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Research report

Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats

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Abstract

The effects of postweaning social isolation (pwSI) on the morphology of the pyramidal neurons from the medial part of the prefrontal cortex (mPFC) and hippocampus were investigated in rats. The animals were weaned on day 21 postnatal (P21) and isolated 8 weeks. After the isolation period, locomotor activity was evaluated through 60 min in the locomotor activity chambers and the animals were sacrificed by overdoses of sodium pentobarbital and perfused intracardially with 0.9% saline solution. The brains were removed, processed by the Golgi-Cox stain and analyzed by the Sholl method. The locomotor activity in the novel environment from the isolated rats was increased with respect to the controls. The dendritic morphology clearly showed that the pwSI animals presented a decrease in dendritic length of pyramidal cells from the CA1 of the hippocampus without changes in the pyramidal neurons of the mPFC. However, the density of dendritic spines was decreased in the pyramidal cells from mPFC and Hippocampus. In addition, the Sholl analyses showed that pwSI produced a decrease in the number of sholl intersections compared with the control group only in the hippocampus region. The present results suggest that pwSI may in part affect the dendritic morphology in the limbic structures such as mPFC and hippocampus that are implicated in schizophrenia.

Theme: Disorders of the nervous system

Topic: Neuropsychiatric disorders

Keywords: Early social isolation; Locomotor activity; Medial prefrontal cortex; Hippocampus; Golgi-Cox stain; Schizophrenia; Neurodevelopmental

1. Introduction

Rat animals live in social groups, pre-pubertal and pubertal steps are critical to establish social organization in a group. During these periods, young rats spent much of the time in social play behavior [28]. The occurrence of social play follows an inverted U-shape during their development. It emerges at about 18 days postnatally (P18), giving peaks during P28 to P35 and decreases with age until sexual maturity [2,37,58]. The social play is crucial for establishing social organization in a group [59]. In addition, social isolation produces a well characterized syndrome consisting in hyperactivity, such as increased locomotor activity in response to a novel environment and dopamine (DA) agonist [25,33], decreased pain thresholds [44], deficit in the prepulse inhibition [11,19,61,64,65] and altered response to neuroleptic drugs [50]. These behavioral effects implicate alterations in DA function, in particular, in the DAergic mesolimbic system [19,25,33,64]. In addition, in vivo microdialysis data has revealed that social isolation produces an increase in the DA levels in caudate-putamen and nucleus accumbens [33].

Social dysfunction has been implicated in the development and course of schizophrenia [1]. Before schizophrenic symptoms initiate, the patients tends to social isolation; however, when the schizophrenic symptoms appear, the social isolation is exacerbated [3,26,31,62]. The medial part of the prefrontal cortex (mPFC) and hippocampus (Hc) appear to be critical sites of dysfunction in schizophrenic patients [40,63]. Recent data have shown a decrease in the dendritic spine density on the hippocampus and the medial part of the prefrontal cortical pyramidal

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neurons in subjects with schizophrenia [21]. Furthermore, evidence has been accumulated which implicates cortical neuronal error in the development in schizophrenia [63]. Studies on mPFC or ventral Hc lesions [4–6,14,15,36] induced in the neonatal rat brain indicated that these regions might be have participated in the development of subcortical dopaminergic activity. In addition, schizophrenic symptoms appear in adolescence or early adulthood, after the social play period. Given the importance of social functioning in schizophrenia, it is critical to understand factors with underlie deficits in this area. The postweaning social isolation (pwSI) model has been related with the schizophrenia [17,18,55].

In the present investigation, we have compared the developmental consequence of pwSI in rats 8 weeks after being weaned. At adult ages, dendritic longitude and spine density on pyramidal neurons from mPFC and Hc were evaluated in pwSI rats and controls. The results suggest an important role of the pwSI on the development and maturation of the mPFC and Hc pyramidal neurons in adult rats.

