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Neural mechanisms underlying strain preference behaviour and plasticity in mice

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All social animals, including humans, have different social identities that generate unique social interactions. Social preference behaviours, including social integration, prosocial behaviour, and cooperation, have a wide impact on an individual's social life. However, the neural mechanisms underlying social preferences are not yet clear. In this study, using mice as model animals, we investigated strain preference, which is a social preference based on social identification. We revealed, for the first time, that the social behaviour strain preference of mice is heterogeneous; that is, C57 mice prefer to interact with mice of the same strain, whereas KM mice prefer to interact with mice of a different strain. We further confirmed that strain preference in mice can be plastically altered by changing the nurturing environment and increasing social exposure to mice of other strains. Finally, we screened brain regions related to mouse strain preference and revealed that the activity of the periaqueductal grey (PAG) was not only consistent with the social preference of both C57 and KM mice but also coordinated with the alteration in social preference. We subsequently used muscarine to inhibit the PAG in C57BL/6J mice and found that the strain-specific social preference in C57 mice disappeared. These results showed that the PAG is a key brain region for regulating strain preference and its plasticity. This work fills a gap in the study of strain preferences in social preference research.

Social preferences, which arise based on social recognition, are defined as an individual's tendency to preferentially interact with one social stimulus over another[1,2]. These preferences affect the social activities of animals and even determine their survival[3,4]. Social preferences are evident in rodent behaviour. For example, mice display social novelty preference, showing a greater inclination to interact with novel conspecifics, as evidenced by increased social time with a new social partner than with a familiar one[5]. In human society, another form of social preference that has attracted much attention is the preference towards ingroup members over outgroup members. As a result, individuals tend to show stronger empathy and altruism towards ingroup members[6–8]. Social grouping is a ubiquitous phenomenon in human societies rooted in disparities among individuals belonging to various social groups and is defined by factors such as nationality, gender, occupation, and educational background. The intricate network formed by these multiple social identities gives rise to significant heterogeneity in interpersonal interactions. Although existing research has demonstrated the profound impact of social groupings on individual social behaviours, neural mechanisms, particularly at the level of brain circuits, remain largely unexplored.

In this study, mice were employed as model organisms to investigate the neural mechanisms underlying the differential social interactions influenced by social groupings at the brain circuit level. This choice is grounded in several considerations. First, rodents are now recognised to exhibit a rich repertoire of social behaviours[9–12]. Consequently, mice have been extensively used as experimental animals to explore the neural underpinnings of diverse social behaviours, such as novelty preference, social memory, social aggression, and social hierarchy[2,13–15]. Second, as inherently social and gregarious animals, mice exhibit strain-specific differences that can be considered a form of social grouping. The variations in social interactions observed between different mouse strains thus serve as a paradigm for studying the impact of social groupings on social behaviour. Therefore, this study proposes a research paradigm utilising mice to examine the neural mechanisms underlying social interaction differences induced by social groupings, as well as their plasticity.

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Social behaviour can be modified by a nurturing environment, which is another important means to support adaptation to changes in living conditions. For example, increasing exposure to outgroup members, especially positive outgroup members, can decrease one's implicit bias towards them[16,17], indicating that social preferences are highly plastic. For mice, cross fostering provides a nurturing environment with enhanced exposure to mice of other strains. Cross-fostering is a method used to assess the influence of the rearing environment on the behavioural phenotypes of mice in adulthood[18]. Cross-fostering can influence a mouse's sex preferences, water maze performance[19], autism-like symptoms[20], and aggressive behaviour[21]. However, whether and how it alters strain preference is still unclear. More importantly, the degree to which strain preference can be plastically modified by the nurturing environment is still unknown.

The neural mechanism of social activity has been extensively studied in recent years, and several brain regions, including the basolateral amygdala (BLA), prefrontal cortex (PFC), posterior intralaminar thalamic nucleus (PIL), CA2 region of the hippocampus (CA2), supramammillary nucleus (SUM), and periaqueductal grey (PAG), have been identified to be vital for different aspects of social behaviour[22–27]. Therefore, we hypothesised that these brain regions might be involved in regulating strain preference and its plasticity in mice.

In this study, we first investigated the existence of strain-dependent differences in social interactions between two distinct mouse strains, C57BL/6J (C57) and Kunming (KM) mice. Upon confirming these strain-specific differences, we further explored whether cross-fostering, a manipulation that increases exposure to conspecifics of a different strain, could attenuate these behavioural disparities. Importantly, we excluded the potential influence of anxiety and depression, which could be induced by cross-fostering. We subsequently conducted a screening process to identify brain regions potentially involved in these behaviours. Based on neural activity patterns, PAG emerged as the brain region that most consistently correlated with the observed behavioural differences. Furthermore, transient inactivation of the PAG using muscimol abolished the strain-specific differences in social interactions, providing compelling evidence for the pivotal role of the PAG in mediating these behaviours.

Materials and methods
Ethics information
The animal experiments and animal handling procedures were approved by the Animal Ethics Committee of Kunming Medical University (project number kmmu20220082). The experiments followed the relevant guidelines of the National Institutes of Health (NIH) and strictly followed internationally recognised animal welfare and ethical guidelines, aiming to manage and use experimental animals in a humane and scientific manner. The animals used in the experiments (C57 and KM mice) were provided by the Experimental Animal Center of Kunming Medical University (experimental animal licence number: SCXK9 (Dian) k2015-0002). After the experiment, the mice were anaesthetised by an intraperitoneal injection of sodium pentobarbital, euthanised, and killed by cervical dislocation. The experimental procedures followed the ARRIVE guidelines.

Animals
C57BL/6J mice and Kunming (KM) mice were used in this study. The mice were housed under standard laboratory conditions, with a temperature range of 22–25 °C, humidity between 40 and 70%, and a 12-hour light/dark cycle (9:00 AM to 9:00 PM, automatically controlled). Specifically, all female mice, including C57BL/6J and KM female mice, were maternal mice used for cross-fostering, and the breeding mating ratio was 1:2. During the late stages of pregnancy and before the pups were born, the female mice were placed alone. The breeding cages were raised in nests before the mice reached cage age (3 weeks). Cross-fostering mice (test mice) were housed with their mothers prior to weaning. After weaning, male mice were selected and raised at a density of ≥ 96.7 cm². According to the Guide for the Care and Use of Laboratory Animals, when the mice weighed less than 20 g after being separated into cages, a maximum of 8 mice were placed in each cage; when the mice weighed ≥ 20 g, a maximum of 6 mice were placed in each cage. Eight-week-old adult male mice were used directly for both normally reared mice and stimulated mice. All the mice used for the social interaction test were 8-week-old male mice. All the male test mice and stimulated mice underwent half an hour of adaptation training in the behavioural laboratory three days before the behavioural experiments. All mice were kept in uniform cages. The rearing conditions remained consistent, and the main indicators were as follows: the overall cage box dimensions were $403 \times 165 \times 175$ mm (length×width×height), the bottom area was 665 cm², and the cage height was 13 cm. The bedding material used was SPF-irradiated mouse bedding material, the food used was SPF-irradiated mouse feed, and the bedding material was changed every 4 days. The lighting intensity in the animal housing rooms was maintained between 15 and 30 lx, and that in the behavioural laboratory was between 150 and 200 lx. The noise level in the housing rooms was kept below 60 db. The mice were provided ample food and water throughout the experiment.

The control mice in this study were normally reared mice, including normally raised C57 mice and KM mice. The treatment group consisted of cross-fostering mice, which included C57 mice and KM mice grown in a mixed-bred environment. The cross-fostering mice were handed over to the lactating dam for mixed rearing within 3 days after birth. When the mice reached 8 weeks of age, behavioural experiments were conducted. All the mice used in the behavioural experiments were male. The only female mice in this study were the maternal mice. Information on the number of litters and mice used in the behavioural experiments has been compiled in Supplemental Table 1.

Power size calculation
Sample size selection was based on references in the same field. The replicate sample size for the mouse experiments was six or more. For the main experimental results, sample size calculations and power analyses were performed for C57 and KM mice using G*Power 24 software (version 3.1.9.7). For the behavioural experiments, a three-chamber social preference experiment was performed to calculate sample sizes and to conduct power

analyses between strains. The paired t test results of C57 mice revealed that the required total sample size of C57 mice was $n = 19$ (output parameters: effect size dz = 0.79, noncentrality parameter = − 3.44, critical t = − 2.1, actual power = 0.90); the required total sample size of KM mice was $n = 5$ (output parameters: effect size dz = 2.15, noncentrality parameter = 4.80, critical t = 2.78, actual power = 0.94). For the immunofluorescence experiments, using the PAG brain region in a normal rearing environment as an example, the results of one-way analysis of variance revealed that the required total sample size of C57 mice was $n = 24$ (output parameters: effect size f = 0.84, noncentrality parameter = 17.00, critical F = 3.46, actual power = 0.93); the required total sample size of KM mice was $n = 9$ (output parameters: effect size f = 1.73, noncentrality parameter = 27.06, critical F = 5.14, actual power = 0.94). For the drug administration experiment, the total sample size of C57 mice required for the paired t test was $n = 7$ (output parameters: effect size dz = 1.41, noncentrality parameter = 3.74, critical t = 2.44, actual power = 0.87). In summary, as mentioned above, the selection of experimental samples meets the experimental design requirements.

