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Adult resocialization restores sociability, reduced by adolescent social isolation in rats

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We aimed to assess the effect of 6-week adolescent social isolation on sociability and social novelty preference in male Wistar rats. In addition, we examined whether isolation effects would persist after adult resocialization. Our findings demonstrate that social isolation resulted in decreased social interest (the time spent interacting with an unfamiliar rat of the same sex and age decreased from 115.4 ± 13.3 s in group-housed animals to 82.3 ± 11.0 s in isolated, $P < 0.05$), while social novelty preference was not significantly different between isolated and group-housed rats. We also report that 6-week resocialization was sufficient to restore social interaction levels in isolated rats (time spent with an unfamiliar rat was 117.9 ± 12.6 s in isolated animals and 102.5 ± 10.9 s in group-housed). These findings may be useful for better understanding the impact of social deprivation on human subjects, which has recently become especially relevant due to the restrictions of the COVID-19 pandemic.
Key words: social isolation; adolescence; resocialization; sociability; social novelty.

INTRODUCTION

Recently, social isolation has become widespread around the world due to COVID-19 pandemic restrictions. Adolescents have been particularly affected by the lack of social contacts since peer interactions at this age are of great importance for normal development. By definition, adolescence is a gradual transition between childhood and adult age, during which substantial behavioral and neurobiological changes occur [1, 2]. In the male rat, the most commonly used time frame of adolescence lies between PND 21-59 [3]. Behaviorally, adolescence is characterized by increased peer-oriented socialization, risk-taking and novelty seeking [2, 3]. During the adolescent period, the neuronal networks continue to undergo structural reorganization, refinement, and myelination – particularly in the regions referred to as the “social brain” [1]. Furthermore, adolescents have increased stress vulnerability compared to adults [4]. Thus, adverse experiences in adolescent age may interfere with normal development, causing long-lasting consequences in adulthood [5]. Indeed, in humans, the onset of many affective disorders is peaking at the age of 14 years, which is probably related to the interplay between adolescent maturation processes and psychosocial factors [2, 6]. Adolescent social isolation is one of such adverse experiences that can have long-term consequences in adulthood [7]. Animal studies have shown that isolation at juvenile and adolescent periods decreases hippocampal neurogenesis, alters synaptic plasticity, and induces changes in brain neurochemistry [8–10]. Behavioral effects of isolation rearing include cognitive impairments, increased aggressiveness, anxiety- and depressive-like behaviors, and decreased sociability [8, 9, 11–17]. Most of the literature, however, focuses either on immediate effects or long-term consequences of social isolation. Particularly, there are very few studies evaluating social behavior both before and after rehousing in groups [17]. In addition, the resocialization procedure is carried out mainly before the adolescent period is over [13–15, 17]. In the present study, we aimed to examine the immediate effects of adolescent social isolation on sociability and social novelty preference in male Wistar rats, as well as its long-term consequences after resocialization in adulthood.