2. Material and methods

Male Sprague–Dawley rats used in this study were obtained and bred in our animal facilities (University of Puebla). On day 21 postnatal (P21) the animals were weaned and were randomly divided into two rearing conditions: nine rats were reared in isolation during 8 weeks and a similar number in social groups. Isolation-reared rats were housed individually in cages (45×20×20-cm high). While socially reared rats were housed three at a time in similar cages. All subjects were housed in the same room with 12 L:12 D and ad-lib food and water. Isolation only prevented physical contact since all subjects could see, hear, and smell other rats. All procedures described in this study were in accordance with the guidelines of the Laws and Codes of Mexico in The Seventh Title of the Regulations of the General Law of Health Regarding Health Research.

After 8 weeks (P77) of social isolation, the control of locomotion in social isolation rats was evaluated in eight-photocell activity boxes connected to a counter (Tecnología Digital, México). The locomotion following exposure to a novel environment in unacclimatized rats was evaluated and the rats were placed in an activity box for a 60-min period. Immediately after this was measured, the rats were deeply anesthetized with sodium pentobarbital and perfused intracardially with 0.9% saline. The brains were removed and processed by Golgi-Cox staining, using procedures described previously [20]. The brains were first stored in the dark for 14 days in Golgi-Cox solution followed by 3 days in 30% sucrose. The brains were sectioned 200-μm thick on the coronal plane at the level of mPFC and ventral Hc [43] (Fig. 1) using a vibrotome. Sections were collected on cleaned, gelatin-coated microscope slides (four sections/slide) and stained with ammonium hydroxide for 30 min, followed by Kodak Fix for film for another 30 min and finally were washed with water, dehydrated, cleared and mounted using a resinous medium.

The Golgi-impregnated pyramidal neurons from the mPFC or the Hc were readily identified by their characteristic triangular soma shape, apical dendritic extension toward the pial surface, and numerous dendritic spines. The following criteria were used to select pyramidal neurons for reconstruction: (1) localization of the cell soma in layer 3 of the mPFC (Fig. 2a) or in the CA1 from the ventral Hc (Fig. 2b) and within the middle of the thickness of the section; (2) full impregnation of the neurons; (3) presence of at least three primary basilar dendritic shafts each of which branched at least once; (4) no morphological changes attributable to Golgi-Cox staining. Five neurons in each hemisphere were drawn using a camera lucida at a magnification of ×250 by a person blind to treatment conditions. For each neuron, the basilar dendrite, including all branches, was reconstructed and the dendritic tracing was quantified by Sholl analysis [53]. In addition, the dendritic surface was quantified by counting the number of branches at each order from the cell body and by Sholl analysis [34], counting the number of ring intersections using an overlay of concentric rings. The density of dendritic spines was estimated by drawing at least 10-μm long segments from close to the cell body and from the terminal tips at high power (×1000) and counting the number of spines.

Data from the Sholl analyses and the spines densities were analysed using two-tailed Kruskal–Wallis and Mann–Whitney tests.

3. Results

3.1. Locomotor activity

The effects of the pwSI on locomotor activity in a novel environment are illustrated in Fig. 3. Both, control or social isolation animals exhibited active exploratory behaviour when placed in a novel environment. A significant increase in the locomotor behaviour was clearly observed in the pwSI animals group ($P=0.02$), when compared with the control group (Fig. 3b).

3.2. Golgi-Cox staining

Dendritic branching and density of dendritic spines on neuron from mPFC and Hc were measured by Golgi-Cox stain between pwSI and control rats. Maximum branch order, spine density and total dendritic length obtained were similar to previous reports [46,47]. The Golgi-Cox