Mouse cross-fostering experiment

Cross-fostering of newborn mice was used to investigate whether the rearing environment can alter strain-dependent social preference behaviour. Specifically, the cross-fostering experiments were conducted in a similar manner. First, the time point for cross-fostering was within 1–3 days after the birth of the offspring. That is, to carry out cross-fostering, one litter of KM newborn mice and one litter of C57 newborn mice born within 1–3 days were needed for each round of cross-fostering (two litters of mice cannot be born more than 3 days apart). After having age-matched offspring, we replaced half of the total number of C57 pups with the same number of KM pups. That is, half of the C57 pups were given to the KM lactating dam (the C57 pups in the cross-fostered environment are represented by C57$_{LD: KM}$, where $_{LD: KM}$ means the lactating dams were KM), and half of the KM pups were given to the C57 lactating dam (the KM pups in the cross-fostered environment are represented by KM$_{LD: C57}$, where $_{LD: C57}$ represents the lactating dams were C57). Specifically, when conducting cross-fostering experiments, first, the bedding materials of the KM dam or C57 dam were placed in two cleaned boxes. The KM pups were then loosely covered with bedding material from the C57 dam, and the C57 pups were loosely covered with bedding material from the KM dam. This bundled arrangement was then placed in an air-conditioned environment at 28 °C for 30 min, allowing the odour from the lactating dam C57/KM bedding to thoroughly mask the original scent of the KM/C57 pups. The KM or C57 pups were subsequently introduced into the cages of lactating C57 or KM dams for cross-fostering, respectively. Observations were made to determine whether the lactating dam exhibited retrieval behaviour. The above experimental procedure ensured that the lactating dam could successfully retrieve and raise heterogeneous mice raised via mixed breeding. When the pups reached 3–4 weeks of age, they were separated into cages after weaning. Male C57 and KM mice from the same litter were selected and raised together. Behavioural experiments were conducted after the animals were raised for 8 weeks.

Strain social interaction test

Eight-week-old male mice were used for this test. The three-chamber social interaction test is commonly employed to assess social interaction behaviour in mice[5]. In this experiment, we utilised a modified version of the classical three-chamber social interaction test, referred to as the strain social interaction test. Male KM and C57 mice underwent three-day adaptation in the behavioural laboratory before testing. During adaptation, cupping hands were used to reduce the stress experienced by the mice, that is, the mouse was placed in the hands of the experimenter for 3 min to adapt (linen gloves were used to prevent them from being bitten). The mouse was allowed to adapt for three minutes every day. If the mouse showed anxiety while being held, the time was increased until the mouse remained quietly in the experimenter's hands and exhibited grooming or face-washing behaviour. Prior to the experiment, the mice were allowed approximately 1 h of acclimatisation in the behavioural laboratory and then gently placed in the central empty chamber for 5 min to explore the three-chamber apparatus freely. The mouse was first moved to the experimental area on the hand of the experimenter and then gently placed into the empty chamber.

The experiment consisted of three phases. In the first phase, a mouse from one strain, termed Stranger 1, was placed in a metal confinement cage on one side, while the other side remained empty. The partitions between the chambers were opened, and the subject mice were allowed to explore freely for 10 min. In the second phase, a mouse from another strain, referred to as Stranger 2, was placed in the metal confinement cage on the opposite side. The partition corresponding to the area where Stranger 1 was located was closed. The subject mouse was then allowed to freely explore the three chambers for 10 min. To prevent recognition bias caused by order, the order in which the same or other strain of mouse was presented was randomised in phases one and two. Additionally, to mitigate spatial location effects, mice from the same-strain (SS) and other-strain (OS) groups were randomly placed on the left or right side. After 10 min of familiarisation with each stranger, in the third phase, both partitions were removed, allowing the subject mice to freely engage in social interaction for 10 min. The entire session was recorded using video equipment, and behavioural data for the last 10 min were analysed. Key behaviours, such as social preference, were reflected in the times (in seconds) the mouse spent in the left, middle, and right compartments of the three-chambered apparatus, as well as the investigation time (the time the subject mouse spent within 1 cm of the restraining cage), and interactions with strangers of the same and other strains were measured and analysed statistically. We continuously recorded the entire behavioural experiment and then, based on the experimental requirements, selectively timed the test mouse's presence in the left, middle, and right compartments or the direct interaction time with mice of the same or other strains. All behavioural data were statistically recorded manually using a single-blind method.

Two-chamber free social interaction test

The two-chamber free social interaction test is a modified version of the three-chamber social interaction test[5]. Male KM and C57 mice underwent a three-day habituation period in the behavioural laboratory prior to testing. Before each experimental phase, the mice were allowed approximately one hour to acclimate to the laboratory environment. During the first phase, both C57 and KM mice were gently placed in separate chambers divided by a transparent barrier, and they were allowed to explore and adapt for 10 min. In the second phase, the barrier was removed, allowing the mice to freely engage in social interactions for 10 min, with video recordings made of their behaviour. Key behaviours, including latency to interact, frequency of attacks, duration of attacks, and proactive social interactions (physical contact, sniffing), were quantified to prevent any cross-contamination of odours between tests, and all equipment was thoroughly cleaned with 75% alcohol between each experimental step.

Two-chamber social interaction test

The two-chamber social interaction test is a modification of the three-chamber social interaction test[5]. The aim of this study was to examine the expression of c-Fos neurons in specific brain regions following social interactions in test mice. Employing a two-chamber social interaction setup, the aim is to assess the neural responses of mice following interactions with different strains. Male KM and C57 mice underwent three-day adaptation in the behavioural laboratory before testing. Prior to each phase, the mice were allowed approximately one hour of acclimatisation in the laboratory. In the first phase, the subject mice were placed in the central empty chamber with the door to the left chamber open, allowing free exploration for 5 min. In the second phase, strangers (SS/OS) were placed in the metal confinement cage on the left side, and the subject mice were allowed to explore freely for 10 min. Before each experimental step, the equipment was cleaned with 75% alcohol to eliminate any odours left by previous test subjects.

Forced swimming test

The forced swimming test is commonly employed to assess depressive-like behaviour in mice[28]. Male KM and C57 mice underwent three-day adaptation in the behavioural laboratory before testing. The water level in the container (transparent cylindrical, height: 30 cm, diameter: 16 cm) was maintained at a depth of approximately 20 cm, and the water temperature was approximately 24–26 °C. The mice were placed in the water for a duration of 6 min during each trial. Two indicators, latency to immobility and total immobility time, were used to represent the depression level. That is, if a mouse shows increased depression, it should show a significant decrease in latency to immobility and an increase in total immobile time.

Elevated plus maze

The elevated plus maze is commonly used to assess anxiety-like behaviour in mice. The mice were acclimated to the behavioural laboratory for approximately 1 h before testing. Following preparation, the mice were placed in the centre of the maze with their head facing the closed arm, which was elevated approximately 60 cm above the ground. The maze featured opaque open arms approximately 76 cm in length, closed arms also approximately 76 cm in length, and a central area of approximately 5 cm² serving as the intersection of the open and closed arms. The mice were allowed to explore freely for 10 min. Behavioural recording was then conducted for 10 min. Exploration was recorded using a video recording system positioned directly above the centre of the maze. Prior to each experimental step, the equipment was cleaned with 75% alcohol to eliminate any residual odours from previous experimental mice. The time spent in the open arms, closed arms, and central area was recorded in seconds (s), and then each metric was divided by the total time to yield a percentage (%). These measures reflect the anxiety-like state of the mice.

Immunofluorescence assay

Following the two-chamber social interaction test, the mice were given a 1.5-hour rest period before being anaesthetised with an intraperitoneal injection of pentobarbital sodium at a dose of 60 mg/kg. Anaesthesia was considered successful if no corneal reflex was observed when the cornea was gently touched with a cotton swab. The mice were then perfused to collect brain samples. Following brain extraction, the tissue was fixed in 4% paraformaldehyde solution for 24 h and then dehydrated in 30% sucrose solution for 48 h. The brain tissue was subsequently embedded in optimal cutting temperature (SOLARBIO, 4583) compound and stored at −80 °C until further processing by frozen sectioning. These slices (25 μm) were placed in a humid chamber, PBS was dripped on them, and this washing procedure was repeated three times for 5 min each to remove embedding agents from the brain slices. After washing, a 10% concentration of bovine serum blocking solution was added for a 2-hour incubation period to block nonspecific binding sites. After the blocking step, primary antibody (RRID: AB_2747772, Abcam, diluted at 1:200) was subsequently applied, and the samples were incubated at 4 °C overnight. Following primary antibody incubation, the slices were washed three times for 15 min each. After washing, secondary antibody (RRID: AB_955591, Abcam, diluted at 1:500) was applied, and the samples were incubated for 2 h in the dark. After secondary antibody incubation, the slices were washed three times for 15 min each. Finally, mounting was performed using a light-avoiding mounting reagent containing DAPI (ab104139; Abcam).

To prevent potential errors during the experimental process that could result in sample loss, we collected five consecutive sections from each brain region of each mouse (Zeiss fluorescence microscope, Axio Observer. Z1/7; objective: EC Plan-Neofluar 10x/0.30 Ph 1; image pixel size: 1104 × 1138). The c-Fos-positive cells were manually marked on three consecutive sections using the multipoint function in FIJI software.

