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Sex-specific role of body weight in mediating stress susceptibility through MeA Tac2-Nk3R signaling

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Abstract

Weight fluctuations, such as weight loss or gain, are hallmark features of major depression. However, the potential bidirectional relationship between body weight and depressive-like behaviors remains poorly understood. While the prevalence of major depression is approximately twice as high in women compared to men, the role of body weight in mediating sex differences in depression susceptibility has yet to be fully explored. In the current study, we reported that exposure to two weeks of social isolation during adolescence induced depressive-like behaviors in female mice, while male mice exhibited resilience and did not develop these behavioral abnormalities. Interestingly, female mice displayed slower weight gain during social isolation compared to control mice and isolated male mice. Furthermore, 20% calorie restriction, which suppressed weight gain, mimicked social isolation-induced depressive-like behaviors in both male and female mice. Conversely, a high-fat diet that compensated for the slowed weight gain rescued the depressive-like behaviors in socially isolated female mice. We also found that social isolation led to an increase in the peptide tachykinin 2 (Tac2) within the medial amygdala (MeA) region, which contributed to both the slowed weight gain and the development of depressive-like behaviors. Pharmacological blockade or genetic knockout of the Nk3 receptor (Nk3R), the receptor for Tac2, reversed both the weight gain deficits and the associated behavioral abnormalities. Our findings highlight a sex-specific vulnerability to stress during adolescent social isolation and suggest that slowed weight gain, mediated by MeA Tac2-Nk3R signaling, plays a crucial role in this process. These insights open new avenues for potential therapeutic interventions for depression.

1. Introduction

Depressive disorder is a prevalent mental health condition and a major contributor to global disability, imposing significant economic and healthcare challenges [1–3]. Management of depression remains problematic, as more than 50% of patients fail to achieve remission, and 30–50% do not respond to antidepressant therapy [4, 5]. The underlying causes of treatment resistance remain unclear; however, evidence suggests that the prevalence of treatment-resistant depression is higher in obese and overweight individuals compared to those with a normal weight [6], which prompts the critical role of body weight in the development of depression.

Depression is associated with changes in body weight, manifesting as either gain or loss, driven by physical or behavioral mechanisms [7–10]. However, the influence of body weight on depression remains inconclusive, as findings from existing research are inconsistent. Some studies provided limited evidence supporting the notion that body weight significantly impacted depression [11], indicating that the relationship between obesity and depression may be minimal. Conversely, other studies suggested a stronger association, with obesity linked to an increased likelihood of depression in the general population [12–17]. For instance, one study reported the highest prevalence of depression among individuals with severe obesity [15], and another identified obesity as a risk factor for developing

depressive symptoms [17]. Although prior research has explored the connection between obesity and depression in various demographic groups [18–20], comprehensive causal analyses examining the impact of body weight on depression across genders remain scarce.

Women are diagnosed with stress-related psychiatric disorders, including major depression, at twice the rate of men [21–25]. Research suggested that females may exhibit different stress responses and adopt distinct coping strategies compared to males [26–29]. Although rodent models of depression have significantly advanced our understanding of the neural mechanisms underlying behavioral responses to stress, these studies predominantly rely on male subjects [30–32]. Consequently, the mechanisms driving sex differences in stress vulnerability remain poorly understood.

Extensive research has demonstrated that early life stress, such as social isolation, is a strong predictor of developing psychiatric conditions, including depression and anxiety [33–37]. As a critical period of physical and psychological maturation, adolescence is particularly sensitive to the detrimental effects of social isolation, which impair brain function and behavior in adulthood across mammalian species [38–42]. In our previous studies, four weeks of social isolation initiated during adolescence enhanced fear memory retention and reduced social interaction in mice [43]. Additionally, a two-week period of social isolation during adolescence induced anxiety-like behaviors [36]. Therefore, the social isolation paradigm serves as an effective model for investigating sex differences in stress vulnerability.

Neuropeptides have been implicated in mediating stress responses in a variety of systems [44]. An unbiased screening of peptidergic neurons identified DTK (Drosophila tachykinin)-expressing neurons and the DTK peptide as essential for the effects induced by social isolation [45]. In rodents, the tachykinin gene family consists of Tac1 and Tac2 [46]. Tac1 encodes the peptides substance P (SP) and neurokinin A (NkA), whereas Tac2 encodes neurokinin B (NkB). These peptides exhibit the highest binding affinities for the G-protein-coupled receptors Nk1, Nk2, and Nk3, respectively [47]. Both Tac1 and Tac2 are expressed across various brain regions associated with emotion and social behavior [48]. However, the logic underlying their actions in social isolation is not yet clear.

This study aimed to explore the influence of body weight on depressive-like behaviors in adolescent mice subjected to social isolation. Our findings revealed that female mice were more susceptible to developing depressive-like behaviors compared to male mice. Notably, female mice exhibited slower weight gain during the social isolation paradigm, a factor contributing to their heightened stress vulnerability. Finally, the elevated neuropeptide Tac2-Nk3R signaling pathway was identified as a mediator of these observed changes in female mice.

2. Materials and methods

Animals. Male and female C57BL/6 mice were obtained from the Southern Medical University Animal Center (Guangzhou, China) and housed in standard laboratory cages (five mice per cage) on a 12-hour

light/dark cycle (lights on at 8:00 A.M.) in a temperature-controlled room (21–25°C), with ad libitum access to food and water. At post-natal day 21 (P21), littermate pups were either housed individually or in groups of five per cage. Although isolated animals could hear and smell other mice within the facility, they were not physically exposed to other animals. Isolation occurred prior to the commencement of any experimental procedures. Behavioral testing was conducted during the light phase of the cycle, between 10:00 A.M. and 5:00 P.M. All procedures adhered to the guidelines set by the Chinese Council on Animal Care, with efforts made to minimize animal suffering and reduce the number of animals used.

Behavioral analysis. Mice were handled by investigators for three days prior to any behavioral testing. To minimize stress, the number of investigators handling the isolated animals during weekly cage changes was kept to a minimum. The mice tested at both 1 and 2 weeks social isolation are not the same but using different batch of mice.

Sucrose Preference Test (SPT): The SPT was performed as described previously [49]. Mice were first habituated for 2 days using two 50-ml bottles (A and B). On day 1, both bottles contained water (w/w), and on day 2, both bottles were filled with a 1% sucrose solution (s/s). Following the habituation period, bottle A was filled with 1% sucrose solution and bottle B with water (s/w). The sucrose preference was assessed for 1 hour after a 22-hour period of food and water deprivation. The volume of fluid consumed from each bottle was measured, and the sucrose preference was calculated as $100 \times (Vol_A / (Vol_A + Vol_B))$.

Forced Swimming Test (FST): The FST was conducted in a clear glass cylinder (height 45 cm, diameter 19 cm) filled with water (22–25°C) to a depth of 23 cm. Mice were subjected to a 6-minute test, and the duration of immobility during the final 4 minutes was recorded by an investigator who was blinded to the experimental conditions.

Social Interaction (SI) Test: The SI test was performed in darkness. Mice were placed in a novel area with a small, empty cage at one end. After a 2.5-minute baseline movement tracking period, a caged, sex- and age-matched mouse was introduced into the arena, and social interaction was recorded for an additional 2.5 minutes. The time spent in a predefined interaction zone was measured using EthoVision XT software (Noldus, USA). After each trial, the apparatus was cleaned with a 70% ethanol solution to remove olfactory cues.

Novelty-Suppressed Feeding (NSF) Test: As previously described [49], the NSF test was conducted after 24 hours of food deprivation (water was not restricted). Mice were placed in a testing box (50 × 50 × 20 cm) with a single pellet of food placed on a white piece of paper at the center. The floor of the box was covered with 2 cm of thick bedding. Latency to bite the food was recorded using a stopwatch, with the test lasting for 5 minutes. Once the mouse bit the food, it was immediately returned to its home cage.

Open Field Test (OFT): The OFT was conducted in a rectangular chamber (60 × 60 × 40 cm) made of gray polyvinyl chloride. The center of the chamber was illuminated by 25 W halogen bulbs (200 cm above the field). Mice were gently placed in the center of the chamber, and their movements were tracked for 5

minutes using an automated video tracking system. The paths traveled during the 5-minute period were analyzed using the DigBehv animal behavior analysis program.