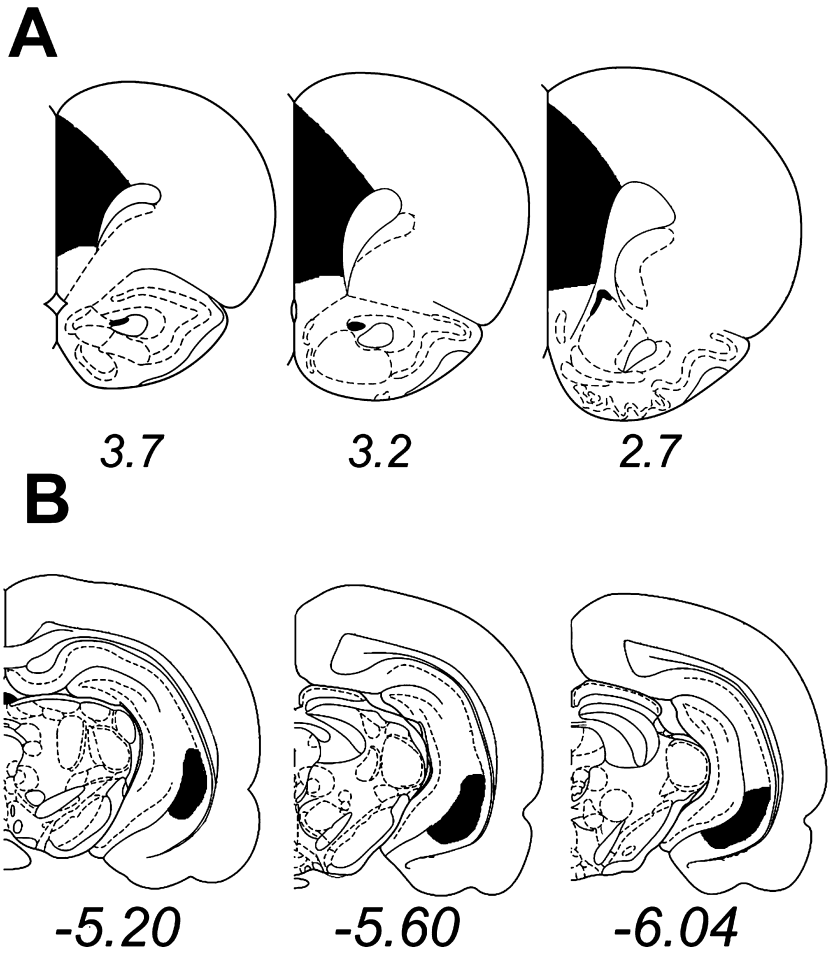

Fig. 1. Regions of interest used to analyze the dendritic branching and density of dendritic spines on neuron from medial part of the prefrontal cortex (A) and CA1 of hippocampus (B) by Golgi-Cox stain between postweaning social isolation and control rats (black areas). Reproduced from Paxinos and Watson's Atlas [43].

impregnation procedure clearly filled the basilar dendritic shafts and spines of layer 3 pyramidal neurons and CA1 pyramidal cells (Fig. 2). Comparisons between pwSI and control animals showed that the mean spine density on the basilar dendrites of pyramidal neurons from layer 3 of mPFC and CA1 the pwSI animals were lower than their controls (48–51% decrease in the close and far spine, respectively, from mPFC neurons and 70–58% decrease in the close and far spine density, respectively, from CA1 neurons) (Figs. 4 and 5). In addition, the maximum branch order in the social isolation was not different compared with the control group (Table 1).

As measured by Sholl analysis, total dendritic length from CA1 neurons differed significantly ($F=1.807$, $P=0.02$) between pwSI and controls rats (Fig. 6). However, at the level of mPFC pyramidal neurons, there were not significant differences between pwSI rats with the control group (Fig. 6). The analysis of intersection per radius of shell shows that pwSI animals were not different from the control rats (Fig. 7).

4. Discussion

The major aim of the present study was to investigate the consequences of 8 weeks of social isolation immediately introduced after weaning (P21) on the basilar dendritic structural morphology from layer 3 pyramidal cells of the prefrontal cortex and pyramidal neurons from the CA1 region of the hippocampus. We report here that the pwSI induces major reductions in dendritic spine density in layer 3 pyramidal neurons from the mPFC and pyramidal neurons from the Hc, these data may be linked in part to early social isolation presented in schizophrenia. In the same animals, pwSI caused an increase in locomotor activity in a novel environment as described previously [27,33].

