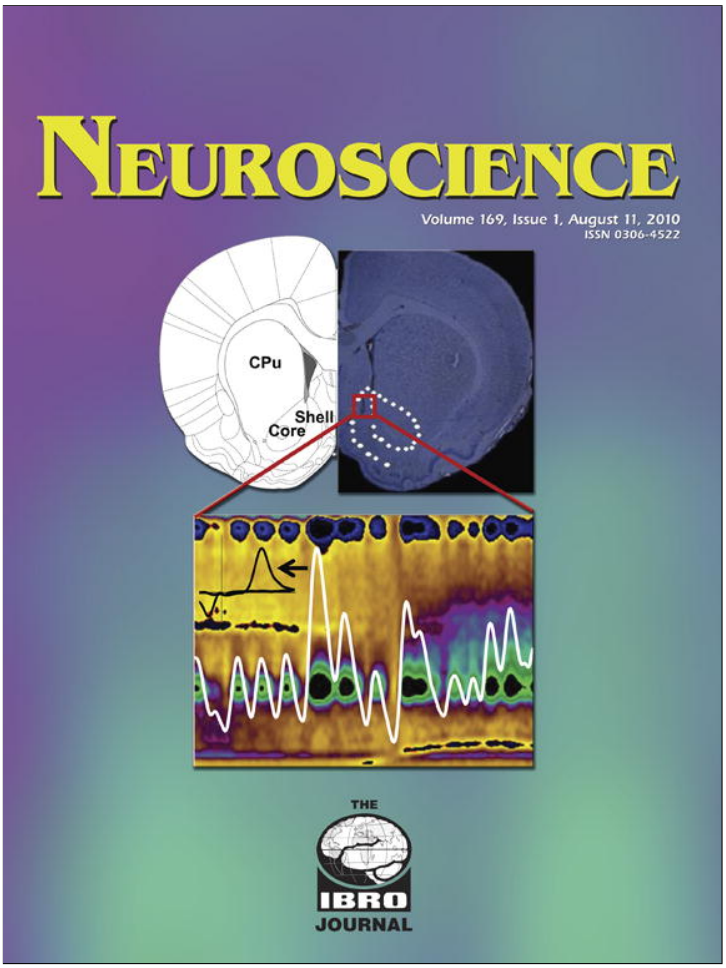

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POST WEANING SOCIAL ISOLATION INFLUENCES SPATIAL COGNITION, PREFRONTAL CORTICAL SYNAPTIC PLASTICITY AND HIPPOCAMPAL POTASSIUM ION CHANNELS IN WISTAR RATS

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Abstract—Post weaning isolation-reared rats show deficits in learning and memory, which are also seen in many psychiatric disorders like schizophrenia. The present study utilized behavioral and electrophysiological tests to further characterize cognitive disorders in this rat model, and to explore possible neurobiological mechanisms associated with them. Isolation rearing was performed in male Wistar rats from weaning for 8 weeks. Spatial memory and reversal learning were assessed using Morris water maze (MWM); synaptic plasticity was assessed by recording long-term potentiation (LTP) from thalamus to prefrontal cortex; and potassium ion channel currents were tested using the cerebrospinal fluid (CSF) of different groups in hippocampal slices by patch clamp. The results of MWM showed that isolation-reared rats performed worse in probe trials and memory retention tests. The LTP tests showed that the prefrontal cortical postsynaptic potential slopes were significantly lower in isolated rats than group housed ones. The patch clamp recording showed that the amplitudes of hippocampal voltage-dependent transient outward K^+ currents (I_A) were enhanced, and the steady inactivation curve of I_A was shifted towards positive potential by CSF of isolated rats. These data suggested that isolation rearing can impair the spatial cognition of rats, with the possible mechanisms of affecting prefrontal cortical synaptic plasticity and hippocampal potassium ion channel currents.

Key words: isolation rearing, spatial cognition, LTP, prefrontal cortex, potassium ion channel, hippocampus.

There is substantial evidence that social isolation from weaning influences brain development and plays an important role in the causation of certain psychiatric disorders such as schizophrenia. In rats, following isolation rearing from weaning, the following aspects can be observed: disrupted prepulse inhibition and raclopride reversed prepulse inhibition deficit (Geyer et al., 1993; Roncada et al., 2009); enhanced amphetamine-induced locomotion and dopamine release (Weiss et al., 2000; Fone and Porkess, 2008); impaired novel object recognition and attentional set-shifting (McLean et al., 2010); increased social interaction and aggression (Ferdman et al., 2007); reduced prefrontal cortex (PFC) volume and altered accumbal protein expression (Day-Wilson et al., 2006; Schubert et al., 2009). These behavioral, morphological and neurochemical abnormities strongly resemble the core features of schizophrenia, which suggest that examination of the behavioral and neurophysiological changes induced by social isolation in rats may shed light on the neurobiology and treatments of schizophrenia.

Schizophrenia is associated with deficits in a wide variety of cognitive domains including executive function, attention, memory and language (Weickert et al., 2000). Cognitive dysfunction is estimated to occur in 75%–85% of patients with schizophrenia, often precedes the onset of other symptoms (Reichenberg et al., 2006), and persists even after other symptoms have been effectively treated (Heinrichs, 2005). The cognitive deficits have been linked to functional outcome and are relatively independent of the psychotic symptoms (Gold et al., 1995; Green, 1996). Thus it is important to further explore the characteristics and mechanisms of cognitive deficits in order to find better therapeutic targets.

