IBRO Neuroscience Reports 15 (2023) 203–208

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Short communication

Enhanced social motivation in briefly isolated male rats

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ARTICLE INFO

Keywords:
Anxiety
Loneliness
Rats
Social isolation
Social motivation

ABSTRACT

Loneliness and anxiety are associated with psychiatric disorders in humans. Although brief social isolation in adult rats and mice has been proposed as a rodent model of loneliness, its socioemotional characteristics are not well known. In this study, we evaluated the social and emotional behaviors of adult male rats subjected to brief social isolation. Isolated rats frequently showed sniffing behavior toward empty cylinders where conspecifics had previously existed, as well as conspecifics themselves. Furthermore, social motivation correlated with anxiety levels, as indicated by the elevated plus-maze test performance in isolated but not in non-isolated rats. These results suggest that high social motivation is associated with anxiety in briefly isolated rats.

1. Introduction

Loneliness, which is defined as a distressed state accompanied by the perception that the quantity or quality of one's social relationships does not meet social needs (Hawkley and Cacioppo, 2010), is a common social problem in modern developed countries. In aging populations, older adults frequently feel lonely because of the death of their close acquaintances. Additionally, the coronavirus disease-2019 pandemic has restricted face-to-face communication, increasing loneliness among people. Indeed, social isolation and loneliness are associated with mental health problems, including depression and anxiety, across one's lifespan (Cacioppo, 2011; Cacioppo et al., 2006; Heinrich and Gullone, 2006; Lim et al., 2016; Moeller and Seehuus, 2019) as well as physical health (Cacioppo, 2011; Cacioppo et al., 2002; Segrin and Passalacqua, 2010). Despite the severe consequences of loneliness on health, there are limited findings predicting potential characteristics associated with loneliness-induced emotional disorders. This is mainly due to a lack of appropriate animal models, which hinders early pharmacological treatments and clinical interventions for various psychiatric diseases linked to loneliness. Therefore, biobehavioral characteristics of a loneliness-like state and its neural mechanisms in rodents need to be urgently studied.

In rodent studies, socially isolated housing has been used to establish animal models of several psychiatric and developmental disorders, including schizophrenia (Liu et al., 2017; Sakurai et al., 2021) and autism spectrum disorder (Caruso et al., 2022; Matsumoto et al., 2019). Thus, rats or mice are often subjected to isolation from their siblings at weaning and then chronically housed in isolation during the developmental period. In contrast, the early studies demonstrated that acute social isolation enhances social play behaviors (Panksepp and Beatty, 1980) and social interaction (Niesink and Van Ree, 1982). Interestingly, in some studies, brief social isolation in adulthood has been addressed as a rodent model of loneliness (Lee et al., 2021; Matthews et al., 2016). Matthews et al. (2016) first reported increased dopaminergic activity in the dorsal raphe nucleus during social interactions in mice that had been socially isolated for 24 h. They further demonstrated that artificial activation of dorsal raphe dopamine neurons induced social approach behavior and place avoidance, whereas their inhibition decreased social behaviors only in isolated mice, concluding that neural activities following brief social isolation represent a loneliness-like state. Other studies have revealed the neural mechanisms by which brief social isolation promotes social behaviors (Choi et al., 2022; Ferrara et al., 2021; Fukumitsu et al., 2022).

In contrast to the accumulated neurobiological findings of brief social isolation, behavioral phenotypes, including emotional behaviors during brief isolation and their associations with social motivation, have not yet been fully examined. Brief isolation did not influence anxiety-like behaviors in an open-field test in male rats (Alshammari et al., 2019) and mice (Choi et al., 2022) and failed to show an apparent relationship between sociability and anxiety-like behaviors in isolated mice (Choi et al., 2022; Musardo et al., 2022); however, loneliness was found to be correlated with anxiety levels in humans (Lim et al., 2016; Moeller and Seehuus, 2019). In addition, isolated female mice have been found to exhibit more contact-seeking behaviors that reflect social

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https://doi.org/10.1016/j.ibneur.2023.08.2195
Received 26 June 2023; Received in revised form 9 August 2023; Accepted 30 August 2023
Available online 11 September 2023

motivation than males (Fukumitsu and Kuroda, 2023), and these behaviors have not been demonstrated in briefly isolated males.