The novelty induced locomotion data are consistent with previous reports [11,16,17,27,50,56,64] using the same pwSI paradigm. In those studies, isolation rats after weaned showed increased locomotor activity in response to a novel environment. Exactly how early SI came to

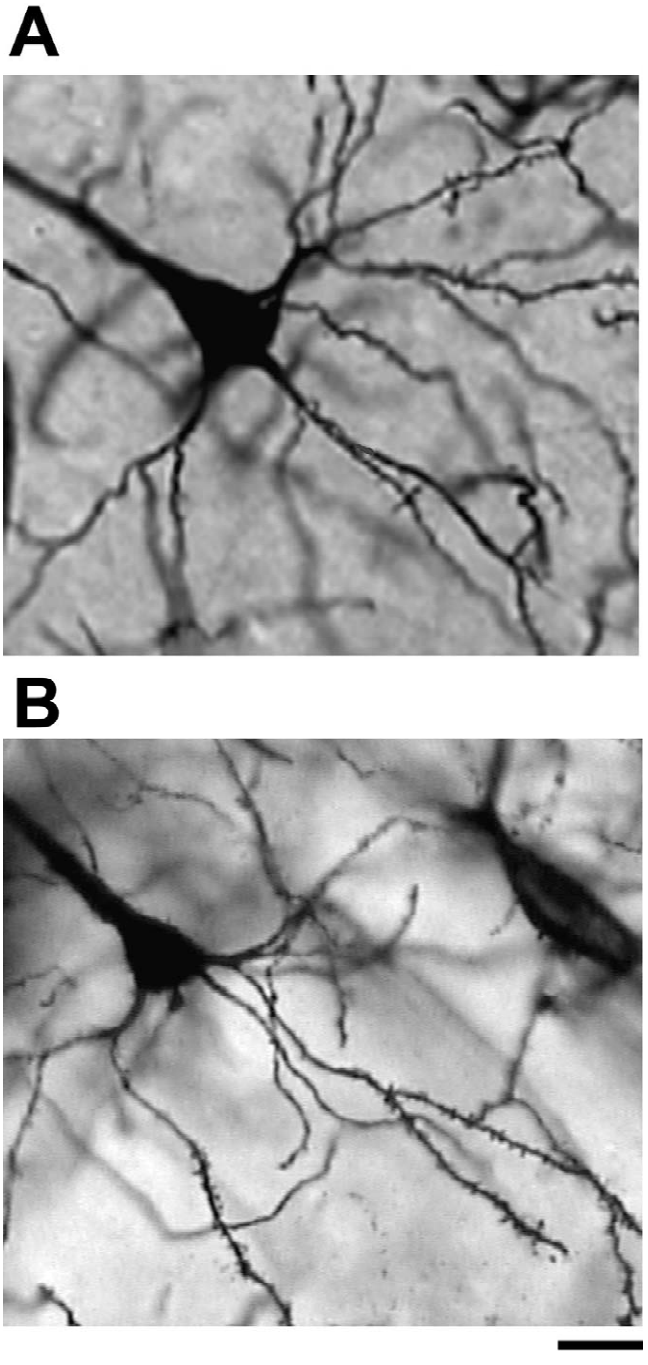

Fig. 2. Photomicrograph illustrating Golgi-Cox-impregnated pyramidal neurons from layer 3 of medial part of the prefrontal cortex (A) and from CA1 of hippocampus (B). Scale bar, 15 μm.