Elevated Plus Maze (EPM) Test: The EPM consisted of two opposing open arms (30 × 5 × 0.5 cm) and two enclosed arms (30 × 5 × 15 cm) connected by a central platform (5 × 5 cm), forming a plus sign shape. The maze was placed in a dimly lit room, and mice were acclimatized for at least 30 minutes before testing. The time spent in the open and closed arms was recorded over a 5-minute period. The maze was cleaned with a 70% ethanol solution between sessions.

Contextual Fear Conditioning (cFC) Test: The cFC test was performed as previously described [50]. Mice were first habituated to the behavioral room and allowed to explore the conditioning chamber (MED-VFC-NIR-M; Med Associates) for 3 minutes. During the training phase, mice were placed in conditioning chamber A and exposed to tone-foot-shock pairings (tone: 30 seconds, 80 dB; foot shock: 1 second, 0.4 mA), with an 80-second interval between each pairing. Twenty-four hours later, the mice were returned to chamber A to assess contextual fear learning. Freezing behavior during both training and testing was recorded using Med Associates Video-Tracking and Scoring software.

Y-Maze Test: The Y-maze consisted of three symmetrical arms (30 × 10 × 20 cm) made of gray polyvinyl chloride and evenly illuminated. Mice were placed in the center of the maze and allowed to explore freely for 5 minutes. An alternation was defined as a consecutive entry into all three arms.

Determination of blood composition. Serum samples were collected by centrifugation and subsequently analyzed for leptin (Crystal Chem, Inc., Downers Grove, IL), insulin (Crystal Chem, Inc.), and corticosterone (ICN Biomedicals, Inc., Costa Mesa, CA) levels using commercially available assay kits. Glucose levels were measured in tail vein blood samples prior to sacrifice using a glucose assay kit (Fisher Scientific, Morris Plains, NJ).

Calorie restriction. All mice were provided with ad libitum access to food for a period of three days to determine the average daily food intake. Subsequently, mice were randomly assigned to equal groups and given either ad libitum access to chow or a caloric restriction (CR) regimen consisting of 10%, 20%, or 40% of their average daily food intake (10%, 20%, 40% CR). Calorie-restricted mice were fed once daily, just before the onset of the dark cycle at 19:00, to minimize disruption to their circadian rhythms.

Microdialysis. Each mouse was deeply anesthetized and positioned on a stereotaxic frame (Stoelting). A guide cannula (CMA/7, CMA/Microdialysis) was implanted into the right medial amygdala (MeA) at the following coordinates: AP = ± 1.8 mm, ML = 2.5 mm, and DV = 4.5 mm. A microdialysis probe (CMA/7, membrane length: 1–2 mm, molecular weight cut-off: 6,000 Da, outer diameter: 0.24 mm) was inserted through the guide cannula and connected to a syringe pump (CMA 402). The artificial cerebrospinal fluid (ASCF) was continuously perfused through the microdialysis probe at a constant flow rate of 1 μl/min, with sampling conducted 1 hour after probe insertion. Two 30-μl samples were automatically collected from each mouse every 30 minutes over a 60-minute period using the CMA 142 microfraction collector. To minimize the degradation of background monoamines, each collection tube was pretreated with

antioxidative agents (8 μl), containing 100 mM acetic acid, 0.27 mM Na2EDTA, and 12.5 μM ascorbic acid (pH 3.2). The interstitial fluid 5-HT levels were then measured immediately using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD).

Quantitative Real-Time PCR. As described in previous studies [51], brains were removed and sectioned on ice in a brain block (1 mm thick) using Brain grinding tool (RWD, 68707) and bilateral micropunches (15 gauge) of meA were snap-frozen on dry ice and stored at -80°C until use. Tissue samples were immediately stored in TRIzol (Invitrogen), and RNA was extracted according to the manufacturer's protocol. Genomic DNA was removed using gDNA eraser treatment (Takara), and 1.0 μg of RNA was used for first-strand complementary DNA (cDNA) synthesis (Takara). Quantitative reverse transcription PCR (qRT-PCR) was performed on a Stratagene Mx3000P thermal cycler with Universal qRT-PCR master mix (Takara) for the specified genes. The primers used in the analysis were designed and synthesized as follows:

Tac1 sense 5'-GATGAAGGAGCTGTCCAAGC-3', antisense 5'-TCACGAAACAGGAAACATGC-3'; *Tac2* sense 5'-GCCATGCTGTTTGCGGCTG-3', antisense 5'-CCTTGCTCAGCACTTTCAGC-3'; *BDNF* sense 5'- AAAACCA TAAGGACGCGGACTT-3', antisense 5'-GAGGCTCCAAAGG CACTTGA-3'; *GDNF* sense 5'-TGACTCCAATATGCCTGAAG ATTATC-3', antisense 5'-TCAGTCTTTTAATGGTGGCTTGAA- 3'; *IGF-1* sense 5'- CCCGTCCCTATCGACAAACA-3', antisense 5'-TTCCTGCACTTCCTCTACTTGTGT-3'; *VEGF* sense 5'-GCAGGCTGCTGTAACGATGA-3', antisense 5'-TT GATCCGCATGATCTGCAT-3'; *FGF-2* sense 5'-GCGACCCA CACGTCAAACTA-3', antisense 5'-GGTACCGGTTGGCACA CAT-3'; *β actin* sense 5'-CAGATGTGGATCAGCAAGCAGG- 3', antisense 5'-TTGTCAAAGAAAGGGTGTAAAACG-3'.

Western Blots. Western blot analysis was conducted following the described protocol [51]. Briefly, protein concentrations were determined using the BCA Protein Assay Kit (Thermo). Samples were mixed with 2× SDS loading buffer, heated at 95°C for 10 minutes, and then loaded onto a 10% or 4–20% gradient polyacrylamide-SDS gel. Proteins were transferred to a PVDF membrane (Millipore, Billerica, MA) for 2 hours at 350 mA, after which the membranes were incubated in Odyssey Blocking Buffer (LiCor) for 2 hours at room temperature. The membranes were then incubated overnight at 4°C with primary antibodies: rabbit polyclonal anti-Tac2 (OACD05986; 1:500; ProSci) and rabbit polyclonal anti-Nk3R (A7220; 1:1000; ABclonal), or monoclonal mouse anti-β-actin (1:1000; Bostor, China) and monoclonal mouse anti-GAPDH (3683S; 1:1000; Cell Signaling). Following three 15-minute washes in TBS containing 0.1% Tween-20, the membranes were incubated for 1 hour at room temperature with peroxidase- or IRDye-conjugated secondary antibodies in TBS with 0.1% Tween-20. Immunoreactivity was detected using chemiluminescence with an ECL reagent or by using a LiCor imaging system.

Stereotaxic Microinjection. As described earlier [52], the mice were anesthetized and positioned in a stereotaxic frame. A brain infusion cannula (PlasticsOne) was implanted unilaterally into the MeA (coordinates: AP = ± 1.8 mm; ML = 2.5 mm; DV = 4.5 mm). Seven days following the implantation, osanetant (0.3 mL vehicle containing 375 ng per site) was administered into the MeA using an automatic injector (catalog no. 53311, Stoelting). Behavioral testing was performed 30 minutes after the infusion.

The Nk3R antagonist, osanetant (Axon Medchem, Axon 1533), was dissolved in saline with 0.1% Tween 20 as the vehicle.

Statistical analyses. All data are presented as means ± standard error of the mean (s.e.m.). Statistical analyses were conducted using SPSS 30.0 software. Differences in mean values for comparisons among multiple groups were assessed using two-way analysis of variance (ANOVA), followed by the Bonferroni's post hoc test. Independent-sample t-tests were employed to compare differences between two groups throughout the study, unless otherwise noted. A significance level of $P < 0.05$ was applied for all tests.