Surgery

The mice were anaesthetised by intraperitoneal (i.p.) injection of sodium pentobarbital (60 mg/kg). Successful anaesthesia was confirmed by the absence of a corneal reflex when the cornea was gently touched with a cotton swab. The fur on the scalp was shaved, and each mouse was secured in a stereotaxic apparatus. Under sterile conditions, a scalpel was used to incise the scalp, and the skull was levelled by aligning bregma and lambda at the same height using a positioning needle. Based on the mouse brain atlas, the coordinates for the periaqueductal grey (PAG) were identified (A/P: −3.28 mm, M/L: 0.3 mm, D/V: −2.86 mm, angle: −20 degrees). A burr hole was drilled at this location, with additional holes made to secure the screws. A cannula was then implanted into the brain and fixed in place with dental cement. After the mouse fully regained consciousness, it was returned to its home cage. Postoperative care included subcutaneous injections of ibuprofen (0.05 mg/kg) every 12 h for two days to provide analgesia.

Cannula infusions

The mice were anaesthetised using isoflurane. Initially, they were placed in an induction chamber with the airflow set to a maximum and the isoflurane concentration adjusted to 1.5–2%. Once anaesthetised, the mice were transferred to a face mask with the airflow reduced to 0.1. A Hamilton syringe (10 µL) was filled with liquid paraffin to expel any air bubbles, and then FCM (commercially designated Muscimol-TMR-X, product #M23400) was drawn into the syringe. The mouse was secured in a stereotaxic frame, and the guide cannula cap was removed. The infusion cannula was inserted into the guide cannula and fixed using a locking nut. The drug mixture (concentration: 0.08 mmol/L) was slowly infused into the periaqueductal grey at a rate of 0.1 µL/min, with a total volume of 100 nL, using a microinjection pump. After the injection, a 5-minute waiting period allowed for complete absorption before the infusion cannula was removed and the guide cannula cap was replaced. The behavioural experiments commenced 40 min postinfusion. After all the behavioural tests, brain slices (25 µm) were collected for histological analysis to confirm the cannula tip locations using a Zeiss fluorescence microscope (Axio Observer. Z1/7; objective: EC Plan-Neofluar 5x/0.16 M27; image pixel size: 904 × 732). FCM exhibited intrinsic fluorescence with excitation and emission peaks at 543 nm and 572 nm, respectively.

Data analysis

Statistical analyses were conducted using GraphPad Prism software (version 9.5.0). All the data were subjected to tests for normality and homogeneity of variance. The Shapiro−Wilk test was employed to assess normality, and Bartlett's test was used to assess the homogeneity of variances between the groups. The data are presented as the means ± standard errors of the means (SEMs). For the behavioural data, statistical comparisons were performed using unpaired Student's t tests or paired t tests. One-way ANOVA was used for c-Fos staining analysis. The quantification of c-Fos fluorescent signals in brain regions was carried out using FIJI software (version: 1.54f), and statistical analysis of these data was conducted via one-way ANOVA. A significance threshold of $P < 0.05$ was considered statistically significant. For all the experiments, data collection and analysis were conducted manually using a single-blind method.

Results

Strain-dependent social interaction preference in C57 and KM mice altered by cross-fostering

We initially conducted a modified social preference test on mice under normal rearing conditions to assess whether they exhibited specific interaction preferences when engaging with mice of the same strain or with mice of other strains; this test is referred to as the strain preference test. The test mice were first arranged to interact individually with mice of the same or other strain for 10 min to become familiar with each strain; then, they were allowed to choose their social partner, as representatives of both the same strain and the other strain were shown in the device for an additional 10 min (Fig. 1A−C). The interaction times with the mice of the same strain and other strain were recorded separately for each mouse for the last 10 min of the test. The results revealed significant differences in the interaction times of both C57 and KM mice with different strains under normal rearing conditions. Compared with the mice of the other strains, the C57$_{ctl}$ mice presented a significantly longer interaction time with the same strain (paired t test, C57$_{Ctl}$: SS vs. OS, t$_{10}$ = 2.621, p = 0.0256; Fig. 1D). For the KM mice reared under normal rearing conditions, a significant difference in interaction time was also observed; however, unlike the C57 mice, the KM mice presented a shorter interaction time with mice of the same strain than with those of the other strain (paired t test, KM$_{Ctl}$: SS vs. OS, t$_7$ = 6.074, p = 0.0005, n = 8; Fig. 1E). Moreover, KM$_{Ctl}$ mice spent significantly more time in the central chamber than C57$_{Ctl}$ mice did, whereas no differences were observed within each strain (Fig. 1F). These results indicate that both C57 and KM mice exhibit different strain-dependent social interaction behaviours. However, their interaction preferences were opposite, with C57 mice favouring interactions with mice of the same strain and KM mice preferring interactions with mice of other strains. Furthermore, these findings suggest that both C57 and KM mice can discriminate between mice belonging to the same strain and mice belonging to other strains and show strain-specific social preferences.

Next, we explored whether strain preference in mice is influenced by the nurturing environment. Cross-fostering was used as an environmental stimulus. C57 and KM mice were cross-fostered by lactating dams (LDs) within 3 days after birth and reared together until adulthood to investigate whether this approach could mitigate their strain-specific preferences (Fig. 1A−C). In the strain preference test, C57$_{LD: KM}$ (C57 offspring nurtured by lactating KM dams) no longer exhibited differences in social interactions with mice of different strains (Fig. 1D). Similarly, the strain preference was also absent for KM$_{LD: C57}$ (KM offspring nurtured by lactating C57 dams) after cross-fostering. These results indicate that manipulating the nurturing environment through cross-fostering could modify the social preference of mice. We also noted that there was no significant difference in the time spent in the middle chamber (Fig. 1F), indicating that sociability did not change. This result revealed
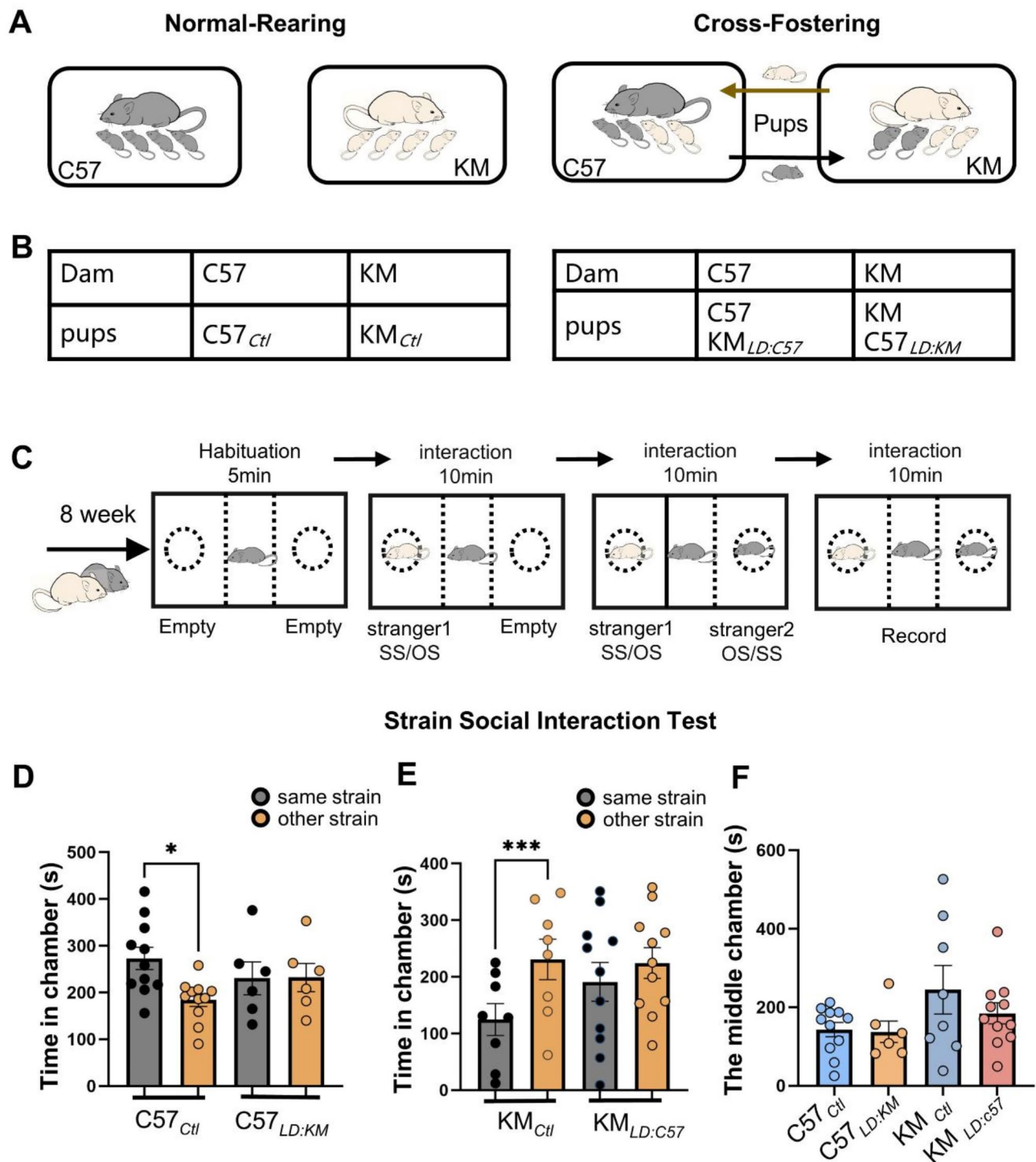
Fig. 1. Social preference of mice towards same and other strains in normal-rearing and cross-fostering environments, demonstrating plasticity in preferences. (**A**) Mice were divided into two groups: normal-rearing and cross-fostering environments, represented by gray and white icons for C57 and KM mice, respectively. (**B**) In the normal-fostering environment, the offspring served as the control group (Ctl). The treatment groups included the cross-fostering environment with $C57_{LD: KM}$ and $KM_{LD: C57}$. (**C**) Paradigm for strain preference in three-chamber social interaction of mice. (**D**–**F**) Statistical analysis of mice's social interaction time in the three-chamber social interaction experiment with same and other strains. The black bars represent the same strain, while the white bars represent the other strain. Social interaction time (S) is shown. (**D**) statistical results for social behaviour in C57 mice under normal-fostering and cross-fostering conditions(Paired t-test, $C57_{Ctl}$: SS vs. OS, $t_{10} = 2.621$, $p = 0.0256$, $n = 11$; $C57_{LD: KM}$: SS vs. OS, $t_5 = 0.0273$, $p = 0.9788$, $n = 6$). (**E**) KM mice under normal-fostering and cross-fostering conditions (Paired t-test, KM_{Ctl}: SS vs. OS, $t_7 = 6.074$, $p = 0.0005$, $n = 8$; $KM_{LD: C57}$: SS vs. OS, $t_{10} = 0.5960$, $p = 0.5645$, $n = 11$). (**F**) Time Spent in the middle chamber by Mice (Unpaired t-test, $C57_{Ctl}$ vs. $C57_{LD: KM}$, $t_{15} = 0.1784$, $p = 0.8636$; KM_{Ctl} vs. $KM_{LD: C57}$, $t_{17} = 0.9873$, $p = 0.3374$). Data are presented as means ± s.e.m, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