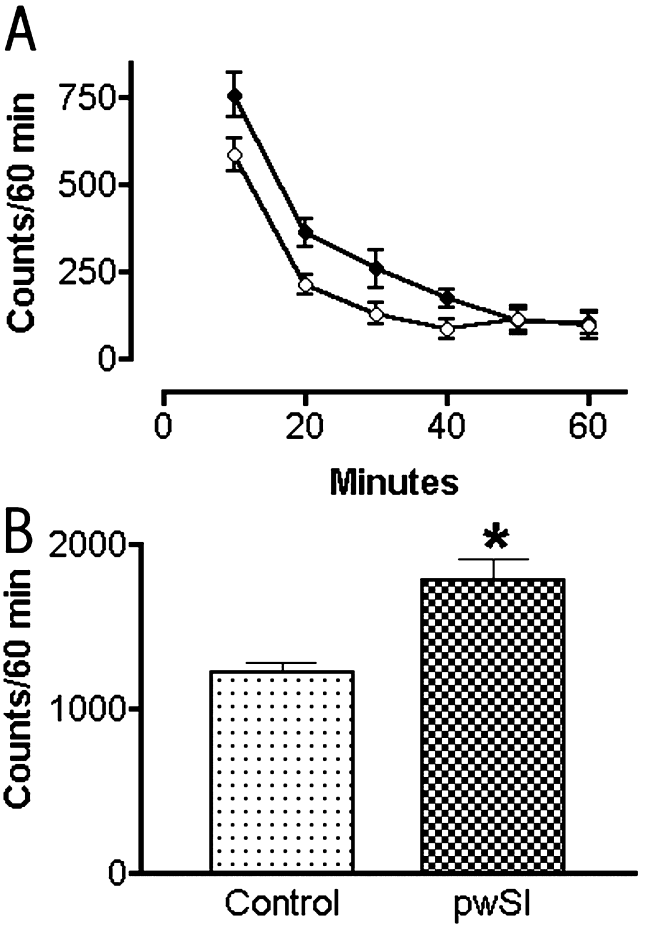

Fig. 3. Locomotor activity in a novel environment of control or social isolation animals tested at P70 ($n=10$ per group). (A) Temporal profile of locomotor activity (means number of beam interruptions per 10 min±S.E.M.). (B) Analysis of total activity scores (means number of beam interruptions per 60 min ±S.E.M.). *$P<0.05$, significantly different from the control group. pwSI, postweaning social isolation rats.

enhance locomotor activity is not clear. However, several evidences suggest that the DA system may in part participate in this altered behavior of this paradigm. Furthermore, DA agonists, like amphetamine or cocaine increase in locomotion in the pwSI animals [25,33]. In addition, social isolation increase locomotion in response to novel environment [33] and in vivo microdialysis data suggests that social isolation produced increase in the DA levels from the nucleus accumbens in basal conditions [33] and in response to *d*-amphetamine [33]. There is enough evidence to suggest that hyperactivity induced by pwSI is primarily mediated by DAergic mechanism of the nucleus accumbens.

It is interesting to note that pwSI animals present reduction in the density of dendritic spines more than alteration in the dendritic arborization in these two regions. Thus, it is possible that our finding of a decrease in the dendritic spines in the mPFC with reduction in the dendritic spines and the total length of dendritic in neurons from the Hc together with the changes in locomotion [27] may in part be correlated with DA levels in the nucleus accumbens [25]. In addition, the importance of the pwSI in regulating the dendritic morphology of the pyramidal neurons from the mPFC and the Hc and the mesolimbic DA system, may be recognized by examining its connectivity. It is known that the Hc sends excitatory projections to regions of the mPFC [32] and the mPFC projects to ventral tegmental area (VTA), the source of the mesocorticolimbic DAergic projections [52]. Furthermore, the Hc

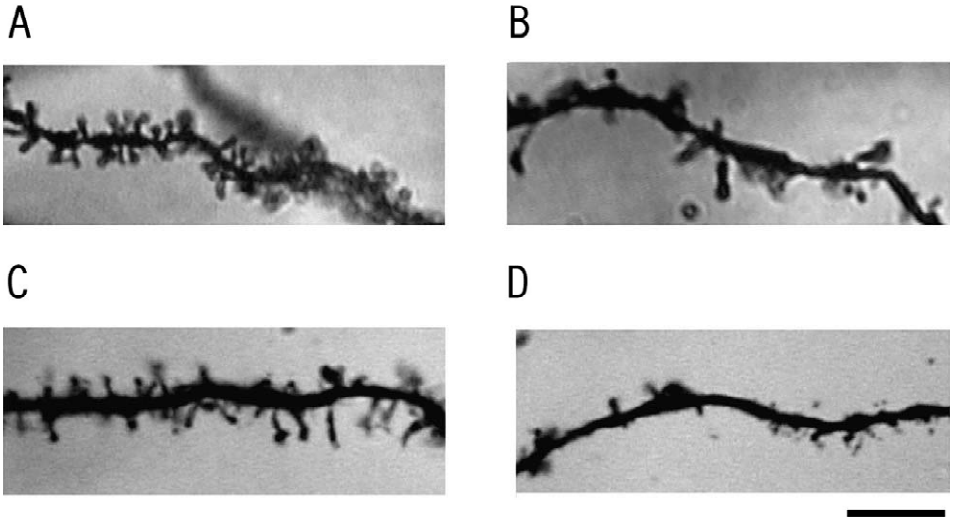

Fig. 4. Photomicrograph illustrating Golgi-Cox-impregnated dendritic spines of the pyramidal neurons from layer 3 of medial part of the prefrontal cortex and from CA1 of hippocampus. (A,C) From control animals, respectively; (B,D) from social isolation rats after weaning at P77, respectively. Scale bar, 5 μm.