3. Results

Female mice are more susceptible in response to social isolation during adolescence

To evaluate potential differences in responses to social isolation between male and female mice, postnatal day 21 (P21) mice were either housed individually (isolated group) or maintained in social groups of five per cage as controls (Fig. 1A). After one week of social isolation, no behavioral change were observed in either male or female mice (Fig. S1). However, after two weeks of isolation, significant differences emerged. Female mice subjected to two-week isolation showed reduced sucrose preference in the sucrose preference test (SPT) (Fig. 1B), increased immobility time in the forced swimming test (FST) (Fig. 1C), and decreased social interaction time in the social interaction test (SI) (Fig. 1D). Additionally, in the elevated plus maze (EPM), female mice spent significantly more time in the closed arms and less time in the open arms compared to control mice (Fig. 1E, F). In the novelty suppressed feeding test (NSF), isolation increased the latency to approach and begin eating the food (Fig. 1G). In the open field test (OFT), isolated females spent less time exploring the center, although their overall locomotor activity was unaffected (Fig. 1H, I). These findings suggest that female mice exhibited both depressive- and anxiety-like behaviors following two weeks of social isolation. In contrast, male mice did not show depressive-like behaviors in the SPT, FST, or SI after two weeks of social isolation (Fig. 1B-D), but they exhibited anxiety-like behaviors in the EPM, NSF, and OFT (Fig. 1E-H). When the social isolation paradigm was extended to three weeks, both male and female mice displayed depressive- and anxiety-like behaviors (Fig. S2). However, these behaviors were more pronounced in females than in males (Fig. S2). To assess potential cognitive differences between control and socially isolated male and female mice, we conducted the Y-maze and contextual fear conditioning tests. After one week of social isolation, no significant differences in cognitive performance were observed across the groups (Fig. S1J-L). However, after two weeks of isolation, male mice maintained normal cognitive abilities, while isolated female mice displayed impaired cognitive performance (Fig. 1J-L).

These findings further support a sex difference in stress susceptibility, with female mice being more vulnerable to developing depressive-like behaviors in response to social isolation.

Social isolation leads to slower weight gain in female mice

During the social isolation period, mice were provided ad libitum access to chow, and their body weights were monitored on a weekly basis (Fig. 1M). Interestingly, while both control male and female mice, as well as isolated male mice, showed typical weight gain over time, isolated female mice exhibited a notably slower rate of weight gain (Fig. 1N). The weight change in isolated female mice was significantly lower compared to all other groups (Fig. 1O), despite no difference in food consumption between groups (Fig. 1P). To assess whether the slower weight gain was associated with altered linear growth, body length measurements were taken (Fig. 1Q). As shown in Fig. 1R and S, isolated female mice were significantly shorter than those in the other groups. Two weeks of social isolation did not result in any gross anatomical changes in either the brain (Fig. 1T, U) or peripheral organs or body composition (Fig. S3).

Blood was collected from the isolated mice, and serum was analyzed for glucose and insulin levels (Fig. 1Q). No difference in serum glucose or insulin levels were observed between the groups (Fig. 1V, W). Similarly, serum leptin levels did not differ across groups (Fig. 1X). However, corticosterone levels were significantly higher in both isolated male and female mice compared to their control counterparts (Fig. 1Y), indicating activation of the HPA axis due to the social isolation stress. Additionally, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated in isolated male and female mice, indicating hepatic oxidative stress (Fig. S4).

Suppression of weight gain mimics social isolation-induced abnormal behaviors both in female and male mice

To investigate whether slowed weight gain plays a key role in the development of behavioral changes, we subjected control male and female mice to varying levels of calorie restriction (CR) for two weeks. Mice were given diets containing 10%, 20%, or 40% of their normal caloric intake, while control group received ad libitum feeding (Fig. 2A, Fig. S5A, Fig. S6A). Notably, while 10% CR had no significant impact on body weight (Fig. S5B-D) and 40% CR induced weight loss (Fig. S6B-D), 20% CR resulted in a slower rate of weight gain (Fig. 2B) and a reduced weight change (Fig. 2C), which was accompanied by decreased food intake (Fig. 2D), closely mimicking the effects of social isolation on body weight.

Following the two weeks of 20% CR intervention, we assessed depressive- and anxiety-like behaviors in the animals. Notably, 20% CR treatment led to cognitive impairments in the contextual fear conditioning test and Y-maze (Fig. 2E-G). Furthermore, CR-treated male and female mice exhibited significant depressive-like and anxiety-like behaviors (Fig. 2H-O). In contrast, 10% CR did not affect behaviors (Fig. S5E-O), while 40% CR had an antidepressant effect without impairing cognitive performance (Fig. S6E-O). However, 40% CR did not improve depressive-like behaviors in two-week isolated female mice (Fig. S7).

High-fat diet rescues slowed weight gain and depressive-like behaviors in isolated female mice

To further investigate whether the slowed weight gain induced by social isolation is directly responsible for the development of depressive-like behaviors, we provided female mice with either a control diet or a

high-fat diet (HFD) during the two-week period of social isolation, starting at postnatal day 21 (P21). Body weight and food intake were monitored throughout the experiment (Fig. 3A). Notably, while the control group exhibited slowed weight gain during isolation, the HFD group showed normal weight gain despite the two-week isolation period without affecting food intake (Fig. 3B-D). Subsequent behavioral assessments revealed that the normalization of weight gain through the HFD rescued depressive-like behaviors in the isolated female mice (Fig. 3E-O).

MeA Tac2 mediates social isolation-induced slowed weight gain and depressive-like behaviors in female mice

The medial amygdala (MeA) is a sexually dimorphic brain region involved in driving many social behaviors [53, 54]. To further characterize the molecular mechanisms underlying sex-specific role for body weight in social isolation-induced depressive-like behaviors in female mice, we analyzed the levels of amino acids, monoamines and neurotrophic factors and found no change among groups (Fig. S8A-C). To determine the involvement of Tac gene expression in the observed behavioral effects, we quantified Tac mRNA levels in select brain regions (Fig. 4A). While Tac1 expression in the MeA was not affected by social isolation (Fig. 4B), we observed a significant increase in Tac2 mRNA levels in isolated female mice, but not in male mice, during the two-week social isolation period (Fig. 4C). Corresponding to the mRNA data, Tac2 protein expression was also elevated in the MeA of socially isolated female mice (Fig. 4D). Immunofluorescence results also found that two weeks but not one week of social isolation induced a decrease in the number of $cFos^+$ cells in the MeA among female but not male mice (Fig. S9). No change in Tac2 expression were detected in other brain regions of isolated female mice (Fig. S8D). HFD did not normalized the increased level of Tac2 in the MeA of isolated female mice (Fig. S10). Moreover, the protein levels of the Tac2 receptor, NK3R, remained unchanged in the MeA following two weeks of social isolation (Fig. S11A).

To confirm the functional role of Tac2 in social isolation-induced depressive-like behaviors, we employed Tac2 knockout (KO) mice. Western blot analysis confirmed the absence of Tac2 in the MeA of KO mice compared to wild-type (WT) controls (Fig. 4E) without affecting the NK3R level (Fig. S11B). WT and KO female mice were either group-housed or socially isolated from P21 onward, and their body weight was monitored weekly (Fig. 4F). While WT female mice exhibited slowed weight gain and depressive-like behaviors after two weeks of isolation, KO female mice displayed normal weight gain and behavior, showing resilience to these effects during the same isolation period (Fig. 4G-N). Importantly, Tac2 knockout did not affect body weight or behavior in group-housed mice (Fig. 4G-N).

To further confirm whether increased Tac2 in the MeA was sufficient to mediate isolation-induced depressive-like behaviors, we employed Tac2 shRNA to specifically knock down Tac2 in the MeA region and measured behaviors. The virus green staining demonstrated that the virus was correctly injected into the MeA (Fig. 4O). Also, the results from western blotting verified that the gene was knocked down (Fig. 4P), without affecting the NK3R level (Fig. S11C). We found that knockdown of Tac2 in the MeA normalized weight gain and behaviors in isolated female mice (Fig. 4Q-W).

Tac2 functions through Nk3R in social isolation-induced slowed weight gain and depressive-like behaviors in female mice

To explore the causal role of Tac2-Nk3R signaling in mediating sex-specific behavioral effects of social isolation, we administered osanetant, a selective Nk3R antagonist that crosses the blood-brain barrier (S12A) [55, 56]. Systemic delivery of osanetant, given 30 minutes prior to behavioral testing after two weeks of social isolation, significantly reduced social isolation-induced depressive-like behaviors in female mice (Fig. S12B-F).

Next, to determine if Tac2-Nk3R signaling is required during social isolation, we administered osanetant daily in the home cage of mice throughout the 2-week isolation period (Fig. S12G). This daily treatment effectively prevented the development of both the slowed weight gain (Fig. S12H-J) and the depressive-like behaviors (Fig. S12K-O) typically observed in socially isolated female mice.