In this study, we aimed to evaluate the behavioral characteristics of briefly isolated male rats. First, we observed social motivation and contact-seeking behaviors because lonely people exhibit high social motivation that is correlated with increased activity of the reward-related brain system (Tomova et al., 2020). Next, we focused on anxiety and its association with social motivation because lonely people are vulnerable to anxiety (Cacioppo et al., 2006).

2. Materials and methods

2.1. Animals

Ninety-three male Sprague-Dawley rats were purchased from Charles River Laboratories (Kanagawa, Japan). They were housed in groups of three per cage under constant temperature and humidity conditions with a 12:12-h light–dark cycle (light on 8:00–20:00) and with food (Oriental Yeast Co., Ltd., Tokyo, Japan) and water available ad libitum. Behavioral experiments were conducted at 9–14 weeks of age. All the experiments were approved by the University of Tsukuba Committee on Animal Research.

2.2. Procedure

2.2.1. Experiment 1

In Experiment 1, the effects of brief social isolation on social motivation were evaluated using a brown semi-transparent acrylic box (80 × 40 × 40 cm) (Medical Workshop, Research Facility Center for Science & Technology, University of Tsukuba, Ibaraki, Japan), at a light intensity of approximately 80 lx (Fig. 1). The behavioral experiments consisted of three phases: habituation (day 1), baseline (day 2), and test (day 3). In Experiment 1–1 (Fig. 1A) and 1–2 (Fig. 1E), after being handled for 5 min, the focal animals were individually habituated to the box in which two empty cylinders were placed at the ends for 10 min on day 1 (habituation). On day 2, the animals were re-introduced into the box, in which two cylinders were placed at the same positions as on day 1 (baseline). One of the cylinders contained an unfamiliar, age- and sex-