and the mPFC send excitatory projections to the NA [10,23,24,51,63]. Therefore the social isolation may affect the neurons from the medial part of the prefrontal cortex and the hippocampus and consequently alter DA-related behaviors as locomotor activity in response to novel environment or DA agonist [27,33].

Consistent with our data, isolated birds showed a decrease in the dendritic spine density in the telencephalic areas [48]. Furthermore, Camel et al. [8], using an enriched environment after weaning, reported an increase in the dendritic length and dendritic spines in the pyramidal neurons from the occipital cortex. However, exposure to an enriched environment, consisting of a combination of increased exercise, social interactions and learning [12], and several reports [9,45,60] have shown that exercise such as running and swimming increase the neurogenesis in the hippocampal dentate gyrus of rats [60] and mice [45]. However, a recent report [12] using an enriched environment with a control of the exercise suggest that the changes in the morphology of the neurons from the Hc are a result of alterations in the animal's environment and not an increase in motor activity. With respect to the pwSI animals, the play behavior, exercise and learning are impaired during the social isolation period [59]. Together, these findings suggest that the observed reductions in dendritic spine density in the mPFC and the Hc may be related to the play behavior and learning deficit associated with a reduction in the exercise during the pwSI period. Finally, the significant decrease in dendritic spines in the mPFC and the Hc induced by social isolation after weaning is also in accordance with previous reports [41,42,54,57] providing further evidence of the effectiveness of the social interaction in the function of these two regions.

Our data clearly show an altered structural dendritic parameter in the pyramidal neurons from the 3 layer of the mPFC and the CA1 of the Hc in the pwSI animals. Spine density was significantly decreased in the Hc and mPFC, which projects to the nucleus accumbens [10,51], the main nucleus implicated in the behavioral responses typically observed in the pwSI [25,27,33]. Furthermore, these alterations are detectable in postmortem brains from subjects with schizophrenia [21,49]. Both sets of data are consistent with the hypothesis that the cortical excitatory inputs to those neurons are altered before puberty in rats or in subjects with schizophrenia [21]. The apparent morphological abnormality in the pwSI animals, together with the behavioral changes previously described [25,27,33], clearly suggest that these morphological and behavioral changes may in part be caused by an alteration in the social interaction processes rather than the other manipulation. In addition, the spine number has been reported to change rapidly in certain brain regions of experimental animals under various conditions [7,29,30,38], because dendritic spines are relatively plastic structures [30,31]. However, our pwSI animals were not submitted to any other manipulation or experimental conditions. In addition, pwSI did not produce changes in the maximum branch order of dendritic spines. Our results are consistent with the hypothesis that the number of excitatory inputs to these neurons is altered in subjects with schizophrenia and this animal model link with schizophrenia. It is not clear whether there are common mechanisms between subjects with schizophrenia and the pwSI animals in relation with the present results; however, it is known that the Hc sends excitatory projections to regions of the mPFC [22,32,63] and the neurodevelopmental abnormalities in the subjects with schizophrenia [63]. Therefore, pwSI may in part affect the medial part of the prefrontal cortical functions during prepubertal and pubertal period, which may in part