To identify the specific region within the MeA where Nk3R signaling is required to mediate the behavioral effects of social isolation, we first pharmacologically inhibited Nk3Rs locally in the MeA. Female mice subjected to two-week social isolation received bilateral microinfusions of osanetant into the MeA region, 30 minutes prior to each behavioral test (Fig. 5A). Local infusion of osanetant into the MeA region selectively improved cognitive performance and reduced depressive-like behaviors in isolated female mice (Fig. 5B-F). Subsequently, we administered daily microinfusions of osanetant into the MeA during the entire 2-week isolation period (Fig. 5G). Chronic infusion of osanetant effectively prevented the isolation-induced slowed weight gain and depressive-like behaviors in female mice (Fig. 5H-O). Lastly, we employed the Nk3R knockout (KO) mice (Fig. 5P, Q). After two weeks of social isolation starting at P21, Nk3R KO female mice did not exhibit the typical isolation-induced slowed weight gain or depressive-like behaviors (Fig. 5R-Z).

4. Discussion

Prolonged exposure to stress increases susceptibility to anxiety, depression, and other psychiatric disorders [57, 58]. Here we show that chronic social isolation during adolescence induced sex-difference in depressive-like behaviors. Isolated female mice exhibit slower weight gain which mediated the sexually dimorphic effects of social isolation. Upregualtion of the neuropeptide Tac2 in the MeA specifically mediates stress-induced slowed weight gain and depressive-like behaviors. Altogether, our results suggest that slowed weight gain during social isolation is a risk factor in mediating sex-difference in stress susceptibility, which is modulated by MeA Tac2-Nk3R signaling.

We demonstrate that a two-week social isolation paradigm induces significant behavioral deficits in female mice, but not in male mice, across a battery of depression-related tests. While the higher incidence of depression in women is well-documented [25], there are few animal models that effectively replicate this increased susceptibility to stress. Many existing stress models, such as learned helplessness, where females exhibit more active escape behaviors [59], and repeated social defeat stress,

which can only be conducted in specific species [60, 61] or in lactating female rodents [62], present challenges for studying females. As a result, much of the research on stress vulnerability mechanisms has been conducted using male rodents, leaving the underlying mechanisms of sex differences in stress susceptibility largely unexplored. In this study, we employed a chronic social isolation paradigm that begins at post-weaning. This model is known to induce depressive-like behaviors, making it a useful tool for investigating sex-dependent differences in stress susceptibility. Our results show that three weeks of social isolation induced depressive-like behaviors in both male and female mice, consistent with previous findings that adolescent social isolation can lead to depression-like behaviors [63, 64]. Interestingly, female mice exhibited greater susceptibility, with just two weeks of social isolation sufficient to trigger behavioral changes, while male mice did not display depressive-like symptoms at this time. Thus, the two-week social isolation paradigm reveals a sex difference in stress susceptibility, offering an opportunity to explore the mechanisms underlying these differences in depression. One limitation of the current study is that we only examined behavioral differences after two weeks of social isolation. However, three weeks of isolation also resulted in gender differences in depressive-like behaviors, with females exhibiting more severe symptoms. This aspect was not deeply investigated in the present study. Future research should focus on understanding the mechanisms behind sex differences in depressive-like behaviors after three weeks of social isolation.

One of the notable findings in our study is that female mice with depression exhibited slower weight gain during social isolation. Moreover, calorie restriction to suppress weight gain replicated the depressive-like behaviors induced by social isolation in both male and female mice, while a high-fat diet reversed the slowed weight gain, thereby rescuing the depressive-like behaviors observed in female mice. These findings suggest that impaired weight gain during social isolation is a critical factor contributing to the development of depressive-like behaviors. However, previous evidence suggested that depression is higher in obese and overweight individuals compared to those with a normal weight [6], which was the opposite of what we observed in the experiments. The relationship between depression and body weight is complex and can be influenced by sociodemographic factors [13, 65]. The mechanisms through which individuals cope with depressive symptoms and obesity are likely to vary across different social and cultural context [66]. Specifically, prior research has highlighted the significant role of gender differences in both depression and body weight, with substantial evidence indicating that females are more likely to experience depression than males [67, 68]. Additionally, it has been shown that obesity is more strongly associated with depression in females than in males [69–71]. For instance, one study reported that the prevalence of depression is more than twice as high among females with a BMI of 30 or greater compared to those with a BMI under 30 [16]. To the best of our knowledge, our study is the first to demonstrate that body weight mediates the sex differences in stress susceptibility. It was also noticed that, while 20% CR treatment induced depressive- and anxiety-like behaviors in male and female mice, 40% CR had an antidepressant effect. In fact, previous studies also reported that 30%–40% CR played a critical role in reducing depressed mood in both humans and animals [72–74]. A large body of evidence demonstrated that CR produced an antidepressant effect through the activation of orexin signaling and

CREB/BDNF signaling, and by releasing endogenous endorphin [72, 75, 76]. Other investigator indicated that CR may have an antidepressant-like effect through astrocyte ATP regulated excitatory synaptic transmission in the mPFC [77]. However, when the antidepressant effect of 40% CR was observed in control female and male mice, it did not improve the depressive-like behaviors in two-week isolated female mice, So these results further indicate that impaired weight gain during social isolation is a critical factor contributing to the development of depressive-like behaviors.

A variety of neuropeptides have been implicated in stress responses [78, 79]. Previous research on tachykinins in stress has primarily focused on Tac1/Substance P/NkA [80–82]. However, pharmacological and genetic studies on NkB's role in stress responses have produced conflicting results [83]. Inspired by findings in Drosophila [84, 85], we identified Tac2/Nk3R as a crucial and previously overlooked mediator of the effects of chronic social isolation on the brain. The observation that tachykinins influence social isolation-induced aggression in both flies and mice supports the idea of an evolutionary conservation of neuropeptide functions in behavioral regulation across species [86]. It is plausible that Tac2/Nk3R may also contribute to the well-documented tendency for solitary confinement to increase aggression in humans [87]. Our social isolation paradigm differs from acute and repeated intermittent stressors (such as footshock, restraint, and forced swim) not only in its nature but also in its prolonged duration and continuous nature. Therefore, the involvement of the Tac2/Nk3R system in chronic social isolation may reflect these distinctive characteristics. Nonetheless, the fact that systemic administration of osanetant or Nk3R knockout effectively blocked social isolation-induced depressive-like behaviors among female mice suggests that Tac2/Nk3R plays a broader role in sex difference of stress response mechanisms. This is not surprising since it has been previously shown that Nk3R antagonism in adult humans reduces circulating sex hormones [88]. Previous studies show no changes on estradiol after a mild acute stressor such as an elevated platform even 6 h after the offset of the stressor [89]. In contrast, other studies using swim stress found increased estradiol concentrations 6 h after stress inoculation in adult females [90]. From these results, it can be concluded that the Tac2/Nk3R pathway may be a key factor in the regulation of sex differences in depression through circulating estradiol. Biochemical studies on Nk3R action indicate that Tac2/Nk3R signaling likely enhances intracellular free calcium through the IP3/DAG pathway [47], potentially amplifying neuronal activation by glutamate or other excitatory transmitters. So activation of Tac2/Nk3R signaling may normalizes neuronal activity in the MeA in isolated female mice. However, further research is needed to provide more conclusive evidence to support this hypothesis.

In conclusion, our study reveals a sex difference in stress susceptibility during adolescent social isolation. Importantly, we are the first to propose that slowed weight gain is a key factor contributing to this sex difference in stress vulnerability, mediated by MeA Tac2-Nk3R signaling. Thus, our findings offer new insights into the mechanisms underlying sex differences in stress susceptibility and suggest a potential novel approach for treatment.

Ethics Approval

Not applicable.

Declarations

Ethics Approval

Not applicable.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Consent for Publication

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. All authors read and approved the final manuscript. The authors consent for publication in *Translational Psychiatry*.

Research Involving Human Participants and/or Animals

All procedures were performed in accordance with the guidelines in the National Institutes of Health Guide for Care and Use of Laboratory Animals, and the study design was approved by the appropriate ethics review board.

Competing Interests

The authors declare that they have no competing interests.

