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Research report

Differential effects of social isolation in adolescent and adult mice on behavior and cortical gene expression

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HIGHLIGHTS

- A model of social isolation stress in mid-adolescence is proposed.
- A wide range of psychopathology-related behaviors and expression of excitatory, inhibitory and myelin-related markers in mPFC were analyzed.
- Mid-adolescent social deprivation is detrimental for normal development and may be particularly relevant as an animal model of developmental psychopathology.

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ABSTRACT

Intact function of the medial prefrontal cortex (mPFC) function relies on proper development of excitatory and inhibitory neuronal populations and on integral myelination processes. Social isolation (SI) affects behavior and brain circuitry in adulthood, but previous rodent studies typically induced prolonged (post-weaning) exposure and failed to directly compare between the effects of SI in adolescent and adulthood. Here, we assessed the impact of a 3-week SI period, starting in mid-adolescence (around the onset of puberty) or adulthood, on a wide range of behaviors in adult male mice. Additionally, we asked whether adolescent SI would differentially affect the expression of excitatory and inhibitory neuronal markers and myelin-related genes in mPFC. Our findings indicate that mid-adolescent or adult SI increase anxiogenic behavior and locomotor activity. However, SI in adolescence uniquely affects the response to the psychotomimetic drug amphetamine, social and novelty exploration and performance in reversal and attentional set shifting tasks. Furthermore, adolescent but not adult SI increased the expression of glutamate markers in the adult mPFC. Our results imply that adolescent social deprivation is detrimental for normal development and may be particularly relevant to the investigation of developmental psychopathology.

1. Introduction

Adolescence is a unique developmental period characterized by significant changes in neurotransmission dynamics, especially in the prefrontal cortex (PFC) [1–3]. Specifically, PFC maturation involves changes to pyramidal cells and interneurons, which lead to alternations in the excitatory/inhibitory balance [4]. Developmental studies also point to accelerated myelination during adolescence [5,6]. Abnormal maturation of PFC neurotransmission and connectivity patterns in adolescence permanently affects cortical function [5] and is associated with the emergence of psychopathology and cognitive dysfunction in adulthood [7,8].

Environmental factors in general, and the social environment in particular, play a major role in PFC development. Exposure to social stress or deprivation in adolescence leads to PFC-related behavioral deficits, alterations in excitatory (glutamatergic) and inhibitory (GABAergic) transmission [9–12], changes in connectivity patterns [13–16] and myelin-related gene expression [17]. Social isolation (SI) is commonly used to induce social stress in rodents. Chronic SI leads to changes in corticosterone levels [18,19], increases baseline and drug-induced locomotor activity in adulthood [20–22] and affects several measures of memory and attention [23].

While many rodent studies have looked at the effects of prolonged social deprivation in adolescence (most commonly from

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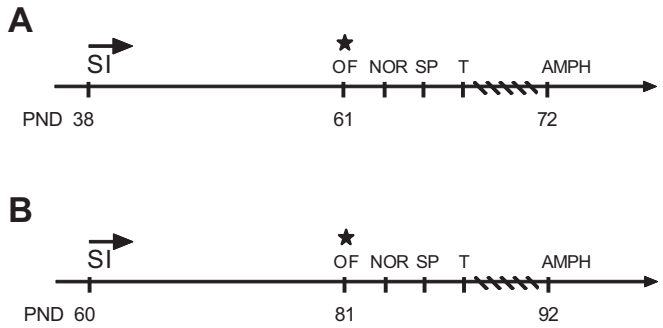

Fig. 1. Study Design. SI in mid-adolescence (A) or in adulthood (B) was followed by a battery of behavioral tests, conducted in the following order: locomotor activity and anxiogenic behavior in the open field (OF), novel object recognition (NOR), social preference (SP), reversal and EDSS in the T-maze (T), and baseline and AMPH-induced locomotor hyperactivity (AMPH). The T-maze and AMPH tests were separated by a 7-day hiatus. ⋆Behaviorally-naïve mice were sacrificed for gene expression studies. PND = postnatal day.

weaning (usually around postnatal day (PND) 21) through adulthood), few studies have examined the effects of SI starting in mid-adolescence, a period of development that overlaps with the onset of puberty and is characterized by accelerated neuronal and glial maturation [24]. Moreover, while SI in adolescence and adulthood have been investigated in separate studies [25,26], few have used the same behavioral and molecular paradigms to test the consequences of SI in these two developmental time periods. Thus, knowledge on the effects of SI is marred by conflicting findings, stemming from different isolation, behavioral and molecular protocols.