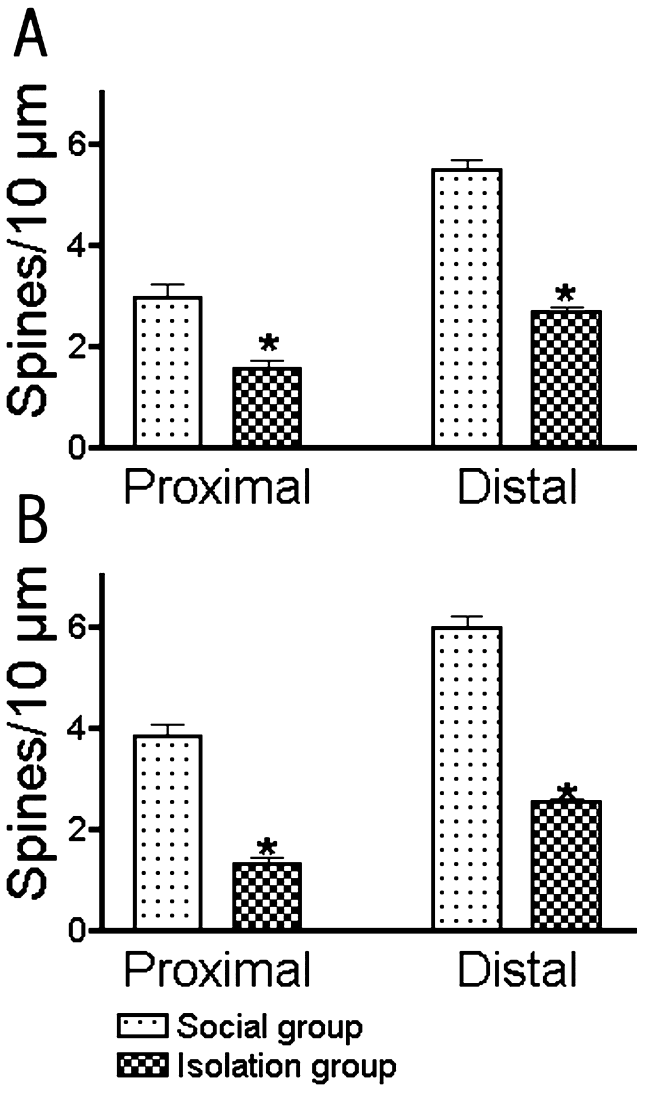

Fig. 5. Spine density in rats with postweaning social isolation or controls. (A) Medial part of the prefrontal cortex. (B) CA1 of hippocampus. Both proximal and distal spine density from medial part of the prefrontal cortex pyramidal neurons or CA1 of ventral hippocampus pyramidal cells decreased in the social isolation animals compared with the controls (*$P<0.01$).

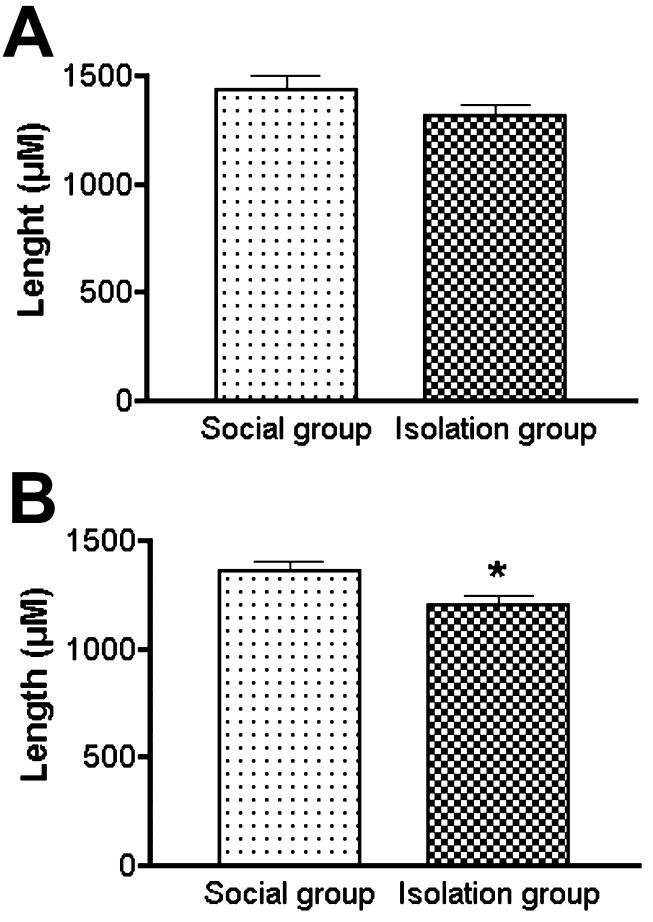

Fig. 6. Graphs of total dendritic length from pyramidal neurons of the layer 3 of medial prefrontal cortex (A) and CA1 of ventral hippocampus (B) of control or social isolation animals. The group of animal that received social isolation after weaning is represented as isolation group and the control group as social group. Total basilar dendritic length was decreased in the social isolation animals (*$P<0.01$).