Code Availability

Not applicable.

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Authors' Contributions

JH.L. designed the study. M.W. and JZ.L. performed the behavior tests and analysis. Q.W. and K.Z. conducted the ELISA measurements. Z.C. and S.D. performed the immunofluorescence and the stereotaxic microinjection. S.L. and X.M. performed the western blotting. JH.L. conceived the project and wrote the manuscript . All the authors approved the final manuscript.

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Figures

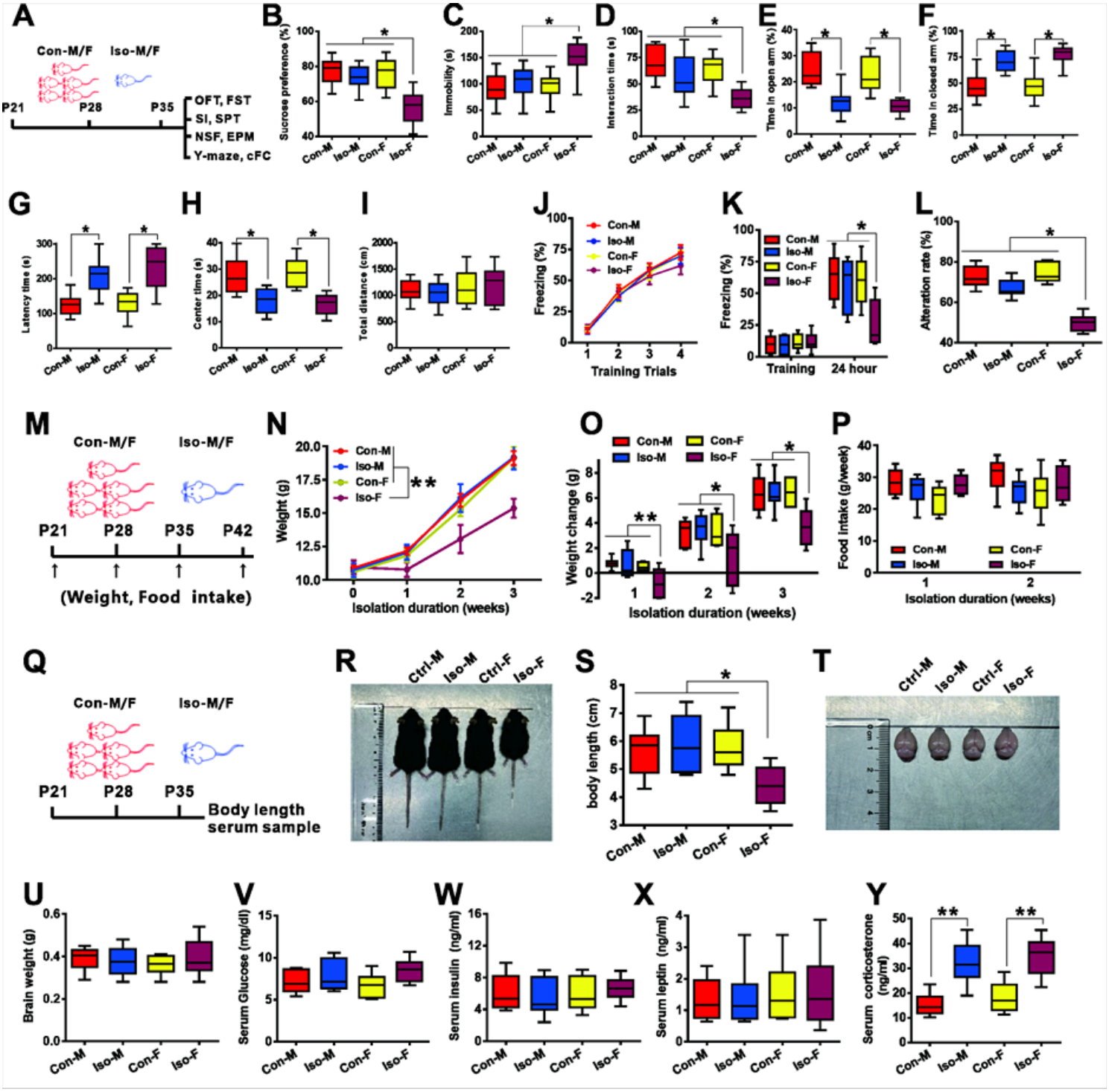

Figure 1

Social isolation induced stress susceptibility and slowed weight gain in female but not male mice. (**A**) Schematic of the depressive- and anxiety-related behavior tests after social isolation during adolescence. One batch of mice underwent the OFT and the FST at one-day intervals, while another underwent the SI test and the SPT at one-day intervals, another underwent the NSF and the EPM at one-day intervals, and another underwent the Y-maze and the cFC at one-day intervals. (**B**) The sucrose preference was decreased in female mice suffering from two-week social isolation during adolescence (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.873, P=0.043). (**C**) Increased immobile time of isolated

female mice in the FST (n=12 mice/group; two-way ANOVA; $F_{(3, 44)}$=4.612, P=0.045). (**D**) After two-week social isolation begun at P21, female mice exhibited impaired social ability (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=5.218, P=0.040). (**E, F**) In the EPM test, isolated mice spent significantly less time in the open arms and more time in the closed arms (n=12 mice/group; two-way ANOVA; for E, $F_{(3, 44)}$=5.634, P=0.037; for F, $F_{(3, 44)}$=5.287, P=0.041). (**G**) In the NSF test, the latency to explore and bite the food was increased in the two-week social isolated mice (n=12 mice/group; two-way ANOVA; $F_{(3, 44)}$=5.378, P=0.042). (**H, I**) Two-week socially isolated mice from P21 spent less time in the central arena without affecting locomotor activity in the OFT (n=12 mice/group; two-way ANOVA; for H, $F_{(3, 44)}$=5.615, P=0.039; for I, $F_{(3, 44)}$=1.792, P=0.376). (**J, K**) The freezing time was decreased among isolated female mice in the contextual fear conditioning test (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.082, P=0.048). (**L**) Two-week isolated female mice showed no alteration in the Y-maze test (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.523, P=0.044). (**M**) Schematic of the body weight experiments. (**N**) Body weight of mice under social isolation paradigm (n=12 mice/group; repeated measures two-way ANOVA; $F_{(3, 176)}$=11.372, P=0.022). (**O**) Isolated female mice exhibited lower weight gain (n=12 mice/group; repeated measures two-way ANOVA; $F_{(3, 132)}$=16.634, P=0.017). (**P**) There was no difference of the food intake among these groups during social isolation (n=12 mice/group; repeated measures two-way ANOVA; $F_{(3, 88)}$=2.092, P=0.626). (**Q**) Schematic of the serum sampling experiments. (**R, S**) The represented image and quantification of body length changes after two-week social isolation (n=12 mice/group; two-way ANOVA; $F_{(3, 44)}$=4.027, P=0.049). (**T, U**) The represented image and quantification of brain weight changes after two-week social isolation (n=12 mice/group; two-way ANOVA; $F_{(3, 44)}$=1.412, P=0.531). (**V-Y**) Serum glucose, insulin, leptin and corticosterone levels after two-week social isolation (n=8 mice/group; two-way ANOVA; for V: $F_{(3, 28)}$=1.235, P=0.875; for W: $F_{(3, 28)}$=1.326, P=0.637; for X: $F_{(3, 28)}$=2.243, P=0.209; for Y: $F_{(3, 28)}$=5.579, P=0.019). Data were presented as mean ± s.e.m; *, P < 0.05, **, P<0.01.