that the strain preference of the mice was highly plastic and that cross-fostering promoted the interaction of the mice with their unpreferred social partner.

Cross-fostering does not elicit depressive or anxiety-like behaviours in mice

To rule out the possibility that changes in social behaviour were due to negative emotions induced by cross-fostering, we conducted a forced swimming test to assess whether cross-fostering led to depressive-like behaviours in the mice (Fig. 2A). The swimming time of each mouse in the water was recorded. Compared with normally reared C57 mice (C57$_{Ctl}$), C57$_{LD: KM}$ mice presented no significant differences in the latency to immobility time or immobility time (Fig. 2B). Similarly, the KM$_{LD: C57}$ mice did not exhibit significant differences in the latency to immobility time (Fig. 2C). These results indicate that cross-fostering does not induce depressive-like behaviours in the forced swim test during adulthood.

Furthermore, we used the elevated plus-maze test to investigate whether cross-fostering induced anxiety-like behaviours in the mice. We recorded the percentage of time spent in each arm (%) as a primary measure of anxiety-like behaviour. For C57 mice, there were no differences in the time spent in the open arms between those reared under normal rearing conditions and those subjected to cross-fostering, and there were no differences in the time spent in the closed arms or in the centre area (Fig. 2D). Similarly, for the KM mice, there were also no differences in the time spent in the open or closed arms or in the centre area (Fig. 2D). These experimental results indicate that cross-fostering does not induce anxiety-like behaviours in either male C57 mice or male KM mice.

Owing to the size difference between C57 and KM mice, which may lead to aggressive behaviour associated with body size differences, we conducted a free social interaction test[29]. Although KM mice have a significantly greater body weight than C57 mice do (C57: average weight 21.22 g; KM: average weight 33.28 g; $n = 7$), the interactions observed showed no aggressive intention. We recorded three key measures of aggression and found no significant differences between C57 and KM mice (Fig. 2E-G). During free interaction, we also recorded proactive social behaviours and reported that KM mice spent significantly more time engaging in social interactions than C57 mice did, indicating a preference for interacting with C57 mice (unpaired t test, C57 vs. KM, t12 = 2.019, $p = 0.0664$; Fig. 2H). These results suggest that the observed social preference may stem from social motivation rather than aggressive intent. These findings further confirm the social preferences of different mouse strains.

Differential activity of the PFC, BLA, PIL, SUM, CA2, and PAG of normally reared C57 mice when interacting with the same or other strain of mice

The preceding experimental results revealed distinct strain preferences of C57 and KM mice. Building on these findings, we aimed to further explore which brain regions are involved in regulating strain preference. We assessed the activity of six brain regions that are crucial for regulating social behaviour—the PFC, BLA, PIL, SUM, CA2, and PAG[23,26,27,30]. The expression of c-Fos is used to reflect the activity levels of these six brain regions[31]. The number of c-Fos-positive (c-Fos$^+$) neurons was quantified for each brain region when the mice underwent no social interaction (control group: Ctl), underwent social interaction with the same strain (same strain social interaction group: SS), or underwent social interaction with another strain (other strain social interaction group) (Fig. 3A).

We first measured neural activity in C57 mice. Low c-Fos expression was observed in the PFC of the control group mice without social interaction. However, both the SS and OS groups of mice presented noticeable c-Fos$^+$ expression in the PFC region (Fig. 3B). The statistical analysis revealed that the expression levels in both the SS and OS groups were significantly greater than those in the control group. However, there was no difference between the SS and OS groups (Fig. 3C). These results suggest that the PFC is involved in the social interaction of C57 mice but may not play a key role in regulating strain preference. The number of c-Fos$^+$ cells in the BLA (Fig. 3B) was similar to that in the PFC: the baseline expression of c-Fos in the BLA was quite low, and after social interaction, c-Fos staining significantly increased. There was no difference between the SS and OS groups (Fig. 3C). These findings indicate that, similar to the PFC, the BLA is involved in the social interaction of C57 mice but may not play a role in regulating strain preference in C57 mice. Recent studies have indicated a close connection between the PIL and the medial preoptic area (MPOA) circuit with social touch[32]. Therefore, we investigated whether the PIL is involved in the social preference of mice (Fig. 3B). Statistical analysis of c-Fos + neurons in this brain region revealed that the number of c-Fos + cells in the SS group was significantly greater than that in the control group and greater than that in the OS group, whereas the number of c-Fos + cells in the OS group did not differ from that in the control group (One-way ANOVA, PIL: F$_{(2, 22)}$ = 11.89, $p = 0.0003$; Fig. 3C). These results indicate the involvement of the PIL region in the social interaction behaviour of C57 mice. The differential activity of the PIL when C57 mice interact with SS or OS mice suggests its potential role in the strain preference of C57 mice. The circuit from the SUM to the hippocampal CA2 region is also considered highly relevant to social memory in mice[26]. Therefore, we examined these two brain regions and found that the results for the SUM were similar to those for the PIL region (One-way ANOVA, SUM: F$_{(2, 22)}$ = 10.87, $P = 0.0005$; Fig. 3B). The number of c-Fos$^+$ neurons in the SUM of the SS groups was significantly greater than that in the control group and greater than that in the OS group (Fig. 3C). Next, we examined the hippocampal CA2 region, and the results for this region were consistent with those of the SUM (One-way ANOVA, CA2: F$_{(2, 24)}$ = 9.177, $p = 0.0011$; Fig. 3C). That is, after interacting with SS or OS mice in the social experiments, the activity in the CA2 region of C57 mice was significantly increased. Statistical analysis revealed significantly more c-Fos$^+$ neurons in the SS group than in the control group or the OS group, with no significant difference between the OS group and the Ctl group (Fig. 3C). These results suggest that, similar to PIL, SUM and CA2 may not only be involved in mouse social behaviour but may also participate in strain preference. Recent research has indicated a close association between the PAG region and social behaviour[33]. Therefore, we examined whether the PAG was also

