr of testing that followed saline injection. This was the only finding that supported our idea that a "double hit", the combination of SI plus subchronic MK-801, would have a greater effect than either treatment alone. The observation that the effect was restricted to locomotor responses to novelty questions the generality of the phenomenon.

Amphetamine augmented locomotor activity but combined SI or MK groups did not differ significantly from combined GH or Sal groups, respectively. A significant housing by time interaction was found in experiment 1 and simple effects analyses showed the combined SI (SI-Sal and SI-MK) groups to be more active than the combined GH (GH-Sal and GH-MK) groups during the first two 5-min bins. This interaction was not observed in experiment 2. Inspecting the activity data for the amphetamine phase in Fig. 2A suggests that the elevated activity of the combined SI (SI-Sal and SI-MK) groups during bins 1 and 2 was driven largely by the SI-MK group although a significant 3-way interaction was not found. Because MK-treated groups were not included in experiment

2, this might account for the apparent discrepancy between experiments 1 and 2 in the effects of housing on the activity response to amphetamine during the first 10 min.

An elevated locomotor response to amphetamine has been reported for SI rats (Smith et al., 1997) but the dose (0.5 mg/kg) and duration of testing (30 min) differed from those used here possibly accounting for the different results. SI rats showed enhanced locomotor responses to cocaine (Phillips et al., 1994; Smith et al., 1997). Subchronic MK-801- or PCP-treated rats have been reported to show elevated responses to amphetamine in some studies (Balla et al., 2001, Beninger et al., 2009; Jentsch et al., 1998) but not others (Egerton et al., 2008; present study). The underlying factors accounting for the differences in results of these studies are unclear. The differences may be accounted for by difference in testing methods, including testing in the home cage or an identical environment to the home cage (Balla et al., 2001, Jentsch et al., 1998), habituation to the testing room prior to activity testing (Jentsch et al., 1998) and testing in a novel environment or following a battery of behavioral tests (Egerton et al., 2008). Thus the effects of subchronic MK-801 or PCP on amphetamine-stimulated activity appear to interact with a number of variables. These observations contributed to our development of the hypothesis that a "double hit", SI plus subchronic MK-801, would produce larger and more reliable effects. However, the present data do not support that hypothesis and there remains a need to clarify the variables that interact with SI, subchronic MK-801, or the two in combination, to increase the locomotor response to amphetamine.

There were no differences in the percentage of time spent in the open arms or the percentage of open arm entries between groups during elevated plus maze testing. Similarly, rats treated with ketamine (30 mg/kg daily for 5 days) or PCP (5 mg/kg for 7 days) did not show significant effect on these variables (Becker et al., 2003; Schwabe et al., 2006). SI rats (12 weeks from P45) entered more and spent more time on an open arm than GH controls in one study (Thorsell et al., 2006). In contrast, SI rats (13 weeks from P21) in a separate study entered the open arms less and spent less time on an open arm (Weiss et al., 2004). The differences in age at which SI began and the length of SI may account for these different findings. From the existing data, post-weaning SI, subchronic treatment with a NMDA receptor blocker or the two in combination does not reliably affect elevated plus-maze behavior.

Subchronic treatment with MK-801 had no significant effects on defensive burying. Increased defensive burying, like decreased open-arm time in the plus-maze, is a reliable index of anxiety (De Boer and Koolhaas, 2003). Thus, subchronic MK-801 appears to have little effect on anxiety. This is the first study to evaluate the possible effects of subchronic MK-801 on shock-probe burying behavior. In the current study SI rats engaged in burying behavior for a significantly shorter duration of time than GH rats. Arakawa (2007) reported a similar finding when juvenile rats were reared in SI but found longer durations of burying in rats isolated during adulthood. This suggests that the effect of SI on burying behavior is sensitive to the developmental period in which SI occurs. The number of shocks received by and the mean reactivity of SI and GH rats did not differ in experiment 2 and therefore the housing group difference in burying is not likely attributable to a difference in pain sensitivity or responsivity to the shocks. SI rats' decreased defensive behavior towards a shock-probe is consistent with the finding that isolation-reared rats are less responsive to attacks from conspecifics (Einon and Potegal, 1990). The decrease in defensive responses of SI rats may reflect the adoption of a passive, rather than active, coping style (De Boer and Koolhaas, 2003). The coping style of humans with schizophrenia has been argued to be similarly passive (Van den Bosch et al., 1992). The increased rearing of SI rats may or may not support this idea.