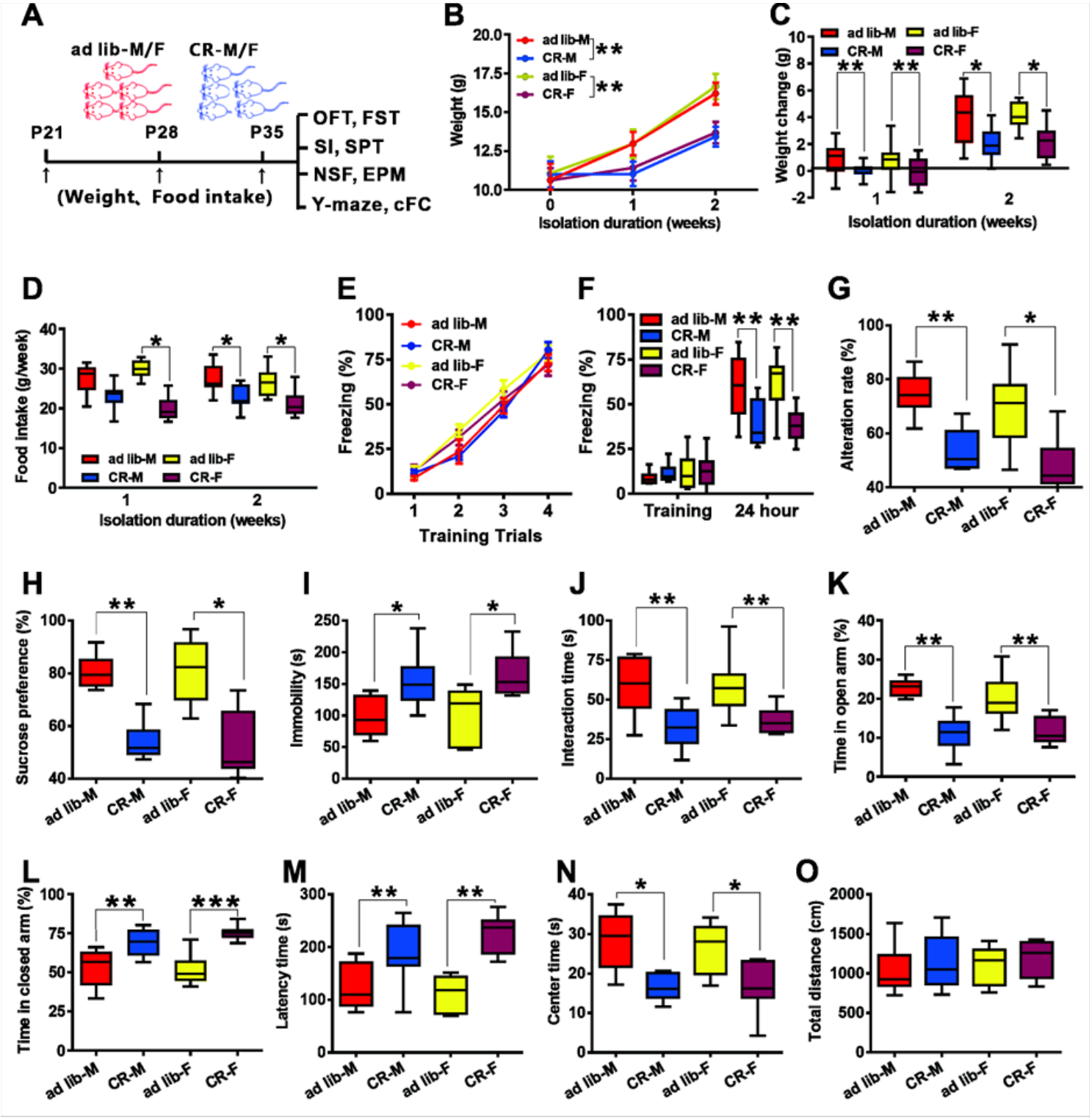

Figure 2

Calorie restriction to suppress weight gain mimics social isolation induced abnormal behaviors both in male and female mice. (**A**) Schematic of the experiments. One batch of mice underwent the OFT and the FST at one-day intervals, while another underwent the SI test and the SPT at one-day intervals, another underwent the NSF and the EPM at one-day intervals, and another underwent the Y-maze and the cFC at one-day intervals. (**B**) Body weight of mice under calorie restriction paradigm (n=10 mice/group;

repeated measures two-way ANOVA; $F_{(3, 108)}$=14.742, P=0.017). (**C**) Calorie restricted male and female mice exhibited lower weight gain (n=10 mice/group; repeated measures two-way ANOVA; $F_{(3, 72)}$=10.025, P=0.011). (**D**) Calorie restriction treatment led to decreased food intake (n=10 mice/group; repeated measures two-way ANOVA; $F_{(3, 72)}$=9.092, P=0.025). (**E, F**) The freezing time was decreased among calorie restricted mice (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.092, P=0.041). (**G**) Two-week calorie restricted mice showed no alteration in the Y-maze (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=5.461, P=0.032). (**H**) The sucrose preference was decreased in calorie restricted mice (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=7.018, P=0.027). (**I**) Increased immobile time of calorie restricted mice in the FST (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=5.561, P=0.035). (**J**) Two-week calorie restricted mice exhibited impaired social ability (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=5.625, P=0.036). (**K, L**) In the EPM test, calorie restricted mice spent significantly less time in the open arms and more time in the closed arms (n=10 mice/group; two-way ANOVA; for K, $F_{(3, 36)}$=6.689, P=0.029; for L, $F_{(3, 36)}$=7.315, P=0.021). (**M**) In the NSF test, the latency to explore and bite the food was increased in the two-week calorie restricted mice (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=5.463, P=0.037). (**N, O**) Two-week calorie restricted mice spent less time in the central arena without affecting locomotor activity in the OFT (n=10 mice/group; two-way ANOVA; for N, $F_{(3, 36)}$=6.093, P=0.037; for O, $F_{(3, 36)}$=2.815, P=0.457). Data were presented as mean ± s.e.m; *, p < 0.05,**, P<0.01, ***, P<0.001.