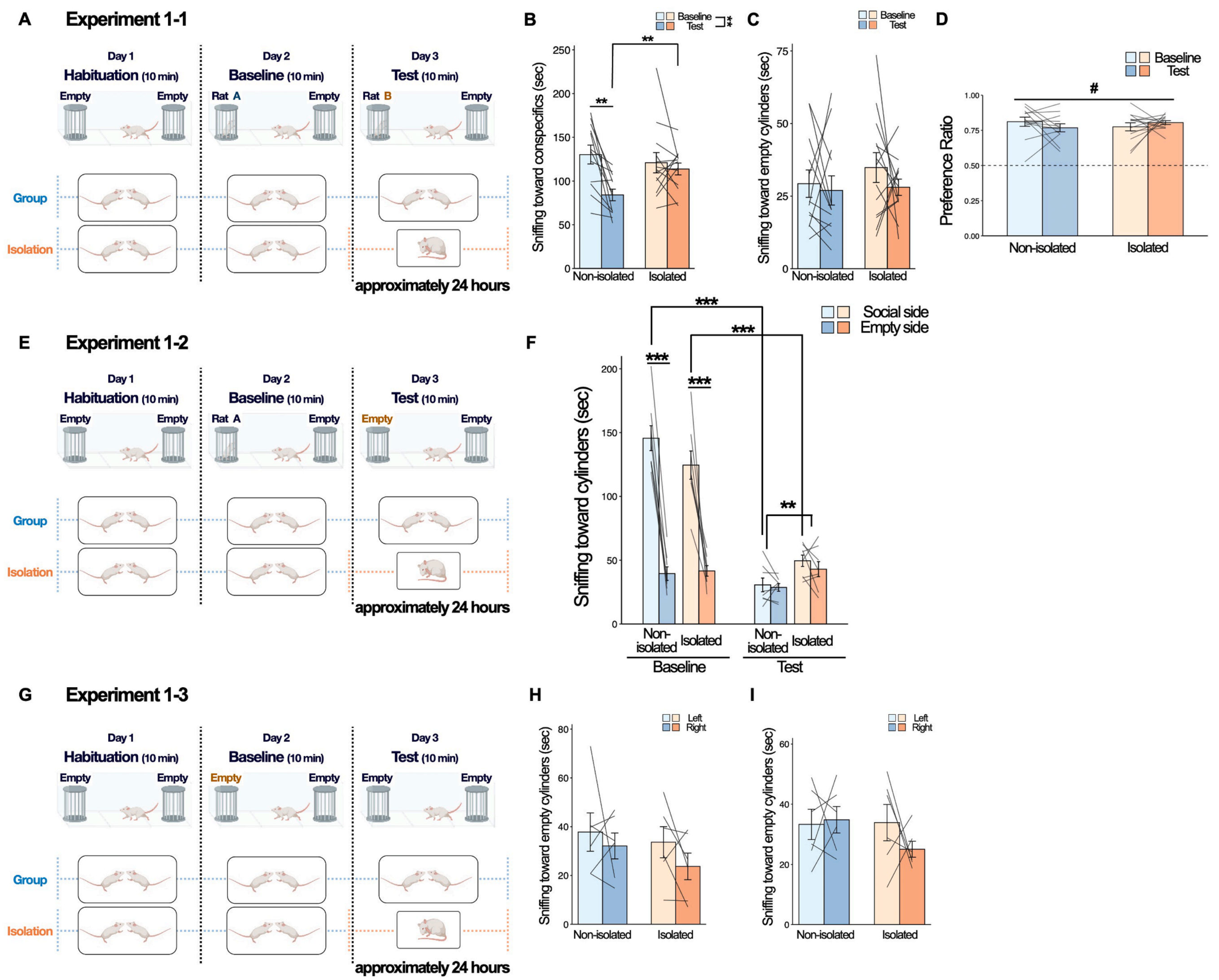

Fig. 1. Effects of brief social isolation on social motivation in male rats. (A, E, G): Diagrams of experimental procedures to assess social approach (A), contact-seeking (E), or general sniffing (G) behaviors. (B–C): Mean time spent sniffing behaviors toward conspecifics (B) or empty cylinders (C) in Experiment 1–1 (± SEM with individual replicates; ** : $p < .01$). (D): Preference ratios in each experimental condition. A dot line represents the chance level (# : $p < .001$ vs. the chance level). (F): Mean time spent sniffing behaviors toward conspecifics or empty cylinders in the baseline and test phases of Experiment 1–2 (± SEM with individual replicates; ** : $p < .01$; *** : $p < .001$). (H–I): Mean time spent sniffing behaviors toward empty cylinders in the baseline (H) or test (I) phase of Experiment 1–3 (± SEM with individual replicates).