METHODS

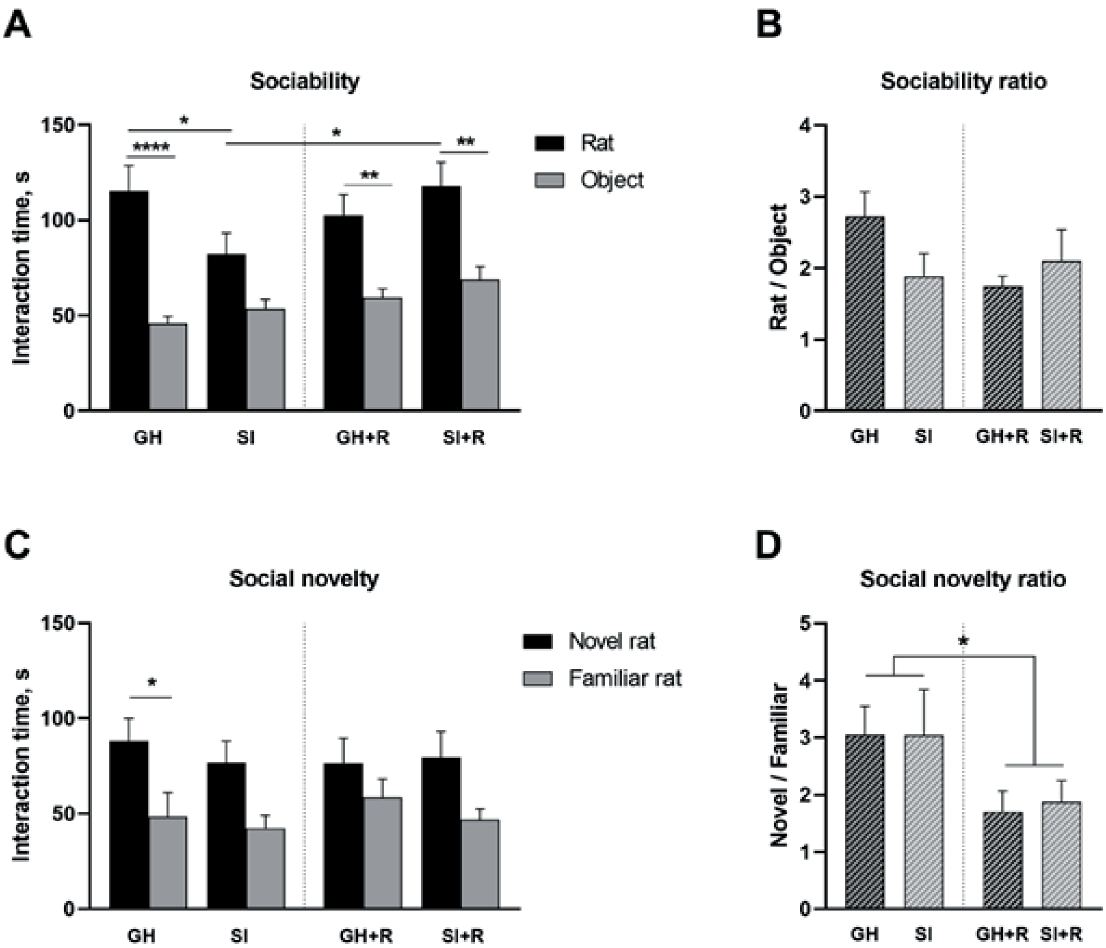
All experimental procedures were performed in accordance with the Directive of European Parliament and Council 2010/63/EU on the protection of animals for scientific purposes. All efforts were made to minimize suffering and to reduce the number of animals used. Experiments were carried out on male Wistar rats, weaned at postnatal days 23-25. Immediately after weaning, rats were randomly assigned to social isolation (SI) or group housing (GH) of 4 animals per cage. SI and GH rats were exposed to the same auditory, olfactory, and visual cues, but SI rats were deprived of social contact with peers by individual housing. Following 6 weeks of different housing conditions, social behavior was evaluated in the three-chamber social interaction test [18], adapted for rats. In the next 2-3 days, SI rats were re-socialized in groups of four per cage (SI+R) for another 6 weeks, after which their social performance was re-evaluated. GH rats remained under unchanged housing conditions and were used as controls for SI+R rats (GH+R).

The apparatus for the three-chamber social interaction test (120×60×50 cm high) consisted of three equal-sized chambers, divided by walls with central openings. Before the experiment, each rat was placed into the empty apparatus for 5-min habituation and then returned to a home cage. After that, an age- and weight-matched male rat unfamiliar to an experimental animal was placed in a wire cage in one of the peripheral chambers of the apparatus. An identical but empty cage was placed in the opposite chamber and considered as an Object. The test consisted of two sessions: sociability and social novelty. For the sociability testing, an experimental rat was placed in the central chamber and allowed to explore the apparatus with both cages for 10 min to evaluate the preference between a Rat cage and an Object cage. After the session, an experimental animal was returned to a home cage and the apparatus was cleaned with 30% ethanol. For the social novelty session, another unfamiliar rat was placed in the Object cage and now labeled as a Novel rat. This time an experimental animal was allowed to interact with a Familiar rat from the previous session and a Novel rat for 10 min. Each trial was video-recorded and the time spent sniffing each cage was noted in a blind manner to the housing condition. Sociability and social novelty ratios were calculated as time spent sniffing a Rat/time spent sniffing an Object, and time spent sniffing a Novel rat/time spent sniffing a Familiar rat, respectively.