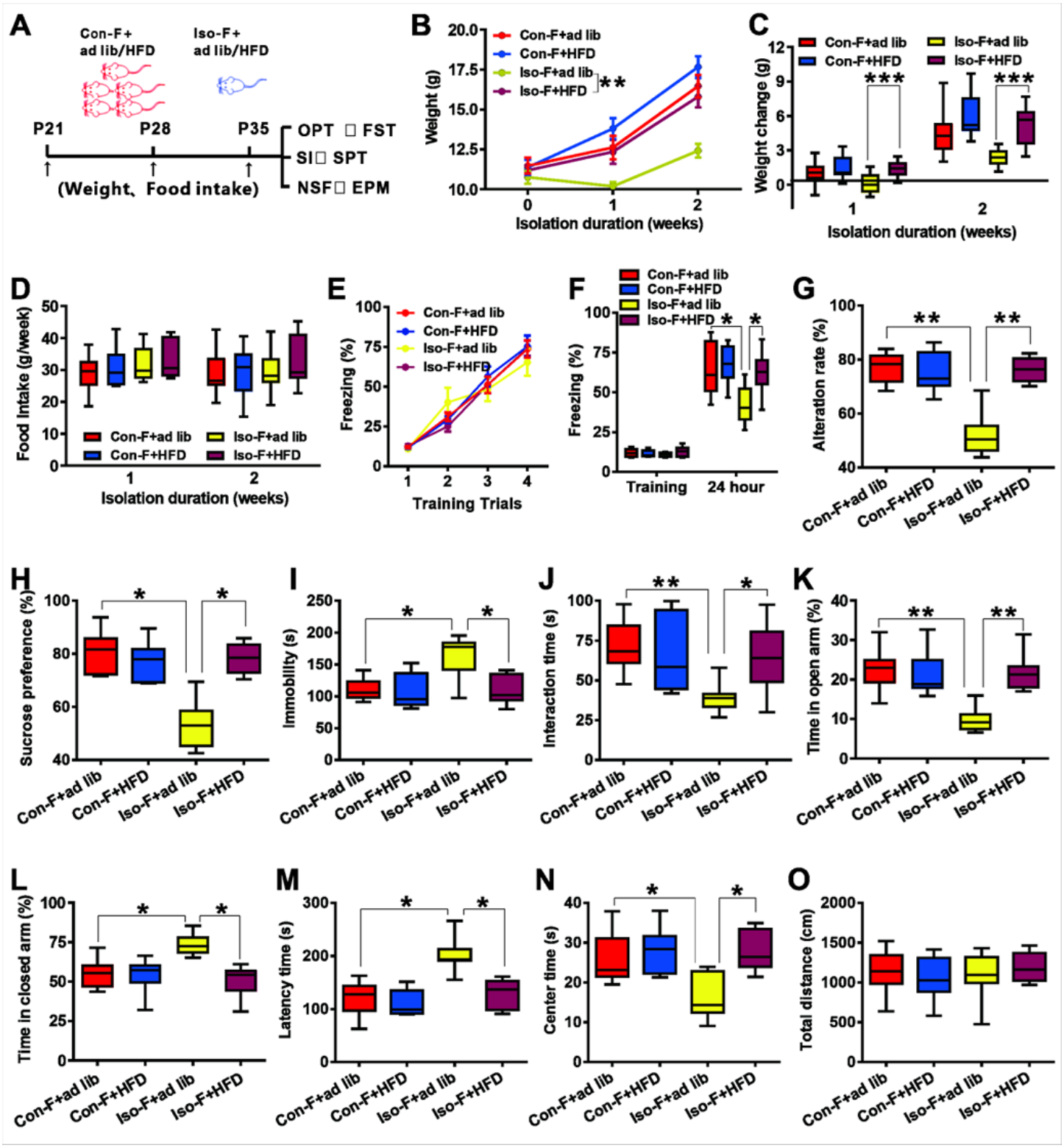

Figure 3

High-fat diet rescues the slower weight gain and depressive-like behaviors in isolated female mice. (**A**) Schematic of the experiments. One batch of mice underwent the OFT and the FST at one-day intervals, while another underwent the SI test and the SPT at one-day intervals, and another underwent the NSF and the EPM at one-day intervals. (**B**) Body weight of isolated female mice under high-fat diet (HFD) paradigm (n=10 mice/group; repeated measures two-way ANOVA; $F_{(3, 108)}$=11.073, P=0.038). (**C**) HFD

normalized the lower weight gain in two-week isolated female mice (n=10 mice/group; repeated measures two-way ANOVA; $F_{(3, 72)}$=16.765, P=0.037). (**D**) There was no difference of the food intake among these groups (n=10 mice/group; repeated measures two-way ANOVA; $F_{(3, 72)}$=2.578, P=0.527). (**E, F**) The freezing time was decreased among isolated female mice, which was rescued by HFD (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=3.875, P=0.045). (**G**) HFD rescued social isolation-induced impaired cognition in the Y-maze test among female mice (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.519, P=0.048). (**H**) HFD rescued decreased sucrose preference in isolated female mice (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.426, P=0.044). (**I**) Increased immobile time of isolated female mice was reversed by HFD in the FST (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.635, P=0.046). (**J**) Two-week HFD reversed impaired social ability in isolated female mice (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.091, P=0.043). (**K, L**) Two-week HFD normalized the anxiety-like behaviors in the EPM test among isolated female mice (n=10 mice/group; two-way ANOVA; for K, $F_{(3, 36)}$=4.837, P=0.042; for L, $F_{(3, 36)}$=4.091, P=0.044). (**M**) In the NSF test, the increased latency to explore and bite the food was reversed by HFD in isolated female mice (n=10 mice/group; two-way ANOVA; $F_{(3, 36)}$=4.316, P=0.047). (**N, O**) Two-week isolated female mice spent similar time in the central arena administrated HFD compared with control mice, without affecting locomotor activity in the OFT (n=10 mice/group; two-way ANOVA; for N, $F_{(3, 36)}$=4.738, P=0.045; for O, $F_{(3, 36)}$=2.413, P=0.498). Data were presented as mean ± s.e.m; *, p < 0.05,**, P<0.01, ***, P<0.001.

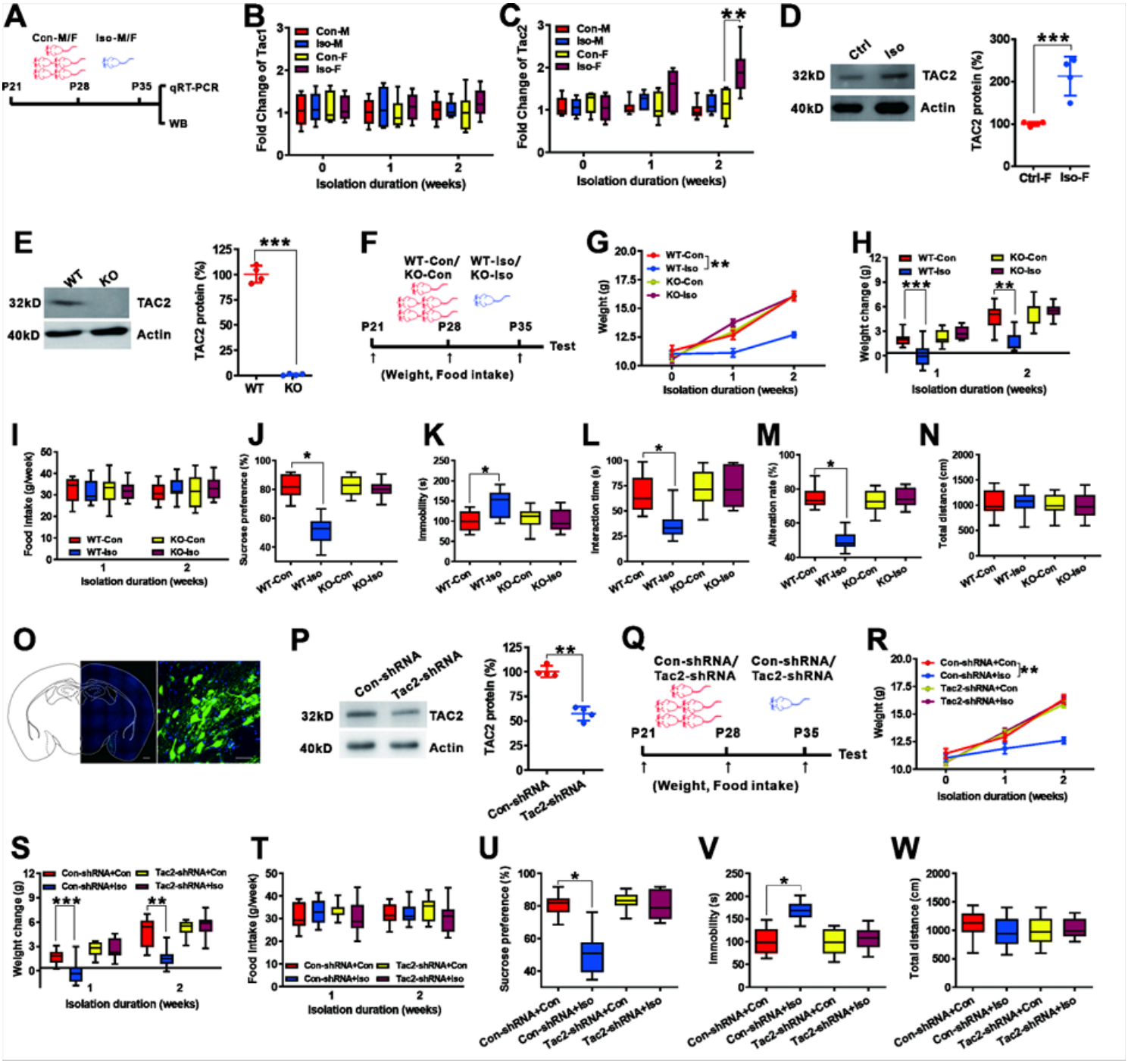

Figure 4

MeA Tac2 mediates social isolation-induced changes in female mice. (**A**) Schematic of the experiments. (**B**) Social isolation did not affect mRNA level of Tac1 in the MeA region (n= 8 mice/group; two-way ANOVA; for 0, $F_{(3, 28)}$=1.302, P=0.412; for 1, $F_{(3, 28)}$=1.641, P=0.454; for 2, $F_{(3, 28)}$=2.742, P=0.213). (**C**) Social isolation led to increased mRNA level of Tac2 in the MeA region (n= 8 mice/group; two-way ANOVA; for 0, $F_{(3, 28)}$=1.258, P=0.438; for 1, $F_{(3, 28)}$=2.964, P=0.108; for 2, $F_{(3, 28)}$=4.125, P=0.028). (**D**) Western blots showing Tac2 protein level increased after two-week social isolation in the MeA of female mice (n = 4 experiments/group; two tailed Student's t test, P = 0.013). (**E**) Western blots of Tac2 in the MeA from Tac2 KO mice and their WT littermates (n = 4 experiments/group; two-tailed Student's t-test; P