Figure (panels A–H)
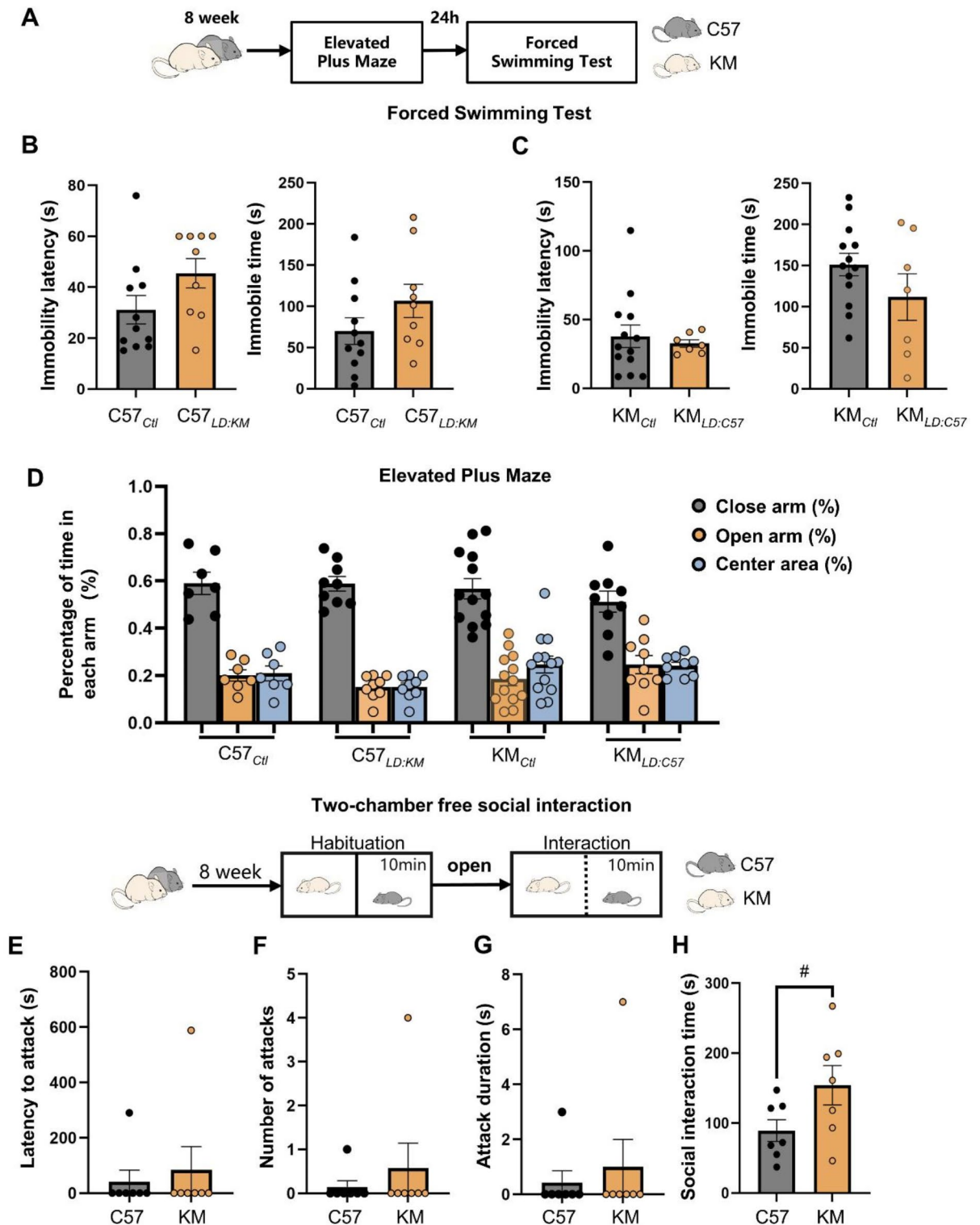
involved in the strain preference of the mice (One-way ANOVA, PAG: $F_{(2, 24)} = 9.562$, $p = 0.0009$; Fig. 3C). The results revealed that c-Fos expression in the PAG of the SS group was significantly greater than that in the control group or the OS group, which was consistent with the activity changes in the PIL, SUM, and CA2 (Fig. 3C). In summary, after social interaction, the number of c-Fos$^+$ neurons significantly increased in all six brain regions examined in C57 mice. Among them, the PIL, SUM, CA2, and PAG showed strain-dependent differences, with higher c-Fos expression in C57 mice when interacting with the same strain.

Cross-fostering attenuates the activity differences in the SUM, CA2, and PAG in C57 mice when socially interacting with mice of the same strain or mice of other strains

According to the above experiments, strain preference was attenuated after cross-fostering. Therefore, we hypothesised that in normal rearing conditions, the number of c-Fos + neurons in the SUM, CA2, and PAG

◀**Fig. 2**. Cross-fostering does not induce depression-like or anxiety-like behaviours in C57 and KM mice, and social interactions are non-aggressive. (**A**,**B**) Forced Swimming Test: Comparison between control group under normal rearing (Ctl) and treatment group under cross-fostering (LD). (**B**) Data recorded immobility latency and duration of C57 (Latency to immobility time(s), Unpaired t-test, $C57_{Ctl}$ vs. $C57_{LD: KM}$, $t_{18} = 1.779$, $p = 0.0921$; Immobility time(s), Unpaired t-test, $C57_{Ctl}$ vs. $C57_{LD: KM}$, $t_{18} = 1.427$, $p = 0.1708$; $C57_{Ctl}$, $n = 11$, $C57_{LD: KM}$, $n = 9$). (**C**) Data recorded immobility latency and duration of KM (Latency to immobility time(s), Unpaired t-test, KM_{Ctl} vs. $KM_{LD: C57}$, $t_{18} = 0.4546$, $p = 0.6548$; Immobility time(s), Unpaired t-test, KM_{Ctl} vs. $KM_{LD: C57}$, $t_{18} = 1.422$, $p = 0.1721$; KM_{Ctl}, $n = 13$, $KM_{LD: C57}$, $n = 7$). (**D**) Elevated Plus-Maze Test: Measurement of time spent by C57 and KM mice in the open, closed arms and center area (%)(Unpaired t-test, closed arms, $C57_{Ctl}$ vs. $C57_{LD: KM}$, $t_{14} = 0.0401$, $p = 0.9687$; KM_{Ctl} vs. $KM_{LD: C57}$, $t_{20} = 0.8632$, $p = 0.3983$. Unpaired t-test, open arms, $C57_{Ctl}$ vs. $C57_{LD: KM}$, $t_{14} = 1.726$, $p = 0.1036$; KM_{Ctl} vs. $KM_{LD: C57}$, $t_{20} = 0.8632$, $p = 0.3983$. Unpaired t-test, center area, $C57_{Ctl}$ vs. $C57_{LD: KM}$, $t_{14} = 1.716$, $p = 0.1082$; KM_{Ctl} vs. $KM_{LD: C57}$, $t_{20} = 0.1156$, $p = 0.9091$. n: $C57_{Ctl} = 11$, $C57_{LD: KM} = 9$, $KM_{Ctl} = 13$, $KM_{LD: C57} = 9$). (**E–H**) Free Social Interaction: C57 and KM mice were placed in two compartments and allowed to acclimate for 10 min before removing the central divider to enable free interaction for 10 min. Behavioural data recorded include latency to attack (Unpaired t-test, C57 vs. KM, $t_{12} = 0.4545$, $p = 0.6576$), number of attacks (Unpaired t-test, C57 vs. KM, $t_{12} = 0.7276$, $p = 0.4808$), attack duration (Unpaired t-test, C57 vs. KM, $t_{12} = 0.5252$, $p = 0.6090$), and active social interaction time (Unpaired t-test, C57 vs. KM, $t_{12} = 2.019$, $p = 0.0664$, $n = 7$). Data are presented as means ± s.e.m. $\#p < 0.067$, $*p < 0.05$.

brain regions would be greater after interaction with SS individuals than with OS individuals and that cross-fostering might lead to alterations in the neural activity of the corresponding brain regions. We then examined the responses of the six brain regions of $C57_{LD: KM}$ mice when interacting with SS mice or OS mice (Fig. 4A-B). The results revealed that the activities of the PFC and BLA were not influenced by the rearing environment, with both the SS and OS groups having higher activities than the control group did; however, there was no difference between the SS and OS groups, similar to normally reared $C57_{Ctl}$ mice (Fig. 4C).

However, the activities of the remaining four brain regions, namely, the SUM, CA2, and PAG, differed after cross-fostering. Unlike $C57_{Ctl}$ mice, after social interaction with mice of a different strain, $C57_{LD: KM}$ mice presented increased activity in the SUM, CA2, and PAG, with no strain-dependent differences (Fig. 4C). However, the activity level in the PIL brain region was greater in the OS group than in the SS group (One-way ANOVA, PIL: $F_{(2, 18)} = 19.98$, $p < 0.0001$; Fig. 4C).

These results indicate that the PAG, SUM, and CA2 undergo plastic changes in response to changes in the social environment and that the direction of the changes in activity was consistent with the behavioural changes in C57 mice. In particular, normally reared C57 mice showed a preference for the SS, and these three brain regions exhibited stronger activity when the mice interacted with other mice of the SS. In cross-fostered mice, the strain preferences disappeared, as did the differences in brain region activity. These findings suggest that these three brain regions not only are involved in social perception but also participate in regulating strain preference.

Differentiated neural activity in the PAG of KM mice following interactions with mice of the same strain or the other strain