SI and GH rats did not differ significantly in the amount of time spent in the interaction zone during the social interaction test. Some studies have reported increased social approach in SI rats (Vale and Montgomery, 1997; Varlinskaya et al., 1999; Wongwitdecha and Marsden, 1996) and others have reported a decrease (Hol et al., 1999; Van den Berg et al., 1999). The discrepancies may be attributable to differences in lighting and familiarity with the testing apparatus (Wongwitdecha and Marsden, 1996) as well as the history of the social target (Hol et al., 1999) and the period of social isolation (Hol et al., 1999; Van den Berg et al., 1999). While in the interaction zone, SI rats orientated towards the social target significantly less than GH rats. This novel finding may reflect the tendency of SI rats to engage in less social exploration, as reported by Van den Berg et al. (1999). Pellis et al. (1999) reported decreased rump orientation towards a conspecific following dodging in SI rats when compared to GH rats. The evidence is consistent with the view that social interaction tendencies develop without the need for social experience, however a lack of juvenile experience with conspecifics may lead to the development of inappropriate social behaviors such as abnormal orientation. Further studies are needed to determine how SI affects the development of social behaviors.

The mechanisms underlying the observed deficits remain unknown. A reduction in NMDA receptor function produced by social isolation rearing may add to the effects of MK-801 in SI-MK rats to produce an increased locomotor response to novelty. NMDA-receptor deficient mice exhibit hyperactivity (Geoff and Coyle, 2001) and NMDA receptor subunit NR1 knockdown mice exhibit hyperactivity in response to novel environments (Mohn et al., 1999). Hall et al. (2002) report reduced NMDANR1A expression in some areas of the striatum and hippocampus of isolation reared Fawn-hooded rats. Similar reductions in the function of NMDA receptors have been reported in people with schizophrenia (Stefansson et al., 2002). NR1 knockdown mice have been reported to exhibit social withdrawal in comparison to controls (Mohn et al., 1999). Changes in NMDA receptor function may account for the decrease in orientation towards a novel conspecific that was observed in SI rats when compared to GH rats.

Reduced GABAergic function may also underlie the deficits reported in this study. Both SI and subchronic treatment with NMDA receptor antagonists have been reported to lead to decreases in GABA neurotransmission. Thus, post-weaning SI reduced GABA transporter-1 (GAT-1)-positive cartridges in the ventral prelimbic region of the prefrontal cortex (pfc) of rats (Bloomfield et al., 2008) and subchronic MK-801 or PCP reduced the density of parvalbumin-immunoreactive GABA cells in the hippocampus and prefrontal cortex of rats (Abdul-Monim et al., 2007; Braun et al., 2007). The observation that locomotor activity during habituation was increased in SI rats and further increased in SI-MK rats suggests that this effect might be related to decreases in hippocampal and/or prefrontal cortical GABA function, although no data currently exist on the effects of combined SI plus subchronic MK-801 on GABA function. People with schizophrenia show similar changes in GABA function (Beasley et al., 2002; Hashimoto et al., 2008; Lewis, 2000; Lewis et al., 2008; Sakai et al., 2008; Torrey et al., 2005; Zhang and Reynolds, 2002) and increased reactivity to novel stimuli (Cortiñas et al., 2008; Dawson and Neuchterlein, 1984). The observation that shock-probe burying and social interaction were affected by SI but not by subchronic MK-801 might suggest that these deficits are not related to changes in GABA function. Further studies are examining the role of GABA in deficits observed in animal models of schizophrenia-like symptoms.

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