< 0.0001). (**F**) Schematic of the experiments. (**G-N**) The impairing effects of two-week social isolation on body weight (G-I, n=10 mice/group; repeated measures two-way ANOVA; for G: $F_{(3, 108)}$=15.975, P=0.038; for H: $F_{(3, 72)}$=9.374, P=0.041; for I: $F_{(3, 72)}$=3.985, P=0.623) and depressive-like behaviors (J-N, n=10 mice/group; two-way ANOVA; for J: $F_{(3, 36)}$=4.357, P=0.047; for K: $F_{(3, 36)}$=4.196, P=0.048; for L: $F_{(3, 36)}$=4.887, P=0.045; for M: $F_{(3, 36)}$=5.091, P=0.039; for N: $F_{(3, 36)}$=2.354, P=0.332) disappeared in Tac2 KO mice. (**O**) Representative fluorescence images showing the locations and cells infected with the pAAV-CAG-Tac2-eGFP (Tac2-shRNA) vector in the MeA region. Scale bar: left: 500 μm, right: 20 μm. Experimental images were obtained from seven mice; three images per mouse were taken, with similar results obtained. (**P**) Western blots showing Tac2 reduction after Tac2-shRNA virus injection (n= 4 experiments/group; two-tailed Student's t-test, P = 0.024). (**Q**) Schematic of the experiments. (**R-W**) Knockdown of Tac2 in the MeA rescued the impairing effects of two-week social isolation on body weight (R-T, n=10 mice/group; repeated measures two-way ANOVA; for R: $F_{(3, 108)}$=12.376, P=0.039; for S: $F_{(3, 72)}$=9.024, P=0.043; for T: $F_{(3, 72)}$=3.145, P=0.665) and depressive-like behaviors (U-W, n=10 mice/group; two-way ANOVA; for U: $F_{(3, 36)}$=4.543, P=0.047; for V: $F_{(3, 36)}$=4.467, P=0.048; for W: $F_{(3, 36)}$=1.356, P=0.745). Data were presented as mean ± s.e.m; *, p < 0.05,**, P<0.01, ***, P<0.001.

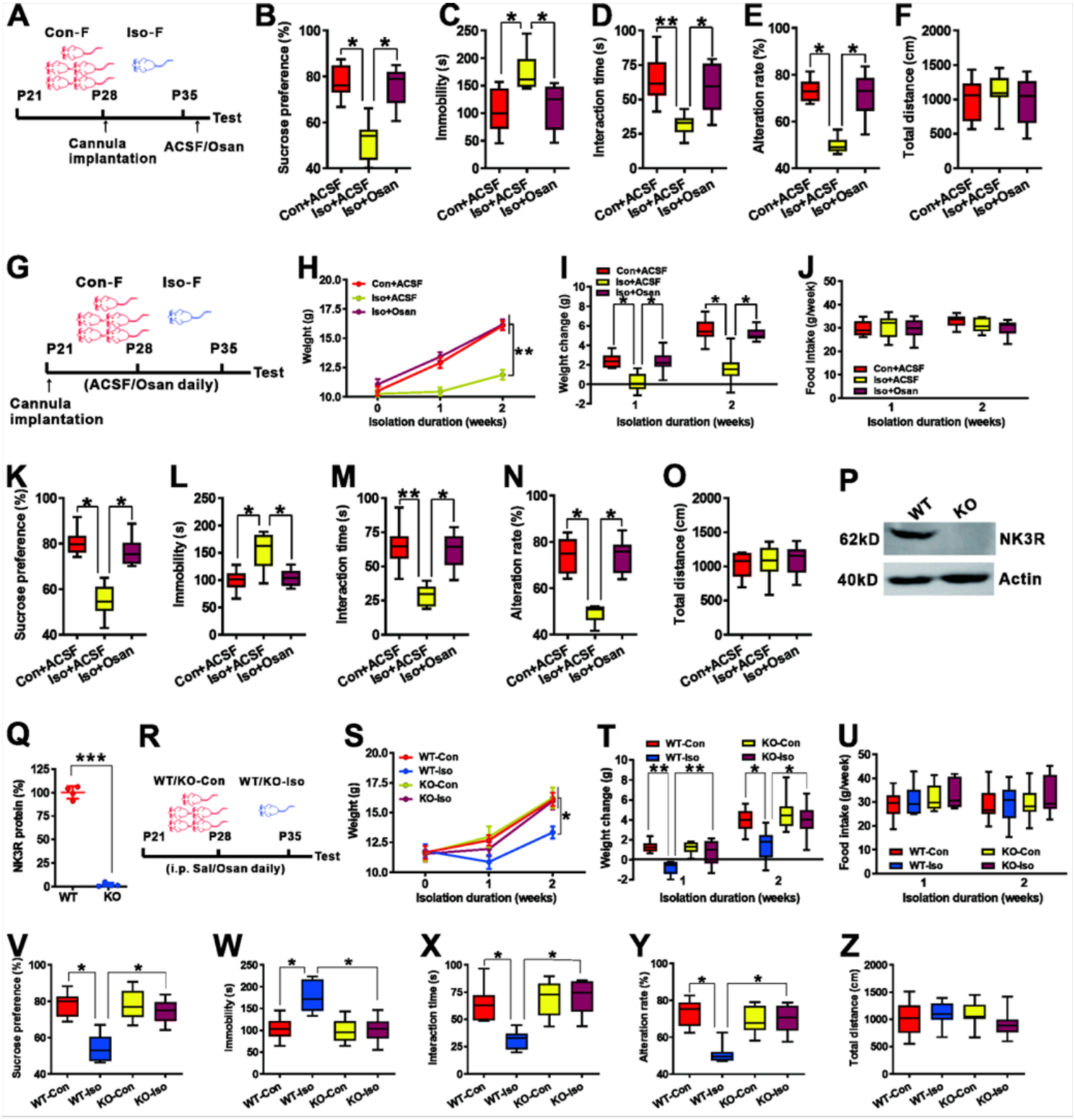

Figure 5

Nk3Rs act in the MeA to mediate the effects of two-week social isolation. (**A-F**) Osanetant infusion into the MeA reversed depressive-like behaviors in isolated female mice (n=10 mice/group; one-way ANOVA; for B: $F_{(2, 27)}$=4.385, P=0.041; for C: $F_{(2, 27)}$=4.569, P=0.043; for D: $F_{(2, 27)}$=5.582, P=0.033; for E: $F_{(2, 27)}$=5.563, P=0.031; for F: $F_{(2, 27)}$=2.092, P=0.583). (**G-O**) Microinjection of osanetant into the MeA daily during social isolation prevented the slower weight gain (H-J, n=10 mice/group; repeated measures two-

way ANOVA; for H: $F_{(2, 81)}$=10.782, P=0.031; for I: $F_{(2, 81)}$=12.018, P=0.030; for J: $F_{(2, 81)}$=3.267, P=0.508) and depressive-like behaviors (K-O, n=10 mice/group; one-way ANOVA; for K: $F_{(2, 27)}$=4.894, P=0.041; for L: $F_{(2, 27)}$=4.560, P=0.045; for M: $F_{(2, 27)}$=5.621, P=0.032; for N: $F_{(2, 27)}$=5.908, P=0.026; for O: $F_{(2, 27)}$=2.192, P=0.453) in isolated female mice. (**P, Q**) Western blots of Nk3R in the meA from Nk3R KO mice and their WT littermates (n = 4 experiments/group; two-tailed Student's t-test; P < 0.0001). (**R**) Schematic of the experiments. (**S-Z**) The impairing effects of two-week social isolation on body weight (S-U, n=10 mice/group; repeated measures two-way ANOVA; for S: $F_{(3, 108)}$=15.636, P=0.040; for T: $F_{(3, 72)}$=9.983, P=0.037; for U: $F_{(3, 72)}$=3.635, P=0.772) and depressive-like behaviors (V-Z, n=10 mice/group; two-way ANOVA; for V: $F_{(3, 36)}$=4.536, P=0.046; for W: $F_{(3, 36)}$=4.502, P=0.047; for X: $F_{(3, 36)}$=5.013, P=0.043; for Y: $F_{(3, 36)}$=5.247, P=0.038; for Z: $F_{(3, 36)}$=2.983, P=0.343) disappeared in Nk3R KO mice. Data were presented as mean ± s.e.m; *, p < 0.05,**, P<0.01, ***, P<0.001.

Supplementary Files

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