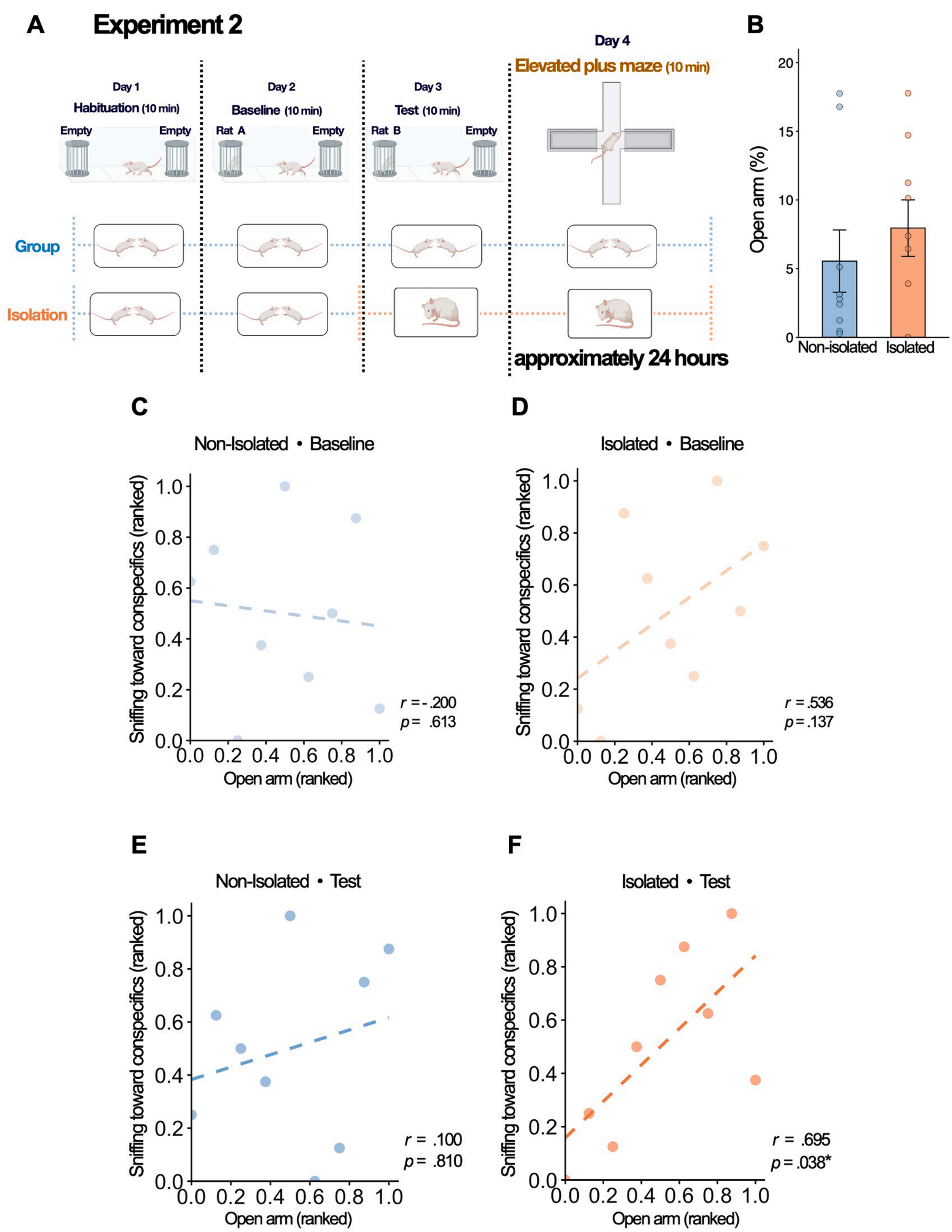

Fig.2. Effects of brief social isolation on social approach and anxiety-like behaviors in male rats. (A): Diagram of an experimental procedure in Experiment 2. (B): Percent of time spent in the open arms in the elevated plus-maze test (± SEM with individual replicates). (C–F): Scatter plots of the time spent in the open arms (ranked) and time spent sniffing conspecifics (ranked). Each dashed line represents a simple linear regression. (C, E): Anxiety and social motivation during baseline (C) or test (E) in non-isolated rats. (D, F): Anxiety and social motivation during baseline (D) or test (F) in isolated rats.

matched conspecific with the focal rats, whereas the other was empty (Fig. 1A, 1E). At the end of the 10-min baseline phase, the focal rats assigned to the isolation group were isolated from their cages and singly housed in a small polycarbonate plastic cage (17 × 33 × 20 cm) until the end of the experiments. The following day, the focal rats were allowed to sniff the cylinders for 10 min (test) freely. During the test phase, the rats were exposed to one empty and one unfamiliar conspecific-containing cylinder in Experiment 1–1 (Fig. 1A) and two empty cylinders in Experiment 1–2 (Fig. 1E). The locations of the conspecific-containing cylinders and the empty cylinders were counterbalanced across subjects to avoid any biases by the experimental groups. Throughout all the phases of Experiment 1–3 (Fig. 1G), the focal rats were introduced into a box with two empty cylinders and allowed to explore it freely for 10 min. In Experiment 1–1, the preference ratio was calculated by dividing the time spent sniffing the cylinder containing conspecifics by the total time spent sniffing both cylinders.

2.2.2. Experiment 2

Experiment 2 was conducted to evaluate the effects of brief social isolation on anxiety-like behaviors in an elevated plus-maze apparatus previously described by (Toyoshima et al., 2018), at a light intensity of approximately 715 lx (Fig. 2). After the 3-day social motivation test conducted in the same way as Experiment 1–1, the focal rats were individually placed on the center platform of the apparatus and allowed to freely explore the maze for 10 min (Fig. 2A). The time spent in the open arms was manually measured by analyzing recorded videos of the elevated plus-maze tests without any knowledge of housing conditions. For the non-parametric correlation analysis, the focal rats were ranked based on the time spent in the open arms. A rat that stayed longer in the open arms (indicating a lower anxiety level) was assigned a lower rank, whereas a rat that stayed for a shorter duration in the open arms (indicating a higher anxiety level) was given a higher rank.