Spatial memory is often used as an index to evaluate cognitive function in animal models, and the growing evidence reflects the continuing importance of hippocampus for spatial memory (Sutherland et al., 2001; Clark et al., 2007; Ibi et al., 2008). Results are inconsistent concerning the impact of isolation on spatial cognition in the Morris water maze. Most groups find no alteration in acquisition (Lapiz et al., 2001; Schrijver et al., 2004), although some have reported improvements (Wongwitdecha and Marsden, 1996) or impairments in learning (Hellemans et al., 2004) accompanied by reduction in long-term potentiation in the CA1 area of rat hippocampus (Lu et al., 2003). Reversal learning, which examines the ability to flexibly adapt the response to a change in learning contingencies (Murray et al., 2008), is also of particular interest in rat models for testing cognitive function (Russig et al., 2003; Abdul-Monim et al., 2006; Li et al., 2007). Reversal learning has been shown to be mediated by medial prefrontal cortex (Birrell and Brown, 2000). In the clinical field, Wisconsin Card Sorting Test is often used to evaluate the reversal learning ability in patients, and schizophrenic patients usually score lower than normal controls, thereby reflecting cognitive rigidity (Rogers et al., 2000). In this

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Abbreviations: aCSF, artificial cerebrospinal fluid; CSF, cerebrospinal fluid; I_A, voltage-dependent transient outward K^+ currents; IT, initial training; I–V, current–voltage; LD, laterodorsal thalamic nucleus; LDDM, laterodorsal thalamic nucleus, dorsomedial part; LTP, long-term potentiation; mPFC, medial prefrontal cortex; MRT, memory retention test; MWM, morris water maze; NE, northeast; NW, northwest; PFC, prefrontal cortex; PrL, prelimbic area; PSP, postsynaptic potential; PT, probe trials; RT, reversal training; SE, southeast; SW, southwest; TEA, tetraethylammonium.

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experiment, we designed an environmental animal model, post weaning isolation-reared rats, to see if spatial memory and reversal learning were affected, and to further investigate the roles of hippocampus in spatial memory and PFC in reversal learning.

Synaptic plasticity, with activity-dependent alterations of excitatory synaptic transmission as its prevalent mechanisms, is widely believed to comprise the cellular basis for memory formation and cognition (Lanté et al., 2006). Long-term potentiation (LTP) of synaptic transmission is one of the functional indices of synaptic plasticity. Growing attention has been focused on LTP of PFC recent years, because of its close relationship with working memory as well as other cognitive problems in schizophrenia (Yang and Chen, 2005; Lopes Aguiar et al., 2008; Coppa-Hopman et al., 2009). The laterodorsal thalamic nucleus (LD) provides massive projections to the anterior cingulate cortex and the frontal cortex, such as the prelimbic area (PrL) and secondary motor cortices (Condé et al., 1990; Van Groen et al., 2002a). These projections may constitute one of the pathways that transmit spatial information to these cortical regions, which are also involved in certain aspects of spatial cognition and behavior (Taylor et al., 2003). Since considerable evidence suggests the contribution of thalamo–cortical pathway to cognitive deficits that are detectable even in early stages of schizophrenia (Lambe et al., 2007), we chose to record LTP from the thalamus to PFC to see if it would be affected in isolation reared rats, and thereby to elucidate a better understanding of PFC function in cognition.

It is well known that much damage is caused by interrupting the function of ion channels in the central nervous system (Calabresi et al., 1995). Potassium ion channel currents contribute to action potential duration, release of neurotransmitters and hormones, Ca^{2+}-dependent synaptic plasticity and epileptiform burst activity (Muller and Bittner, 2002). Under physiological conditions, potassium currents are important for the regulation of neuronal excitability and the maintenance of baseline membrane potential (Storm, 1990). Voltage-dependent potassium currents play crucial roles in modifying neuronal cellular and network excitability and activity (Muller and Misgeld, 1991). Transient outward K^+ current (I_A) is the main neuronal voltage-dependent K^+ currents. It is involved in regulating action potential threshold and plays an important role in repolarization and regulation of firing frequency in many neuron types (Thompson, 1977). The experiments investigated the effects of cerebrospinal fluid (CSF) in different groups on I_A of hippocampal CA3 neurons, in order to find out whether spatial memory was related to the function of potassium ion channel in hippocampus of isolation rats, thus to further explore the mechanism of hippocampal function in cognition.

EXPERIMENTAL PROCEDURES

Subjects

Fourteen male Wistar rats aged 21 days (weaning) were purchased from the Laboratory Animal Center, Academy of Military Medical Science of People's Liberation Army, and were reared in standard rodent cages in animal house of Medical School, Nankai University, under the condition of a constant temperature of 24 °C (±2 °C) and a 12 h light/dark cycle (lights on 7:00h). Food and water were freely available during all phases of the experiment. The rats were randomly divided into two groups: group housed rats (n=7) were housed three or four per cage (48×30×18 cm^3) and isolated rats (n=7) were housed separately with each individual in one cage. Group housed and isolated rats shared the same light, temperature and humidity controlled environment and could see, hear and smell each other. Human contact was restricted to daily visual checks, replenishment of food and water and weekly cage cleaning. All efforts were made to minimize the number of animals used and their suffering. Rats were reared for 8 weeks. The experiments were carried out according to the guidelines of the Beijing Laboratory Animal Center, and approved by the Ethical Commission at Nankai University.