Here, we tested the effects SI starting in mid-adolescence on behavior and gene expression in the mPFC of male mice. As a comparison, we tested the effects of an identical period of SI starting in adulthood on the same behavioral and molecular measures. Since adolescence is a critical time point for the emergence of psychotic, affective and drug-related disorders [27], we assessed disease-relevant phenotypes including locomotor activity and exploration of the open field, social behavior, cognitive function and the locomotor response to the psychogenic drug amphetamine (AMPH). In our gene expression studies, we focused on the mPFC and on markers of glutamate and GABA transmission, and also measured the expression of glial myelination markers. We hypothesized that adolescent SI would have a unique impact on cognitive function and the balance between glutamate and GABA markers in the mPFC.

2. Materials and methods

2.1. Animals

C57BL/6 male mice (Harlan Laboratories, Rehovot, Israel) were used in all experiments. Temperature in the animal facility was maintained at 22 ± 2 °C, with 12 h light/dark cycle (light from 7:00 till 19:00). Animals had ad libitum access to food and water. Experimental procedures were performed in accordance with the guidance for animal experimentation by the United States National Institutes of Health and were approved by the local committee for handling experimental animals in Haifa University (425/16).

2.2. Social isolation procedure

In Experiment 1 (Exp 1), mice were randomly assigned to group housing (GH; n = 22) or SI (n = 22) experimental conditions in adolescence (postnatal day (PND) 38). In Experiment 2 (Exp 2), mice were similarly divided into GH or SI conditions in adulthood (PND60; n = 22/group; see Fig. 1 for study design). In each experiment, 16 mice per group were randomly assigned to behavioral testing and the remaining 6 mice were assigned to molecular analyses. A randomly chosen subset of the "behavioral" mice (n = 7–11/group) were tested in the water T-maze. In both Experiments, mice used for behavioral testing and molecular analyses were kept in separate cages. Mice assigned to the GH groups were housed with unfamiliar mice from the same condition, whereas mice in the SI condition were kept in individual cages for 3 weeks. Interaction with mice from both groups was kept to a minimum (i.e., cages were changed by the experimenter once a week, and the experimenter was the only person who came in contact with the mice in both groups). After 3 weeks, behavioral experimentation began. SI mice remained in individual cages throughout behavioral testing and were not re-grouped [28,29]. Weight before and after the SI/GH procedure was recorded in randomly selected mice (n = 15/group, selected randomly from mice later assigned for behavior or molecular analysis) and weight gain was computed as the difference between the weight before and after the housing manipulation. In the analysis of weight changes, data from 1 mouse (Exp 2, GH) was excluded (statistical outlier; > 2 SD higher than the mean, see 2.6). Mice designated for molecular analyses were behaviorally-naïve.

2.3. Drugs

d-Amphetamine (Tocris Bioscience, BR, UK) was freshly prepared prior to behavioral testing, dissolved in saline (0.9% NaCl) and administrated at a dose of 2 mg/kg, as previously described [30]. Saline was used as vehicle.

2.4. Behavioral testing

All behavioral testing was conducted during the light cycle and in the same dimly lit room. Ethanol 10% was used to clean experimental chambers in between trials. Trials were recorded and analyzed using Ethovision XT9.0 software (Noldus Information Technology, Leeburh, VA).

2.4.1. Locomotor activity in open field

The open field test is commonly used to assess basic locomotor activity as well as anxiety-like behavior [31,32]. The test was performed on PND61 in Exp 1 groups and on PND81 in Exp 2 groups (see Fig. 1). Mice were placed in a white plexiglass arena (50 L*50 W*40 H, cm) for 30 min. The total distance traveled (cm) and the time (s) spent in center of the arena were assessed.

2.4.2. Novel object recognition (NOR)

The NOR test is commonly used to assess recognition and working memory [33]. The paradigm was performed in the open field arena, as previously described [34], 24 h after the open field test. All objects were made of children's plastic building blocks. Briefly, during the *sample* phase of the task, mice were placed in the arena with two identical objects (red- and blue-colored cubes, 5 L*5 W*5 H cm, placed 12 cm away from two diagonal corners and fixed to the arena floor with adhesive tape). The *sample* phase ended when 30 s of object exploration were reached, with a 10 min limit. Following the *sample* phase, mice were placed in a holding cage for a 5 min inter-trial interval (ITI), and one of the objects in the arena was replaced with a novel object (multicolored pyramid, 6 L*6 W*6.5 H cm). In the *test* phase, mice were returned to the arena for 5 min. Novel object location was counter-balanced between animals. Exploration times (s) of the familiar object (A) and the novel object (B) during the *test* phase were assessed. The *exploration index* was calculated as novel object exploration time (s) divided by the total exploration time (s) during the test phase (B/A + B). One mouse from each experiment (GH in Exp 1 and in Exp 2) was excluded