2.3. Statistical analysis

All datasets were analyzed using R (version 4.2.2). The distributions' normality and variances' homogeneity in all data sets were verified using the Shapiro–Wilk and Bartlett tests, respectively. In Experiment 1–1, the amount of time spent sniffing behaviors toward either the conspecific-containing or empty cylinder was analyzed using a two-way mixed analysis of variance (ANOVA) (housing × phase). Preference ratios were analyzed using a two-way mixed ANOVA (housing × phase). Additionally, each preference ratio was analyzed with a one-sample t-test to determine if it significantly differed from chance level, indicating whether the focal rats showed a preferential interest in sniffing the cylinder containing conspecifics or the empty one. In Experiment 1–2, the time spent sniffing the cylinders, either with conspecifics or empty, during the baseline and test phases, was analyzed using a three-way mixed ANOVA (housing × side × phase). In Experiment 1–3, the amount of time spent sniffing the two empty cylinders during either the baseline or test phase was analyzed using a two-way mixed ANOVA (housing × side).

In Experiment 2, the time spent in the open arms of the elevated plus-maze was analyzed by Wilcoxon rank-sum test, owing to the abnormality of the distribution of the data. Furthermore, the correlations between the ranked anxiety level based on the time spent in the open arms by each focal rat and the ranking of the time spent sniffing the conspecifics during either the baseline or test phase were analyzed using Spearman's rank correlation tests to evaluate the relationships between social motivation and anxiety.

3. Results

3.1. Experiment 1

In Experiment 1–1, the briefly isolated rats exhibited more frequent sniffing behavior toward the conspecific-containing cylinder during the test phase than the non-isolated rats (Fig. 1B). Two-way mixed ANOVA indicated a significant main effect of phase ($F_{1,\ 23} = 12.043$, $p = .002$) and a significant interaction between housing and phase ($F_{1,\ 23} = 6.465$, $p = .018$). Post–hoc analysis revealed a significant difference between the phases in the non-isolated rats ($p = .002$). The post-hoc analysis also indicated a significant difference between the non-isolated and isolated rats in the test phase ($p = .005$). Conversely, sniffing behaviors toward empty cylinders were roughly equal between the non-isolated and isolated groups, regardless of the phase (Fig. 1C). The two-way mixed ANOVA revealed no significant main effects or their interactions. A two-way mixed ANOVA revealed no significant differences in preference ratios between housing conditions or phases (Fig. 1D). However, one-sample t-tests demonstrated that the ratios significantly differed from the chance level under all conditions (GH-baseline: $t_{11} = 9.566$, $p < .001$; GH-test: $t_{11} = 9.374$, $p < .001$; SI-baseline: $t_{12} = 9.513$, $p < .001$; SI-test: $t_{12} = 21.72$, $p < .001$). In Experiment 1–2, during the baseline phase, sniffing behaviors towards the conspecific-containing cylinder were more pronounced than those towards the empty cylinder in both housing groups. Moreover, during the test phase, the briefly isolated rats sniffed for longer durations on both sides of the empty cylinders compared to the non-isolated rats (Fig. 1F). A three-way mixed ANOVA revealed a significant main effect of phase ($F_{1,\ 14} = 116.536$, $p < .001$) and side ($F_{1,\ 14} = 109.681$, $p < .001$). There were also significant interactions between housing and phase ($F_{1,\ 14} = 7.996$, $p = .013$) and between phase and side ($F_{1,\ 14} = 148.974$, $p < .001$). Post–hoc analyses revealed significant differences between the phases for both isolated ($p = .006$) and non-isolated rats ($p < .001$). Additionally, there was a significant difference between the non-isolated and isolated rats during the test phase (p = .001). Further post-hoc analyses identified significant disparities between the phases on the social side ($p < .001$) and between sides during the baseline ($p < .001$). In Experiment 1–3, there were no differences in sniffing toward empty cylinders between the two housing condition groups, regardless of the side, in both phases (Fig. 1H, 1I). Two-way mixed ANOVAs revealed no significant main effects or their interactions in the baseline or test phases.

3.2. Experiment 2

Fig. 2B shows the time spent in the open arms by non-isolated and briefly isolated rats. The Wilcoxon rank–sum test showed no significant differences between the two groups. Scatter plots of the rank of time spent in the open arms and the rank of time spent sniffing conspecifics are shown in Fig. 2C–2 F. The Spearman's rank correlation tests revealed a significant positive correlation between the rank of time spent sniffing during the test phase and the rank of time spent in the open arms in the isolated ($r = .695$, $p = .038$) but not in non-isolated rats (Fig. 2E, 2F). Correlations between them during the baseline phase in both housing condition groups were not significant (Fig. 2C, 2D).