KM mice also exhibited strain preference, but unlike C57 mice, they preferred interaction with mice of other strains. We then investigated whether the neural activity in KM mice was similar to that in C57 mice. The six brain regions in KM mice were examined following interactions with SS mice or OS mice in a normal rearing environment (Fig. 5A-B). KM mice without social interaction served as the control group (Ctl). The number of c-Fos + neurons was measured in the Ctl, SS, and OS groups of mice. Statistical analysis of the PFC region revealed significant differences between the control group and OS group, with no difference compared with the SS group (Fig. 5C). The PIL and CA2 exhibited patterns similar to those of the PFC. The BLA and SUM showed similar patterns, with both the SS and OS groups exhibiting greater activity than the control group did, but there was no difference between the SS and OS groups (Fig. 5C). Among the brain regions, only the PAG region showed differences in activity between the OS and SS groups (One-way ANOVA, PAG: $F_{(2, 18)} = 8.828$, $p = 0.0021$; Fig. 5C). In summary, all six brain regions exhibited activity differences between the OS group and the Ctl group. Among them, only the BLA, SUM, and PAG showed activity differences between the SS group and the Ctl group, which was different from the findings in C57 mice, mainly because the neural activity in the Ctl group of KM mice was greater than that in the Ctl group of C57 mice. Importantly, similar to that of C57 mice, the PAG of KM mice also showed greater activity when mice engaged in social interactions with their favoured strain, which was mice of another strain for KM mice.
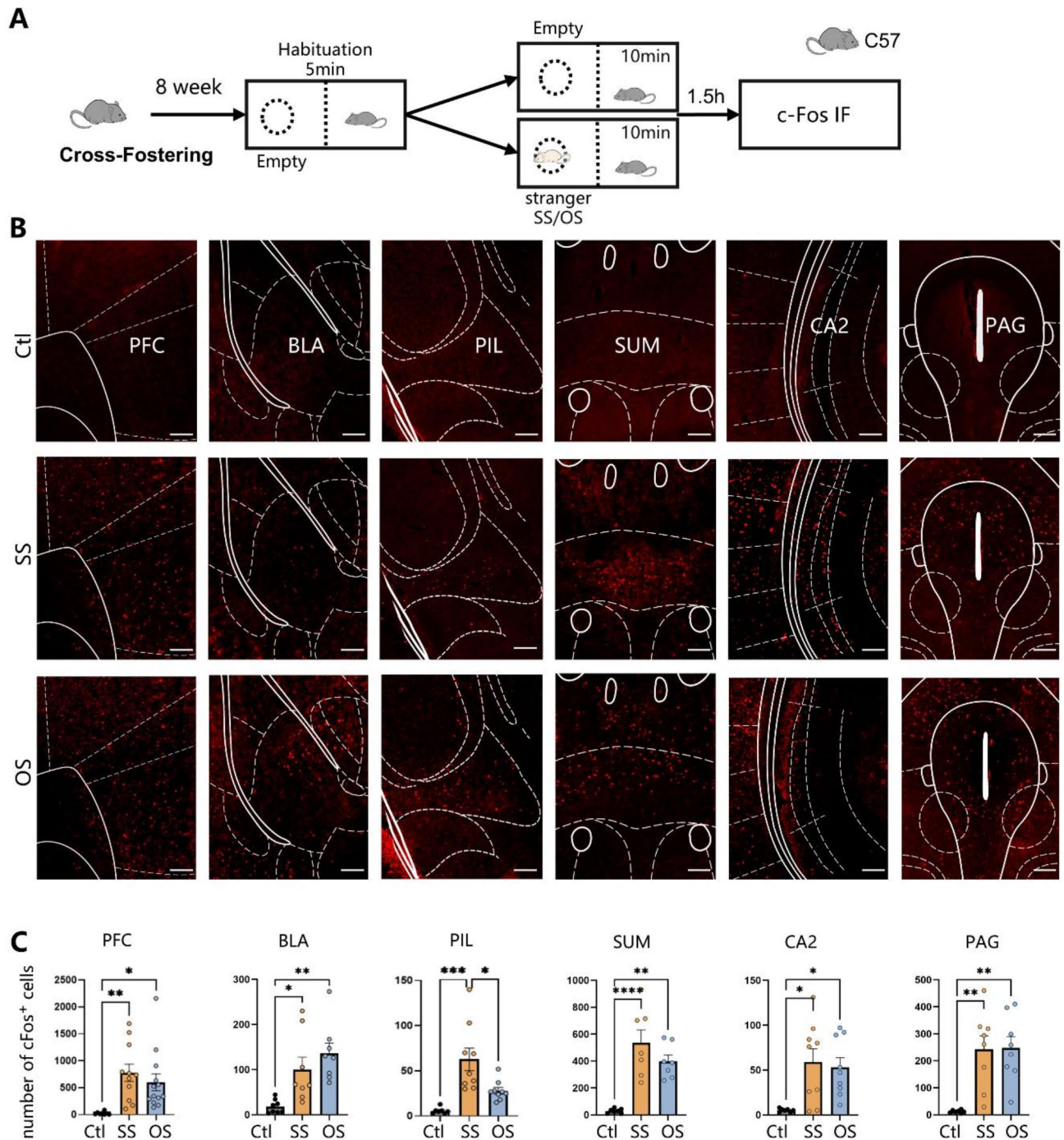
Cross-fostering attenuates the activity difference in the PAG in KM mice when socially interacting with mice of the same strain or mice of the other strain