Behavioral experiments in water maze

Rats of two groups were trained and tested in Morris water maze (MWM) to monitor their spatial learning and memory. The water maze consisted of a tank that was 150 cm in diameter and 60 cm in height. It was filled to the depth of 45 cm with water maintained at 23 °C (±1 °C) by an automatic heater. Black nontoxic ink was added to make the water opaque. The tank was divided into four quadrants by two imaginary perpendicular lines crossing in the center of the tank. The four quadrants were clockwisely named as NE, SE, SW and NW for northeast, southeast, southwest and northwest respectively. A movable black circular platform (10 cm in diameter) was located in the center of SW quadrant. The top of the platform submerged 2–3 cm below the water surface so that the rat could easily climb on it to escape from the water. The swimming path of the rats was recorded using a camera mounted 2.0 m above the center of the pool and analyzed using a computerized video tracking system (Ethovision 2.0, Noldus, Wagenigen, Netherlands). The room was furnished with several extra-maze cues (such as lamp, curtain) immobile throughout the entire experiment process.

Training and testing in the MWM comprised of four consecutive stages: Initial Training (IT); Probe Trials (PT); Reversal Training (RT); Memory Retention Test (MRT) after a 7 day rest period. This protocol was adopted and modified on the basis of previous studies (Dursun et al., 2006; Su et al., 2009). The skeleton of MWM experiment design was shown in Table 1.

The first stage, IT, from the first day morning to the fifth day morning, trains rats to find the hidden platform and examines rats' acquisition of spatial memory. In IT stage, each rat was given nine sessions of trainings. Each session consisted of four trials and each trial lasted for 1 min. The platform was placed in the center of SW quadrant. A rat was placed into the water facing the pool wall at one of the four starting points (north, south, east and west) in a semirandom order so that each point was used only once in one session. The rat was forced to swim and learn to find the hidden platform. The trial terminated when the rat found the platform and stayed for at least 3 s. If the rat did not find the platform within 1 min, the experimenter guided the rat to the platform and forced it to stay on the platform for at least 3 s. The rat was returned to its cage afterwards for a 10 min inter-trial rest, when other rats were trained. All rats received the same order of start positions during trials. Two parameters were recorded: Escape latency, swimming time to locate the hidden platform; Swim distance (path length), the distance (m) swum from the start location to the hidden platform.

The second stage, PT, from the fifth day afternoon to the sixth day morning, examines rats' persistence of spatial memory. In PT stage, each rat was given one session of test, consisting of four probe trials. Each probe trial lasted for 1 min. The platform was taken out. The four trials were started from different points in a

Table 1. The skeleton of Morris water maze experiment design

Stages	Sessions and time points		Parameters
Initial training (IT)	S1−1st day 10 AM S3−2nd day 10 AM S5−3rd day 10 AM S7−4th day 10 AM S9−5th day 10 AM	S2−1st day 4 PM S4−2nd day 4 PM S6−3rd day 4 PM S8−4th day 4 PM	Escape latency and swim distance
Probe trials (PT)	S10−5th day 4 PM (2 trials) and 6th day 10 AM (2 trials)		SW quadrant dwell time and platform crossings
Reversal training (RT)	S11−7th day 10 AM	S12−7th day 4 PM	Escape latency and swim distance
Memory retention test (MRT)	S13−15th day 10 AM (2 trials) and 4 PM (2 trials)		SW, NE quadrant dwell time and platform crossings

Each session (S) of the four stages of Morris water maze (MWM) experiment consisted of four probe trials, and each trial lasted for 1 min.

semirandom order. We use four trials instead of a single trial to rule out the possible effect of different time (morning/afternoon) and different starting locations. Two parameters were recorded: Quadrant dwell time, percentage of time spent in platform quadrant (SW); Platform crossings, numbers passing platform region.

The third stage, RT, from the seventh day morning to afternoon, trains rats to find the hidden platform in an opposite position and examines rats' reversal learning ability. In RT stage, each rat was given two sessions of trainings. The platform was placed in the center of NE quadrant, which was opposite to SW quadrant. The method used and parameters recorded were the same as those in IT stage.

The fourth stage, MRT, from the 15th day morning to afternoon after a week's rest period, examines rats' long term memory of the two platform regions. In MRT stage, each rat was given four trials of test. The method used was the same as that in PT stage. Three parameters were recorded: Quadrant dwell time in NE (new platform quadrant); SW (original platform quadrant); Platform crossings.