4. Discussion

The present study verified the occurrence of social approach, contact-seeking, and anxiety-like behaviors in adult male rats that experienced short-term social isolation. Brief isolation led to an increase in time spent sniffing the cylinder in which a conspecific was present. Furthermore, the isolated rats exhibited more sniffing behavior toward both of empty cylinders regardless of whether a conspecific existed or not in the cylinders during the baseline phase. The rank of time spent sniffing the conspecifics was positively correlated with the anxiety level rank in isolated, but not non-isolated rats. However, the duration spent in the open arms did not show any difference between the two groups.

A previous study has reported that female mice that were separated from their cagemates exhibited biting of the mesh wall when the cagemates were present on the other side of the wall (Fukumitsu et al., 2022). In this study, we showed that brief social isolation increased the sniffing behaviors of male rats toward not only the cylinders in which a conspecific was present (Experiment 1–1) but also the empty cylinders in which a conspecific had previously been present (Experiment 1–2). In addition, social isolation has been found to promote socially conditioned place preferences (Douglas et al., 2004; Nardou et al., 2019) and operant social-seeking behaviors (Olaniran et al., 2022). Furthermore, brief isolation did not influence sniffing behavior when the rats were not exposed to a conspecific in the experimental setting (Experiment 1–3). Given the fact that social isolation disrupts social novelty preference (Okuyama et al., 2016) and does not influence motivation toward a novel object (Musardo et al., 2022), higher sniffing behaviors toward a novel conspecific in the isolated rats during the test phase in Experiment 1–1 can not be attributed to increased general novelty seeking induced by brief social isolation. These findings suggest that sniffing behaviors toward empty cylinders the day after encountering a conspecific present in the cylinder represent contact-seeking behaviors and that brief social isolation increases social motivation.

The present study showed no significant differences in anxiety-like behaviors between the housing condition groups. However, social approach behaviors were positively correlated with anxiety-like behaviors in briefly isolated but not in non-isolated rats. These results demonstrate the differences in the effects of brief social isolation on social and emotional phenotypes. These individual variations in isolation-induced social motivation and anxiety levels may be attributed to social hierarchy. In fact, optogenetic inactivation of dorsal raphe dopamine neurons after brief isolation was found to decrease social approach only in dominant male mice, and changes in social behaviors during inhibition of dorsal raphe dopamine neurons before and after isolation positively correlate with social rank (Matthews et al., 2016). On the other hand, socially dominant males are reported to be less anxious (Davis et al., 2009). Future studies are needed to determine whether social rank contribute to individual variations in anxiety levels and social motivation after brief isolation.

In the current experiment, we isolated the focal rats assigned to the isolated group for approximately 24 h by reference to (Matthews et al., 2016). The length of social isolation may be critical for the establishment of an animal model of loneliness. In terms of face validity for a loneliness model, chronic social isolation may seem more appropriate than acute isolation. However, chronic isolation has been linked to multiple psychiatric disorders and a reduction in social motivation. Loneliness is defined as a distressing state stemming from the perception that one's social relationships—either in quality or quantity—do not meet their social needs (Hawkley and Cacioppo, 2010). Given this definition, we view loneliness as a relatively acute emotional state that can be a precursor to various psychiatric disorders. Moreover, individuals who perceive loneliness might seek contact with others, if possible, to alleviate the discomfort caused by a lack of social interaction. Therefore, 24 h or less of isolation or separation that can induce a loneliness-like neural state of distress and heightened social motivation in rodents (Matthews et al., 2016; Fukumitsu et al., 2022) may be an appropriate rodent model of loneliness.

In summary, brief social isolation enhances social motivation and is associated with anxiety levels. These results imply the presence of common neural mechanisms between social motivation and anxiety and may aid in the development of validated treatments and interventions for loneliness-related emotional disorders.

CRediT authorship contribution statement

Michimasa Toyoshima: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Kazuo Yamada:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

All authors declare that they have no conflict of interest.

Acknowledgement

This study was supported by JSPS KAKENHI Grant Numbers 19K21806, and 19J20173. Illustrations were created with BioRender.com. We would like to thank Editage (www.editage.com) for English language editing.

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