We subsequently examined the levels of neural activity in brain regions associated with strain interactions in $KM_{LD: C57}$ mice raised in a cross-fostering environment to investigate whether plastic changes occurred (Fig. 6A-B). The experimental results revealed similar patterns of activity in the PFC, BLA, SUM, PIL and CA2 of $KM_{LD: C57}$ mice after cross-fostering compared with normally reared mice and the activities of the PFC, BLA, PIL, SUM, and CA2 did not differ between the SS and OS groups (Fig. 6C). The only brain region that exhibited similar alterations in behaviour tests was the PAG. In particular, the activity of the PAG differed between OS and SS in the normal rearing environment, but the difference disappeared in cross-fostered $KM_{LD: C57}$ mice (Fig. 6C). Taken together with the experimental results obtained in C57 mice, these results indicate that the PAG is a key
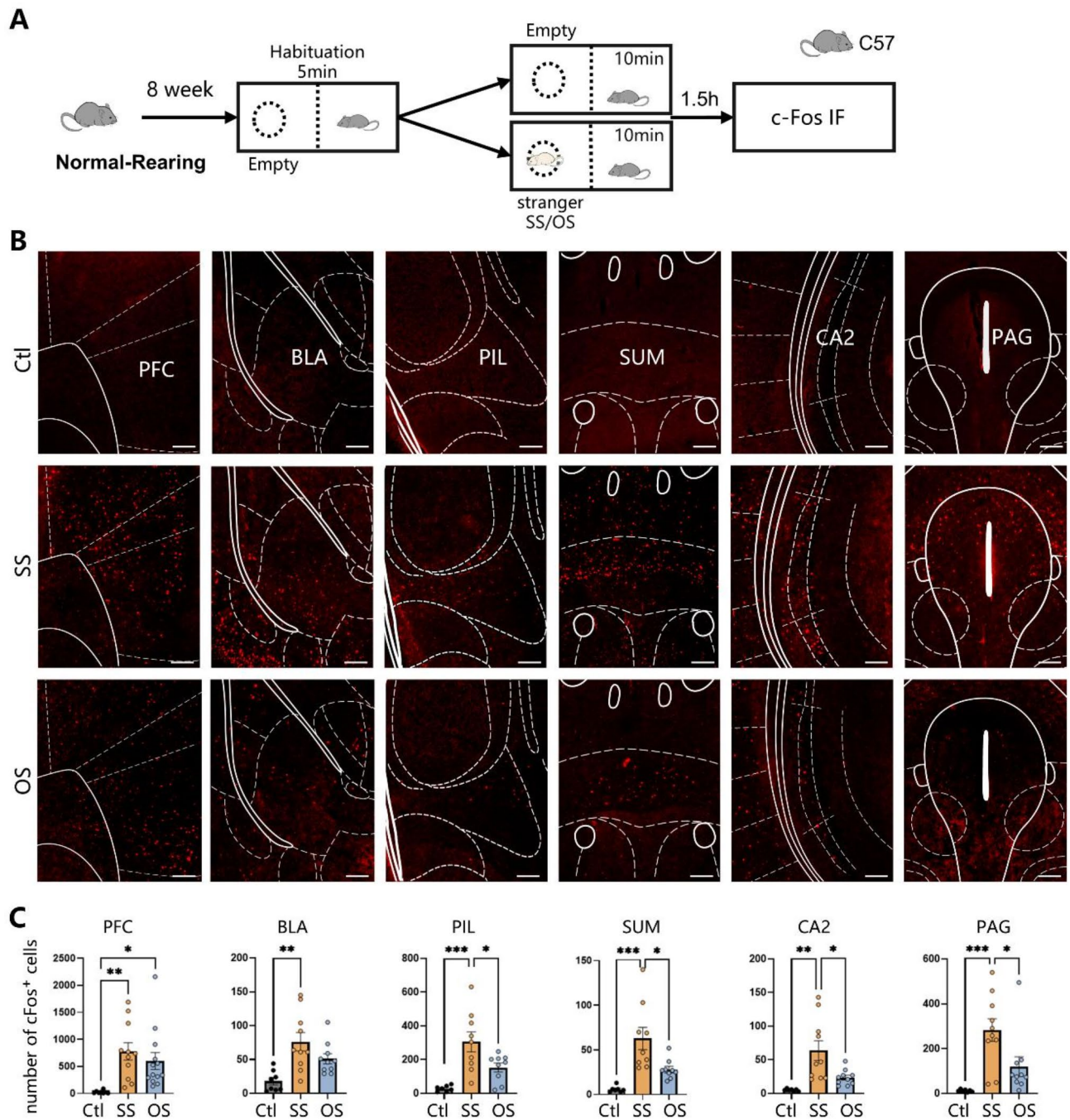
Fig. 3. Differential neuronal activity in PIL, SUM, CA2, and PAG brain regions of C57 mice under normal-rearing social environment. (**A**) Paradigm of the Two-Chamber Social Interaction Test in mice. (**B**) c-Fos neuronal fluorescence staining images with a scale of 1:100 μm. Ctl represents the control group, and the treatment groups include same-strain social interaction (SS) and other-strain social interaction (OS). Brain region localization: PFC (Bregma: 1.78 mm to -1.34 mm), BLA (Bregma: -1.34 mm to -1.58 mm), PIL (Bregma: -3.16 mm to -3.40 mm), SUM (Bregma: -2.80 mm to -3.08 mm), CA2 (Bregma: -2.92 mm to -3.08 mm), PAG (Bregma: -3.08 mm to -3.28 mm), The delineation of brain region ROIs (Regions of Interest) was based on the second edition of *The Mouse Brain in Stereotaxic Coordinates* by Paxinos and Franklin. (**C**) Statistical analysis of the number of c-Fos+ cells. The x-axis represents the control group and treatment groups, and the y-axis represents the count of c-Fos + neurons(ANOVA, PFC: $F_{(2, 29)} = 6.083$, $p = 0.0062$,n: Ctl = 9,SS = 11, OS = 13; BLA: $F_{(2, 25)} = 8.149$, $p = 0.0019$, n: Ctl = 8,SS = 10, OS = 10; PIL: $F_{(2, 22)} = 11.89$, $p = 0.0003$, n: Ctl = 7,SS = 9, OS = 9; SUM: $F_{(2, 22)} = 10.87$, $P = 0.0005$, n: Ctl = 7,SS = 9, OS = 9; CA2: $F_{(2, 24)} = 9.177$, $p = 0.0011$, n: Ctl = 7,SS = 10, OS = 10; PAG: $F_{(2, 24)} = 9.562$, $p = 0.0009$ n: Ctl = 7,SS = 10, OS = 10). Data are presented as means ± s.e.m. Statistical significance was determined using One-way ANOVA, Tukey's multiple comparisons test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Fig. 4. Attenuation of neuronal activity discrepancy in SUM, CA2, and PAG brain regions of C57$_{LD: KM}$ mice under cross-fostering social environment. (**A**) The lactating dam is of the KM strain. c-Fos neuronal fluorescence staining images with a scale of 1:100 μm. Ctl represents the control group, and the treatment groups include the same-strain (SS) and other-strain (OS) social interactions. Brain region localization: PFC (Bregma: 1.78 mm to -1.34 mm), BLA (Bregma: -1.34 mm to -1.58 mm), PIL (Bregma: -3.16 mm to -3.40 mm), SUM (Bregma: -2.80 mm to -3.08 mm), CA2 (Bregma: -2.92 mm to -3.08 mm), PAG (Bregma: -3.08 mm to -3.28 mm). (**B**) Statistical analysis of the number of c-Fos$^+$ cells. The x-axis represents the control group and treatment groups, and the y-axis represents the count of c-Fos expressing neurons(ANOVA, PFC: $F_{(2, 22)} = 13.40$, $p = 0.0002$, n: Ctl = 8,SS = 8, OS = 9; BLA: $F_{(2, 21)} = 8.544$, $p = 0.0019$, n: Ctl = 8,SS = 8, OS = 8; PIL: $F_{(2, 18)} = 19.98$, $p < 0.0001$, n: Ctl = 7,SS = 7, OS = 7; SUM: $F_{(2, 18)} = 17.87$, $p < 0.0001$, n: Ctl = 7, SS = 7, OS = 7; CA2: $F_{(2, 22)} = 5.977$, $p = 0.0084$, n: Ctl = 7,SS = 9, OS = 9; PAG: $F_{(2, 20)} = 10.68$, $p = 0.0007$, n: Ctl = 7,SS = 8, OS = 8). Data are presented as means ± s.e.m. Statistical significance was determined using One-way ANOVA, Tukey's multiple comparisons test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
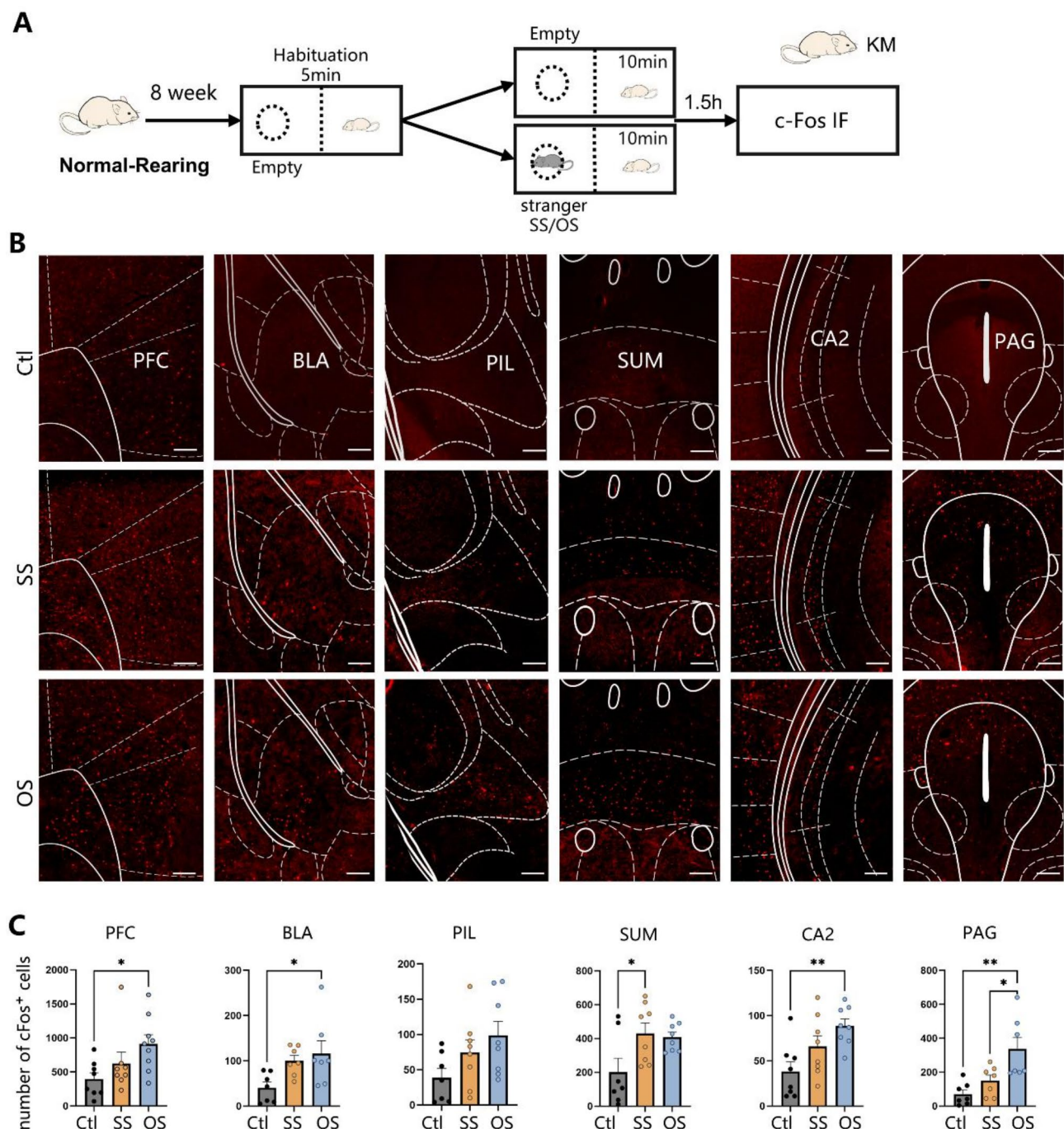
Fig. 5. Interaction between normally raised KM mice and mice from the same or other strains reveals discrepancy only in PAG neuronal activity. (**A**) c-Fos neuronal fluorescence staining images with a scale of 1:100 μm. Ctl represents the control group, and the treatment groups include the same-strain (SS) and other-strain (OS) social interaction groups. Brain region localization: PFC (Bregma: 1.78 mm to -1.34 mm), BLA (Bregma: -1.34 mm to -1.58 mm), PIL (Bregma: -3.16 mm to -3.40 mm), SUM (Bregma: -2.80 mm to -3.08 mm), CA2 (Bregma: -2.92 mm to -3.08 mm), PAG (Bregma: -3.08 mm to -3.28 mm). (**B**) Statistical analysis of the number of c-Fos$^+$ cells. The x-axis represents the control group and treatment groups, and the y-axis represents the count of c-Fos expressing neurons (ANOVA, PFC: $F_{(2, 22)} = 3.778$, $p = 0.0389$, n: Ctl = 8, SS = 8, OS = 9; BLA: $F_{(2, 18)} = 4.302$, $p = 0.0297$, n: Ctl = 7, SS = 7, OS = 7; PIL: $F_{(2, 20)} = 2.830$, $p = 0.0828$, n: Ctl = 7, SS = 8, OS = 8; SUM: $F_{(2, 20)} = 4.287$, $p = 0.0282$, n: Ctl = 7, SS = 8, OS = 8; CA2: $F_{(2, 21)} = 6.170$, $p = 0.0078$, n: Ctl = 8, SS = 8, OS = 8; PAG: $F_{(2, 18)} = 8.828$, $p = 0.0021$, n: Ctl = 7, SS = 7, OS = 7). Data are presented as means ± s.e.m. Statistical significance was determined using One-way ANOVA, Tukey's multiple comparisons test, *$p < 0.05$, **$p < 0.01$.
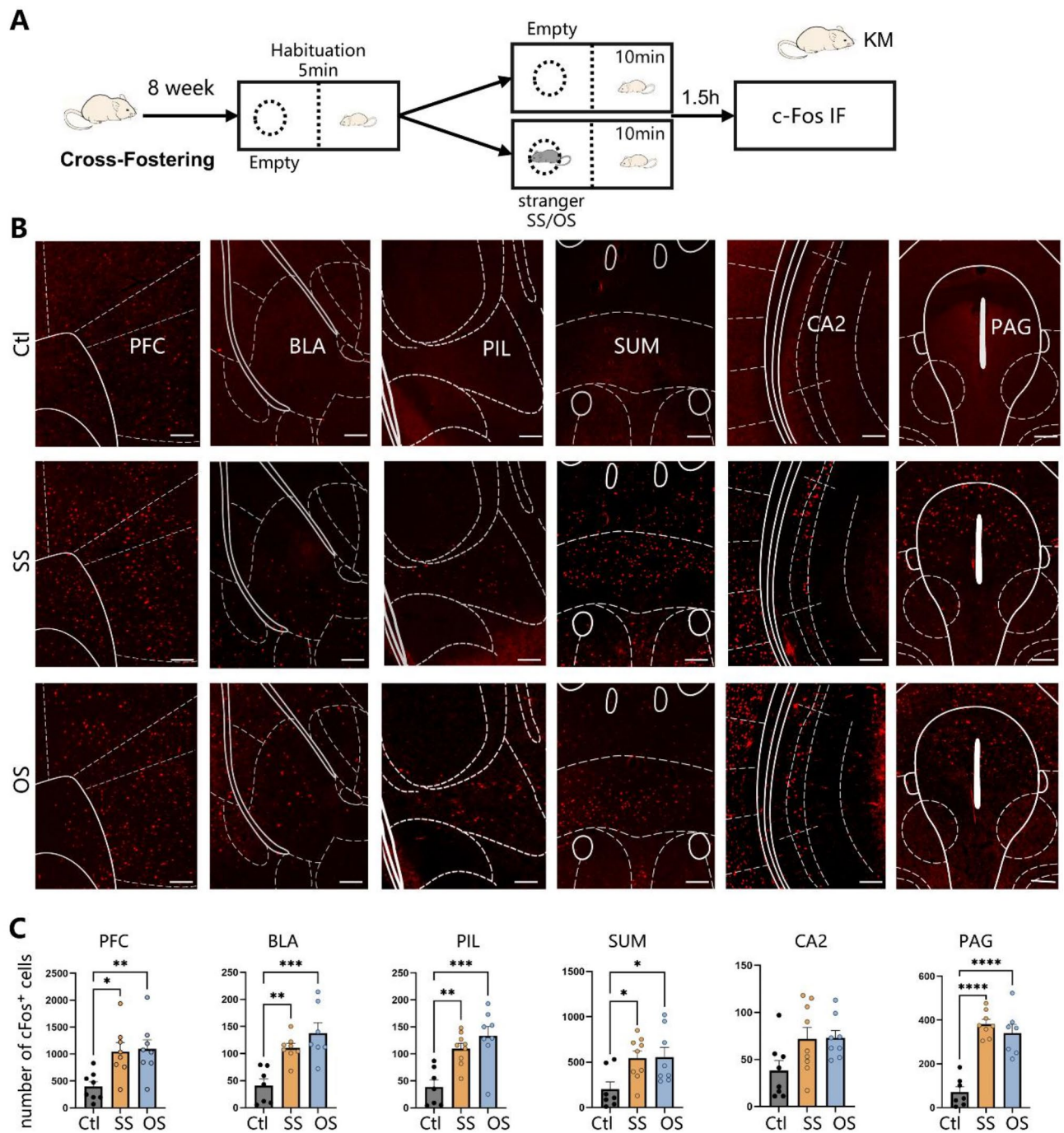
Fig. 6. Disappearance of neuronal activity discrepancy in the PAG of KM$_{LD:C57}$ mice after cross-fostering. (**A**) c-Fos neuronal fluorescence staining images with a scale of 1:100 μm. Ctl represents the control group ($n = 8$), and the treatment groups include the same-strain (SS) and other-strain (OS) social interaction groups. Brain region localization: Prefrontal Cortex (Bregma: 1.78 mm to -1.34 mm), Basolateral Amygdala (Bregma: -1.34 mm to -1.58 mm), Intralaminar Thalamic Nucleus (Bregma: -3.16 mm to -3.40 mm), Nucleus Accumbens (Bregma: -2.80 mm to -3.08 mm), CA2 region (Bregma: -2.92 mm to -3.08 mm), PAG (Bregma: -3.08 mm to -3.28 mm). (**B**) Statistical analysis of the number of c-Fos + cells. The x-axis represents the control group and treatment groups, and the y-axis represents the count of c-Fos expressing neurons (ANOVA, PFC: $F_{(2, 21)} = 6.815$, $p = 0.0052$, n: Ctl = 8, SS = 8, OS = 8; BLA: $F_{(2, 19)} = 13.16$, $p = 0.0003$, n: Ctl = 7, SS = 8, OS = 7; PIL: $F_{(2, 21)} = 11.72$, $p = 0.0004$, n: Ctl = 7, SS = 9, OS = 8; SUM: $F_{(2, 21)} = 4.678$, $p = 0.0209$, n: Ctl = 7, SS = 9, OS = 8; CA2: $F_{(2, 22)} = 3.436$, $p = 0.0503$, n: Ctl = 8, SS = 9, OS = 8; PAG: $F_{(2, 19)} = 33.64$, $p < 0.0001$, n: Ctl = 7, SS = 8, OS = 7). Data are presented as means ± s.e.m. Statistical significance was determined using One-way ANOVA, Tukey's multiple comparisons test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