In vivo LTP test

The day after MWM test, rats were given LTP test. Following administration of 30% urethane (Sigma-Aldrich, St. Louis, MO, USA, 4 ml/kg, i.p.), the rat was placed in a stereotaxic frame (Narishige, Japan). A 1–2 cm long incision was made along the midline of the scalp. At the electrode inputting region of the left side, two small holes (about 5 mm in diameter) were made in the skull with a dental drill. A concentric bipolar stimulating electrode was slowly implanted into the laterodorsal thalamic nucleus, dorsomedial part (LDDM) (2.3–2.8 mm posterior to the bregma, 1.4–2.0 mm lateral to midline, 4.2–4.7 mm ventral below the dura). Recording electrode was implanted into PrL of medial prefrontal cortex (mPFC) (3.0–3.3 mm anterior to the bregma; 0.7–1.0 mm lateral to midline; 2.8–3.4 mm ventral below the dura). Test stimuli were delivered to the LDDM region every 30 s at an intensity that evoked a response of 70% of its maximum (range 0.2–0.5 mA). After a 30 min baseline, high-frequency stimulation to induce LTP consisted of two series of 10 trains (250 Hz, 200 ms) at 0.1 Hz, delivered at test intensity (Hotte et al., 2007). Field potentials were recorded every 30 min after LTP induction ($n=7$ for each group). Evoked responses were stored as averages of four. Postsynaptic potential (PSP) slope was measured as the average slope from 20% to 80% of the first positive deflection of the potential.

In vitro patch clamp recording

After the LTP recording, use a transfusion tube with a needle connecting to the anterior part and a syringe connecting to the posterior part to abstract CSF from the cisterna magna. The CSF was kept in 4 °C fridge after centrifuge for conservation until patch clamp recording. CSF was added to the extracellular solution for extracellular application. The final concentration of CSF used in the experiment was 100 μl/3 ml. The effect of CSF on I_A reached the maximum within 2 min.

Hippocampal slices were prepared as previously described (Liu et al., 2007; Tian et al., 2009). Transverse hippocampal slices (400 μm thick) were obtained from 18 to 21 days old male Wistar rats. Animals were decapitated under deep anesthesia with chloral hydrate (i.p. 350 mg/kg) and hippocampal slices were cut using a vibratome (VT1000S, Leica, Germany). Slices were incubated in room temperature (~25 °C) artificial cerebrospinal fluid (aCSF) containing (in mM) 126 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $MgCl_2$, 2 $CaCl_2$, 10 D-glucose and aerated with 95% O_2 and 5% CO_2 at pH 7.4.

Slices were individually transferred to a recording chamber maintained continuously perfusion with oxygenated aCSF, pH 7.4. For whole cell recording, patch electrodes were prepared from borosilicate glass using a vertical electrode puller (PIP5, HEKA, Germany) to produce tip openings of 1–2 μm (3–5 MΩ). Electrodes were filled with an intracellular solution containing (in mM) 130 KCl, 10 HEPES, 0.5 $CaCl_2$, 10 EGTA, 2 $MgCl_2$, 2 Mg-ATP, and 0.3 Na-GTP, pH 7.3. Whole-cell patch-clamp recordings were made from pyramidal cells in the CA3 region of the hippocampus, visually identified with infrared-differential interference contrast optics (BX51WI, Olympus, Japan) and a television monitor connected to a low light sensitive CCD camera (710M, DVC, USA). Positive pressure was applied to the recording pipette as it was lowered into the medium and approached the cell membrane. Constant negative pressure was applied to form the seal (>1 GΩ) when the recording pipette attached to the membrane. A negative pressure was applied to rupture the cell membrane and access whole cell configuration. Series resistance was 10~30 MΩ, and recordings in which series resistance changed significantly were discarded. All experiments were carried out at room temperature of ~24 °C. Only one slice was used for any given experiment.

To record transient outward potassium currents, 1 μM tetrodotoxin and 0.2 mM $CdCl_2$ and 70 mM tetraethylammonium (TEA) were added to extracellular solution. Total outward potassium current stimulated with 300 ms depolarizing pulses from −60 to +60 mV. Evoked currents were low-pass filtered at 2.9 kHz, digitized at 5 kHz, and stored in PC computer (HP Company) using Pulse version 8.52 software (HEKA, Germany). Capacity transients were cancelled and series resistance was compensated (>70%) using the internal circuitry. The leakage currents were subtracted by the P/4 procedure.

Statistical analysis

All the data were expressed as mean±SEM, and were analyzed by SAS, Igor 5.04 and Origin 7.0 software. Significant level was set at 0.05. Of the MWM test, a two-way repeated measures ANOVA was used with "group" as the between-subject factor and "session" as repeated measure during IT and RT stages. Student's *t*-test was performed on the data from single session during PT and MRT stages for analysis of differences between groups.

Of the LTP test, PSP slopes were expressed as the percentage change of the baseline. Two-way repeated measures ANOVA was applied for analysis of differences between two groups. Of the patch clamp test, N cells in N different slices were examined, and only one neuron per slice was used for any given experiment. The analysis was done by averaging six cells in either group. Statistical comparisons were made using the Independent samples T test as appropriate.