Table 1
Summary of the maximum brain order

Maximum branch	Pyramidal neurons from mPFC		Pyramidal neurons from hippocampus	
order	% Control	% pwSI	% Control	% pwSI
1	0.0	0.0	0	0
2	8	8	7	6
3	26	36	31	35
4	41	32	44	37
5	21	18	15	17
6	2	4	3	4
7 or more	2	2	0	1

cause an alteration in the dendritic process. Furthermore, layer 3 from the mPFC may be regulated by hippocampal projections [13,32,35,51,52] and the activity of the nucleus accumbens neurons may be regulated by synaptic inputs from the Hc and mPFC [23,24,39,40].

In summary, our findings provide evidence for a decrease in excitatory inputs to the mPFC and Hc in early stages that alter the dendritic process with the biochemical and behavioral consequences. Given the interconnection between these two structures and the role of the mPFC in the regulation of dopamine-mediated behaviors, these behavioral, morphological and biochemical changes reported in the pwSI rats may contribute to explain some data reported in schizophrenia.

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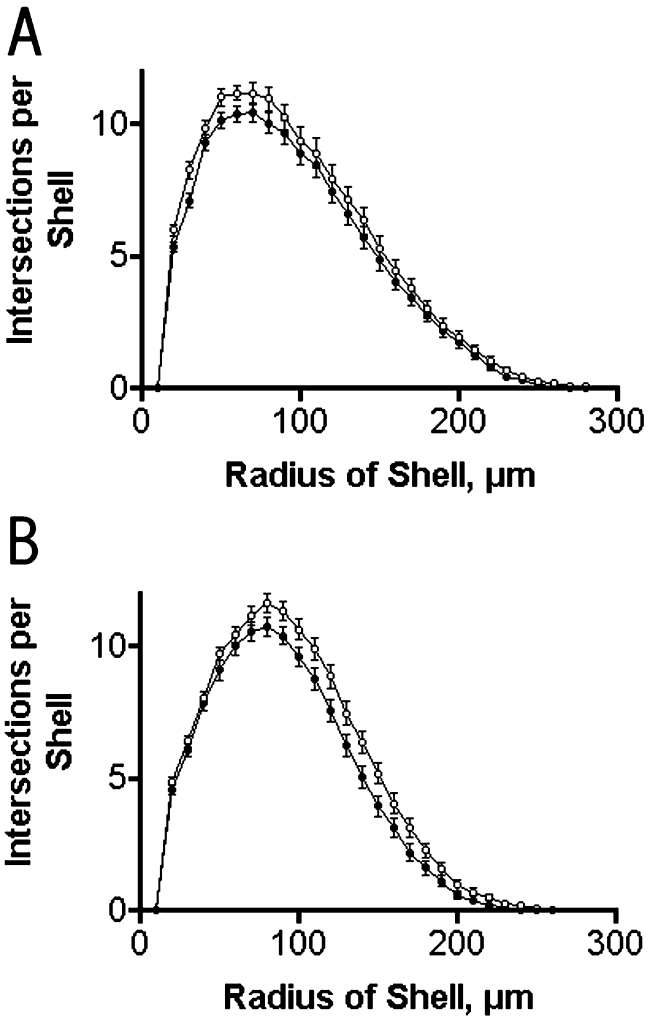

Fig. 7. Sholl analysis of basilar dendrites of medial prefrontal cortex layer 3 pyramidal neurons (A) or ventral hippocampus CA1 pyramidal cells (B). Close circles indicate the mean±S.E.M. (n=10) from post-weaning social isolation rats and the open circles corresponds the mean and ±S.E.M. (n=10) from control rats.

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