Statistical analysis was performed using GraphPad Prism 8 (“GraphPad Software”, USA). Group differences were analyzed using one-way ANOVA followed by Holm-Sidak’s multiple comparisons test or Kruskal-Wallis test followed by a post hoc Dunn’s test, where appropriate. To evaluate the effects of age and housing conditions on sociability and social novelty ratios we used two-way ANOVA and Holm-Sidak’s post hoc test for multiple comparisons. Results are presented as mean ± SEM. A value of $P < 0.05$ was considered significant.

RESULTS

Using the three-chamber social interaction test, we assessed sociability and social novelty reaction in SI rats before and after resocialization (Figure). In the sociability session, we found that isolated animals were significantly less engaged in social interaction than GH rats (GH Rat: 115.4 ± 13.3 s vs. SI Rat: 82.3 ± 11.0 s, $P < 0.05$; Figure, A). In contrast to GH rats, SI animals immediately after isolation procedure didn’t show statistically significant difference in time spent with a Rat or an Object (GH Rat: 115.4 ± 13.3 s vs. GH Object: 45.8 ± 3.8 s, $P < 0.0001$; SI Rat: 82.8 ± 11.0 s vs. SI Object: 53.6 ± 5.0 s, $P = 0.09$). Resocialization of SI



Adolescent social isolation induces changes in social behavior, reversed by resocialization in the adult age. A – sociability session of the three-chamber social interaction test. The time spent sniffing a rat or inanimate object was analyzed. B – sociability ratio between the time spent with the rat and inanimate object. C – social novelty session of the three-chamber social interaction test. The time spent sniffing the familiar rat from the previous session or a newly introduced unfamiliar rat was analyzed. D – social novelty ratio between the time spent with the novel and familiar rat. *P < 0.05, **P <0.01, ****P < 0.0001. Data are presented as Mean ± SEM, n = 16

animals regained their sociability, as indicated by increased interaction with a Rat (SI Rat: 82.8 ± 11.0 s vs. SI+R Rat: 117.9 ± 12.6 s, P < 0.05) as well as significantly higher preference towards a rat than an Object (SI+R Rat: 117.9 ± 12.6 s vs. SI+R Object: 68.8 ± 6.8 s, P < 0.01). Additionally, control to SI+R GH rats did not show any significant changes with age (GH Rat: 115.4 ± 13.3 s vs. GH+R Rat = 102.5 ± 10.9 s, P = 0.33). We also calculated a sociability ratio between the time spent with a Rat and an Object (Figure, B). Two-way ANOVA revealed the tendency towards age × housing interaction effect, which, however, did not reach a level of statistical significance ($F_{interaction\ (1,60)} = 3.39$, P = 0.07). No significant effects of age or housing alone were observed. In the social novelty test, GH rats showed higher interest towards a newly introduced rat than to a familiar one (GH Novel: 88.1 ± 11.8 s vs. GH Familiar: 48.4 ± 12.7 s, P < 0.05; Figure, C). SI animals also exhibited preference towards a Novel rat, but the difference was not statistically significant (SI Novel: 76.8 ± 11.3 s vs. SI Familiar: 42.3 ± 6.9 s, P = 0.15). However, six weeks later the same animals didn’t show any preferences in a social novelty test (GH+R Novel: 76.5 ± 13.1 s vs. GH+R Familiar: 58.5 ± 9.7 s, $P = 0.99$; SI+R Novel: 79.4 ± 13.7 s vs. SI+R Familiar: 46.9 ± 5.5 s, $P = 0.7$). We didn't find any differences in the time spent with a Novel rat between groups. The two-way ANOVA of a social novelty ratio revealed a significant decrease of interest towards a Novel rat with age ($F_{age\ (1,60)} = 5.49$, $P < 0.05$; Figure, D). However, we didn't find any effects of the housing condition ($F_{housing\ (1,60)} = 0.02$, $P = 0.88$) or housing condition × age interaction ($F_{interaction\ (1,60)} = 0.03$, $P = 0.86$).