RESULTS

Changes of spatial cognition in MWM

In MWM experiments, during IT stage, mean escape latency was calculated for each rat on each of nine training sessions. Two-way repeated measures ANOVA conducted on the escape latency for both groups confirmed statistical difference of session ($F_{(8,96)}=73.347$, $P<0.001$), while no statistical difference of session×group interaction ($F_{(8,96)}=1.308$, $P>0.05$) or group ($F_{(1,12)}=0.275$, $P>0.05$), as both groups did improve over the nine sessions of training (Fig. 1A). Similar results were shown on swim distance (figure not shown). During PT stage, SW quadrant dwell time and mean platform crossings were calculated for each rat. Student's *t*-test confirmed that the differences between groups was significant for SW quadrant dwell time ($P>0.05$) and platform crossings ($P<0.001$). Rats in isolated group passed fewer numbers across platform region (Fig. 1B), and spent shorter percentage of time in SW quadrant than group housed rats (Fig. 1C). The results of the first trial were similar to those of four trials average.

During RT stage, mean escape latency was calculated for each rat on each of two training sessions. Two-way repeated measures ANOVA confirmed statistical difference of session ($F_{(1,12)}=5.773$, $P<0.05$), but no statistical difference of session×group ($F_{(1,12)}=1.283$, $P>0.05$), or group ($F_{(1,12)}=2.406$, $P>0.05$), as both groups did improve over the two sessions of reversal training (Fig. 1A). Further analysis for the individual session showed that there was statistical difference between two groups in the second session ($F_{(1,12)}=10.693$, $P<0.05$). Similar results were shown on swim distance (figure not shown). During MRT stage, fewer platform crossings ($P<0.05$), shorter NE quadrant dwell time ($P<0.01$) and longer SW quadrant dwell time ($P<0.05$) were confirmed by Student's *t*-test when comparing isolated rats with group housed ones (Fig. 1B, C). The results of the first trial only were similar to those of four trials average.

Changes of LTP within the LD thalamus–PFC pathway

In LTP test, stimulation of LDDM evoked a basal excitatory PSP in PrL of mPFC and high frequency stimulation induced LTP of the stimulated synapses for at least 2 h. Results representing the time course of PSP slopes nor-

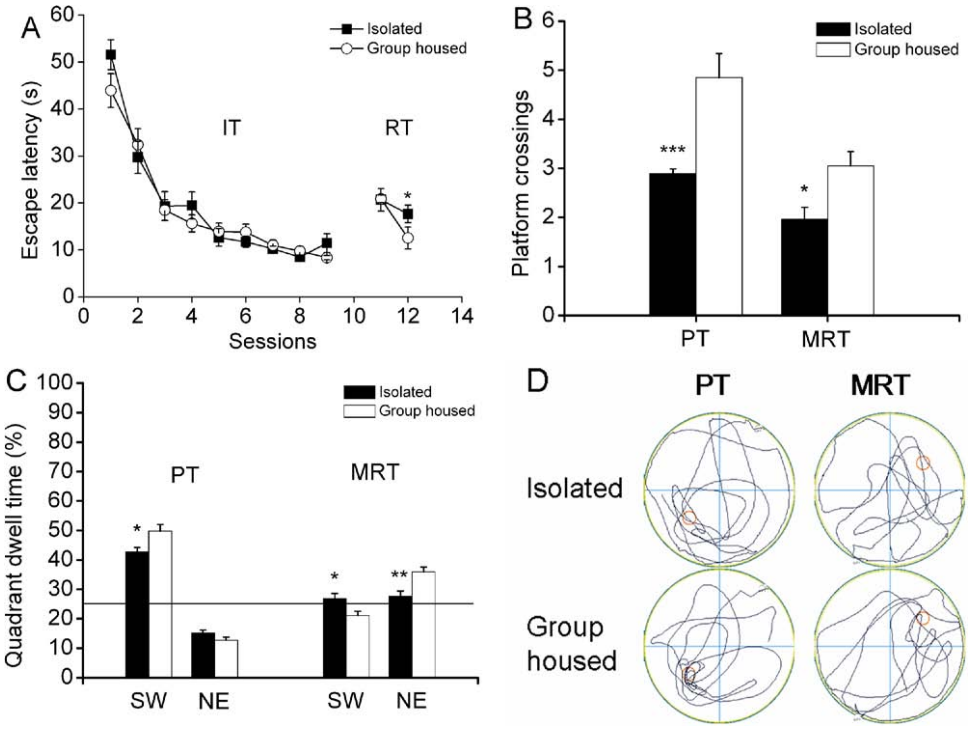

Fig. 1. Measures of spatial memory and reversal learning in the MWM. (A) Mean escape latency calculated for each training session in isolated and control groups in initial training (IT) and reversal training (RT) stages. (B) Mean platform crossings calculated for each session of trials in two groups in probe trials (PT) and memory retention test (MRT) stages. (C) Mean percent of platform quadrant (SW/NE) dwell time calculated for each session of trials in two groups in probe trials (PT) and memory retention test (MRT) stages. Line at 25% represents chance level. (D) Representative swim traces of rats in PT and MRT stages. Data are presented in sessions of four trials as mean (±SEM). Error bars indicate SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ significant difference from group housed, $n=7$ per group. Page: 19For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

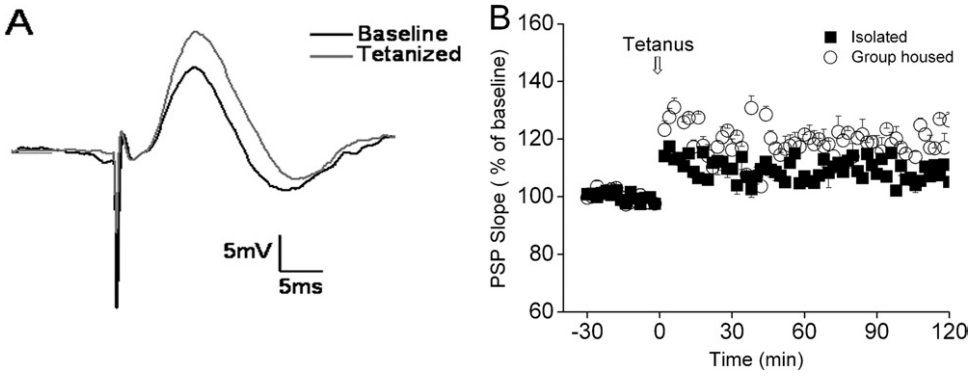

Fig. 2. Representative PSPs evoked in the PFC by stimulation of the LDDM region (A) and time coursing changes in slopes of PSPs in two groups (B). The PSP slope is plotted as a percentage change against the baseline before high-frequency titanic stimulation. Each point represents mean±SEM of averaged to four consecutive evoked responses. Values are expressed as percentage of change relative to baseline (30 min). ANOVA showed a significant difference of PSP slopes in isolated rats compared to group housed ones ($F_{(1,12)}$=7.579, P<0.05). Tetanic stimulation is indicated by arrows. Error bars indicate SEM.

malized to the 30 min baseline period were shown in Fig. 2. The PSP slopes increased immediately after the high frequency stimulation and stabilized to a level above the baseline period. ANOVA showed that PSP slopes were significantly smaller in isolated group compared to that of control group ($F_{(1,12)}$=7.579, P<0.05). The PSP slopes at the time points 30 min, 60 min, 90 min and 120 min after baseline period were calculated for each rat. Each time point represents mean±SEM of averaged to four consecutive evoked responses. In isolated rats, LTP 30 min was 109.70±1.55; LTP 60 min was 107.04±1.76; LTP 90 min was 107.93±1.86; LTP 120 min was 105.18±0.34. In group housed rats, LTP 30 min was 122.96±0.89; LTP 60 min was 121.58±0.05; LTP 90 min was 118.98±1.10; LTP 120 min was 117.09±4.84.

Changes of potassium currents within hippocampal slices

For analysis of the current-voltage (I–V) relationship, neurons were held at −70 mV. I_A currents were obtained by 300 ms depolarizing steps from a command potential of −60 to +60 mV at 10 mV steps (Fig. 3A, B). Upon the application of CSF, the I_A currents were enhanced in a different way at different membrane potentials. Compared with group housed rats, the amplitudes of I_A currents were relatively high in isolated ones (Fig. 3C). To give a representation, the mean I_A current was 1.94±0.10 nA at the command potential of +60 mV in isolated rats, and 1.45±0.12 nA in group housed ones (n=6, $F_{(1,10)}$=0.619, P<0.01).

To investigate the activation kinetics of I_A, cells were held at −70 mV and potassium currents were obtained by 300 ms depolarizing steps from a command potential of −60 to +30 mV at 10 mV steps. Peak amplitudes for I_A currents evoked by the step pulses were converted into conductance by use of the equation $G=I/(V-Vr)$, where G is the conductance, I is the current, V is the membrane potential, and Vr is the reversal potential. Normalized conductance of potassium channels was plotted against the voltages of conditioning pulses, and fitted with a Boltzmann function $G/G_{max}=1-1/\{1+exp[(V-V_h)]/k\}$, where G_{max} is the maximal conductance, V_h is the membrane potential at half-activation, and k is slope factor. There were no differences of V_h or k between two groups (Fig. 3D). The value of V_h was −27.03±5.08 mV in isolated rats, and −29.97±3.20 mV in group housed ones (n=6, $F_{(1,10)}$=0.911, P>0.05). The value of k was 9.24±0.53 in isolated, and 10.82±0.97 in group housed (n=6, $F_{(1,10)}$=2.055, P>0.05).

The steady-state inactivation was studied based on the double-pulse protocols below: neurons were held at −70 mV and currents were elicited with a 100 ms test pulse to +60 mV proceeded by 60 ms prepulses to potentials between −120 and −40 mV. Peak amplitudes for I_A currents were normalized and plotted vs prepulse potentials. The curves were well fitted with Boltzmann equation: $I/I_{max.}=1/\{1+exp[(V-V_h)/k]\}$, where I_{max} is the maximal current. It can be seen that CSF of isolated rats significantly shifted the V_h of I_A inactivation curve to the positive potential, without a significant change of k (Fig. 3E). The value of V_h was −78.67±1.41 mV in isolated, and −88.10±1.87 mV in group housed (n=6, $F_{(1,10)}$=0.871, P<0.01). The value of k was 6.48±0.64 in isolated, and 6.05±1.02 in group housed (n=6, $F_{(1,10)}$=2.455, P>0.05).