DISCUSSION

In the present study, we examined short-term and long-lasting consequences of post-weaning social isolation on sociability and social novelty preference in Wistar rats. We found that SI rats had impairments in social behavior, which were reversed with group housing in adult age. Our data on decreased social interaction immediately after social isolation is in agreement with some previous studies [16]. In line with our results, Meng et al. [17] have also demonstrated improved social preference after resocialization in Sprague-Dawley rats. On the other hand, several other studies have reported impaired sociability after the isolation-resocialization procedure [13–15]. The inconsistent results may be related to the different ages and duration of the resocialization procedure in our animals, as we sought to determine the effects of prolonged resocialization carried out in adulthood. In a social novelty test, SI rats failed to achieve significant preference towards a Novel rat, unlike GH animals. However, time spent with a Novel rat was not statistically different between GH and SI groups, as well as social novelty ratio. We also didn't observe significant social novelty preference in SI or GH rats at the end of the resocialization period. In contrast, Park et al. [15] have shown a loss of social novelty preference in resocialized mice, even though they were of the same age as animals in our study. One possible explanation of these discrepancies might be related to the differences in social behavior between mice and rats. Indeed, a recent study has shown weaker social novelty preference in adult rats compared to mice [19]. We also observed a significant age effect on a social novelty ratio for both experimental groups and a tendency towards decreased sociability ratio with age in GH rats. In the present study, rats were at PND65-67 during the first assessment of social behavior, which corresponds to young adulthood [3]. Therefore, these findings demonstrate that increased social novelty preference persists at the end of the adolescent age but declines in the fully-grown adult rats. In summary, the current study demonstrates that six weeks of post-weaning social isolation in male Wistar rats impairs sociability, but has little effect on social novelty preference. Group housing in adult age, however, fully restores social behavior deficits.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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РЕСОЦІАЛІЗАЦІЯ В ДОРОСЛОМУ ВІЦІ ВІДНОВЛЮЄ РІВЕНЬ СОЦІАЛЬНОЇ ВЗАЄМОДІЇ, ЗНИЖЕНИЙ ВНАСЛІДОК СОЦІАЛЬНОЇ ІЗОЛЯЦІЇ В ПІДЛІТКОВОМУ ПЕРІОДІ У ЩУРІВ

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Оцінювали вплив хронічної соціальної ізоляції в підлітковому віці на рівень соціальної взаємодії та реакцію на соціальну новизну у самців щурів лінії Вістар. Також ми дослідили, чи зберігатимуться ефекти ізоляції після ресоціалізації в дорослому віці. Внаслідок соціальної ізоляції у щурів знижувався рівень соціальної взаємодії (час, проведений у взаємодії з незнайомим щуром тієї ж самої статі та віку, знизився з 115,4 ± 13,3 с у групових тварин до 82,3 ± 11,0 с в ізольованих), тоді як змін в реакції на соціальну новизну не виявлено. Водночас 6-тижнева ресоціалізація в дорослому віці призвела до відновлення рівня соціальної взаємодії (час, проведений із незнайомим

щуром, становив 117,9 ± 12,6 с в ізольованих тварин та 102,5 ± 10,9 с у групових). Отримані результати можуть бути корисними для кращого розуміння проблеми соціальної ізоляції, яка стала особливо актуальною через обмеження внаслідок пандемії COVID-19.
Ключові слова: соціальна ізоляція; підлітковий вік; ресоціалізація; соціальна взаємодія; соціальна новизна.

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