DISCUSSION

No single “ideal” animal model can be expected to represent abnormalities in all human schizophrenia symptoms. With the advent of evermore new models emerging, 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). Schizophrenia can be seen as a disease of memory (Roberts and Greene, 2003). In our study, isolated rats showed impaired spatial cognition in water maze, including spatial memory, reversal learning and long-term retention. These changes resembled the memory problems in schizophrenia. In our study, isolated rats also showed an abnormality in LTP, the most widely accepted cellular model of learning and memory processes. Furthermore, the CSF of isolated

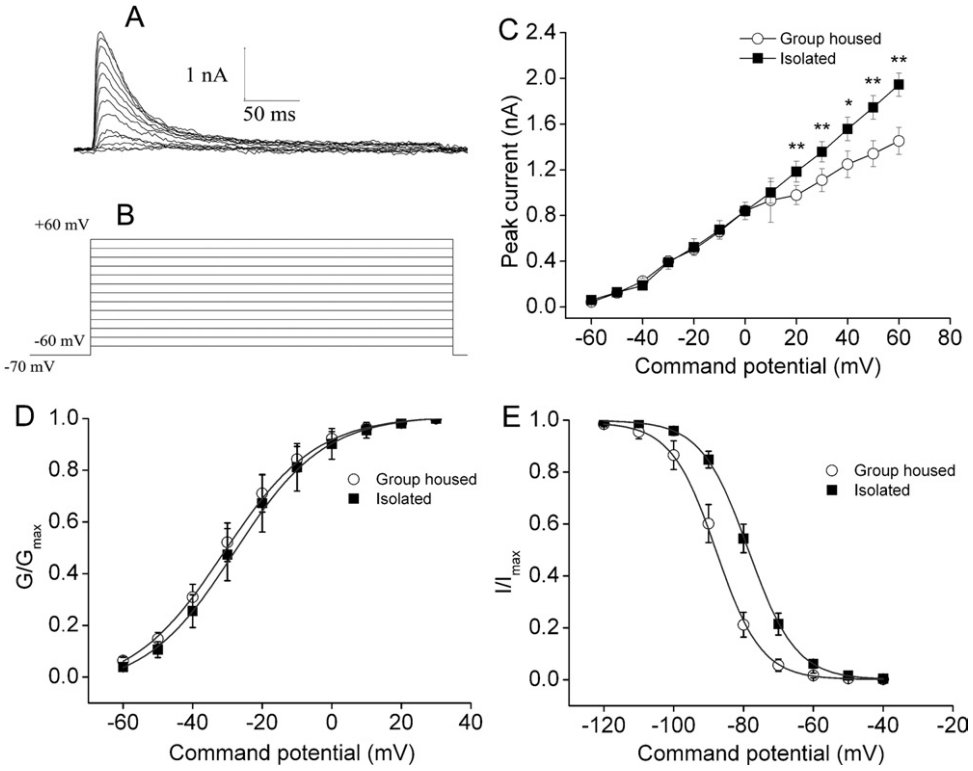

Fig. 3. Effects of CSF on transient potassium currents. (A, B) Representative whole-cell recording of transient potassium currents sample traces and the stimulated protocol. (C) The current–voltage relationship of I_A under different conditions. (D) The effects of group housed and isolated CSF on steady-state activation curve of I_A. (E) Effects of CSF on steady-state inactivation of I_A. Data are presented as mean±SEM (n=6, * P<0.05, ** P<0.01).

rats was used, for the first time, to investigate the influence on certain potassium ion channel currents of hippocampus slices. These results shed light on the mechanisms of learning memory deficits in schizophrenia.

The acquisition of spatial memory was mainly reflected through rats' performances in IT stage. The results showed that with the increase of training days, escape latency was decreasing. That no statistical difference between isolated and group housed rats was seen, either considering nine consecutive sessions or a specific session, indicated that isolation rearing would not affect rats' acquisition of spatial memory. The persistence of spatial memory was mainly reflected through rats' performances in PT stage. The results of platform crossings showed that the rats' performances in isolated group were worse than those in group housed. These results indicated that isolation rearing could impair rats' persistence of spatial memory. Most previous studies find no alteration in acquisition using a fixed platform position in the task (Lapiz et al., 2001; Schrijver et al., 2004), but showed impairments in persistence of spatial memory (Schimanski and Nguyen, 2004; Ibi et al., 2008). Our results agreed with them. Some groups have reported impairments in acquisition (Hellemans et al., 2004). However, the apparent impairment in acquisition compared to group housed controls may be due to the well documented affect of environmental enrichment in the group housed cage rather than any affect of isolation per se (Schrijver et al., 2002). We also observed rats' performances in MRT stage, to measure long-term memory retention. The results of platform crossings showed that rats in isolated group performed worse than those in group housed. This change of the long-term retention resembled certain cognitive deficit in schizophrenia patients during memory retrieval tasks such as item repetition (Heckers et al., 1998). Isolated rats may suffer from stress in a sensitive period during development which may result in alterations in hippocampal neurogenesis, such as reduced expression of development-related genes (Ibi et al., 2008). Some other studies reported reduction in LTP in isolated rats' hippocampus (Lu et al., 2003; Roberts and Greene, 2003). These changes may be associated with impaired spatial memory, as observed in our study.

The reversal learning ability was reflected through rats' performances in both RT and MRT stages. Our results suggested that isolation rearing would impair reversal learning. Though the escape latency of RT stage did not show significant difference, we could see that rats in isolated group spent relatively long time to find the platform in the second session. We used reversal learning here as an index to explore the character of attentional set-shifting ability. Reversal learning is a type of attentional set-shifting, which requires both inhibiting the previously reinforced behavioral strategy and acquiring that of the previously declined (Chudasama and Robbins, 2003). This has been shown to be mediated by mPFC (Birrell and Brown, 2000). Our results of quadrant dwell time showed that rats in

isolated group spent more time in previous quadrant and less time in new quadrant, which suggested that isolation rearing not only interfered in suppressing previously acquired behavior strategies but also interfered in establishing new strategies. These results further indicated that the impairment of attentional set-shifting was not only short term but long term, which revealed the cognitive rigidity, a major feature of cognitive disorder in schizophrenia (Rogers et al., 2000). Previous studies showed that isolation rearing could impair rats reversal learning and attentional set-shifting (Schrijver et al., 2004; Li et al., 2007; Fone and Porkess, 2008; McLean et al., 2010) consistent with the induction of cognitive rigidity, as discussed above. We used a different experiment design to get the same results with theirs.

LTP of synaptic transmission is one of the functional indexes of synaptic plasticity. Glutamate plays a principal role in modulating LTP, and is a likely key cellular mechanism for learning and memory (Malenka and Bear, 2004). The results of LTP test showed that the isolated group affected LTP in a negative way compared to group housed, so we thought there might be change of glutamate transmission in isolation rearing model. The deficiency or hypofunction of glutamate system especially NMDA receptor has been suggested in preclinical models of schizophrenia for decades (Duncan et al., 1999; Morrison and Pilowsky, 2007). Our isolation rearing model was consistent with this hypothesis. However, the isolation rearing model might not only affect glutamate system, but also affected other neurotransmitter systems like dopamine (Shao et al., 2009) and GABA (Serra et al., 2008). It is still unclear whether these are the cause or the result of glutamate abnormalities. There is evidence that lost control of mediation among different neurotransmitter systems may induce schizophrenia (Mathé et al., 1999).

By inducing LTP, we confirmed the presence of synaptic connections or projections from LDDM to PFC. The LD, located caudal to the anterior nuclei, is often treated as part of the anterior thalamic nuclear complex, which constitute part of the Papez circuit and playing important roles in spatial memory and learning (Aggleton et al., 1995; Sziklas and Petrides, 1999; Van Groen et al., 2002a), because of its location and its similar connectivity with the cortex (Steriade et al., 1997). Besides, there is evidence that lesions of lateral dorsal thalamus result in mild impairment of spatial learning and memory (Van Groen et al., 2002b; Mitchell and Dalrymple-Alford, 2006). Our study indicated for the first time that synaptic projections from LD to PFC might have participated in the regulation of spatial cognition. Studies in both animals and humans have implicated PFC and related subcortical afferent connections in mediating executive functions such as prospective coding, set-shifting, and working memory (Hauser, 1999; Birrell and Brown, 2000; Miller, 2000). Our finding gave us a further thought that the synaptic plasticity of the LD to PFC pathway might contribute to reversal learning and set-shifting.

To explore the mechanism of deficits in spatial memory, our study for the first time provided analyses of CSF modulation of transient outward currents in rat hippocampal slice. Employing CSF was to investigate whether the CSF of isolated rats could influence the potassium ion channels function. From our results, it could be seen that CSF of isolated group increased the amplitude of I_A, and shifted inactivation curves to depolarizing direction. Our data suggested that CSF in isolated rats could modulate potassium channels of hippocampal slices. This modulation involved changes of peak amplitude and steady-state inactivation, which may lead to an increase in the excitability and even death of hippocampal neurons. According to previous studies, blocking the currents with TEA or 4-aminopyridine resulted in reducing action potential duration significantly in neurons (Gong et al., 2008). And a decrease in K^+ currents could help mediate an increase in excitability (Desai et al., 1999). I_A modulates action potentials by increasing both the rate of action potential repolarization and accommodation. Our study suggested that CSF of isolated group led to the hyperexcitability, and the influence of CSF might be caused at least in part by changes in hippocampus of isolated group which resulted in the alteration of spatial memory. K^+ is the predominant cation in cytosol. Maintenance of a certain $[K^+]$ in the cytoplasm (140–150 mM) is essential for governing the cell excitability (Hoffman et al., 1997), setting resting membrane potentials (Parsons et al., 2002), regulating apoptotic enzyme activity (Xiao et al., 2002), and controlling cell volume (O'Reilly et al., 2002). Further studies are needed to understand the detailed changes of CSF in isolated rats as well as the mechanisms of CSF on ion channels.

In summary, isolation rearing from weaning, as a proper animal model of schizophrenia, impairs rats' spatial memory, reversal learning, synaptic plasticity in PFC, as well as potassium ion channel currents in hippocampus slices. Further studies investigating the essential mechanisms underlying the development of cognitive deficits during isolation rearing will be necessary for a better understanding of cognitive disorders in schizophrenia.

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