modulating brain region of strain-based social interaction preference and that the PAG is more active when mice interact with their favoured strain than when they interact with their unfavoured strain.

Distinct correlation patterns across six brain regions in C57 mice during interactions with same-strain versus different-strain conspecifics

To investigate whether c-Fos expression in the six selected brain regions reflects distinct connectivity patterns, we chose these regions based on their known involvement in social behaviour and aggression, specifically in modulating interstrain social interactions. The number of c-Fos-positive cells in each region of C57 and KM mice was analysed. Pearson correlation coefficients (r) were calculated between each pair of brain regions based on the c-Fos expression levels, and correlation matrices were generated. The Pearson r values ranged from 1 to -1 and were represented by a gradient spectrum from red to blue. The matrices revealed distinct correlation patterns between the control and treatment groups under normal rearing conditions (Fig. 7A). We further analysed network correlations by selecting nodes with $r > 0.5$ for visualisation. This analysis demonstrated that, under the social task stimulus, SS interactions exhibited functional connectivity among the six brain regions, with the PAG showing three connections with the PFC, PIL and BLA. However, interactions with OS conspecifics led to the disappearance of PAG connections (Fig. 7A). The connection between the PAG and SUM appeared when cross-fostered C57 mice interacted with KM mice, indicating a change in the PAG network (Fig. 7A). The observed changes in connectivity suggest that cross-fostering modulates the integration of social information across brain regions. In contrast, the KM mice exhibited the opposite pattern: under normal rearing conditions, SS interactions presented the disappearance of PAG connections, whereas OS interactions presented connections (Fig. 7B). In cross-fostering KM mice, both the SS and OS interactions presented two and three PAG connections, indicating that the PAG may play a critical role in strain preference and the modulation of social behaviour. Additionally, the neural connection for the OS interaction clearly became more complex after cross-fostering.

Silencing the PAG with muscimol alters social preference behaviour in C57 mice

To further investigate the role of the PAG in modulating social preference behaviour, we employed intracranial infusion of muscimol to transiently silence the PAG in C57 mice to assess changes in social behaviour (Fig. 8A). Muscimol, a GABA A receptor agonist, is commonly used for reversible inactivation of brain regions, with effects that recover within 24 h[34]. The sniffing time was measured after the mice received either muscimol or saline. In the control group, C57 mice received unilateral injections of saline, revealing a clear social preference with a significantly greater sniffing time with SS than with OS (paired t test, saline SS vs. saline OS: $t_5 = 3.468$, $p = 0.0179$; unpaired t test, saline OS vs. muscimol SS: $t_{10} = 2.236$, $p = 0.0493$; Fig. 8B), which means that saline injections did not alter the social preference of the mice. Conversely, following muscimol injection, there was a notable shift in social preference behaviours: the distinction between the SS and OS interactions was diminished, although the overall social ability of the mice remained unaffected (Fig. 8B). To confirm that the changes in social preference behaviour due to PAG silencing were not confounded by negative factors such as depressive-like or anxiety-like behaviours, we assessed depression-like and anxiety-like behaviour via the forced swim test and the elevated plus maze, respectively. The results indicated that muscimol-treated mice did not exhibit significant anxiety- or depressive-like behaviours (Fig. 8C-D). These findings demonstrate that silencing the PAG alters social preference behaviours in C57 mice and highlight the critical role of the PAG in regulating strain-specific social preferences.

Discussion

In this study, we focused on investigating strain-dependent social preference behaviours and their neural mechanisms and plasticity underlying these behaviours. Previous research has indicated the existence of social preferences, including social novelty preferences[2] and sex preferences[35]. These findings suggest that mice can recognise some social classifications and show distinguished interaction preferences based on this social recognition. However, whether strain, an important type of social identification, also influences the social interactions of mice has not yet been studied. For the first time, our study identifies strain-dependent social preferences in mice, revealing heterogeneous strain preferences among mice of different strains. Additionally, we confirmed that this strain-dependent social preference is highly plastic and can be altered by the postnatal rearing environment. Through a comparative analysis of the brain regions involved in strain preference and its plasticity in C57 and KM mice, we propose that the PAG area may play a crucial role in strain-specific social preferences in mice. To validate this correlation, we employed muscimol to transiently inactivate the PAG in C57 mice and observed alterations in social preference behaviours. Our results confirm the pivotal role of the PAG in mediating strain-specific social preferences. Our results revealed distinct social preferences between C57 and KM mice under normal rearing conditions, with C57 mice exhibiting a preference for interactions with mice of the SS, whereas KM mice favoured interactions with mice of other strains. Such heterogeneity has been observed in other social preference studies. For example, CD1 mice showed greater willingness to engage in social interactions than C57 mice did after social isolation[36,37]. Collectively, these studies, together with our study, highlight that different mouse strains manifest distinct social behaviours.

The differences in social preferences observed between C57 and KM mice may be directly influenced by body size. Previous studies have shown that larger CD1 mice prefer to engage in social interactions with smaller C57 mice through lever pressing[29]. In our behavioural experiments, body size appeared to account for the differences in social preferences between C57 and KM mice under normal rearing conditions. However, in the cross-fostering experiments, where the mice had free access to food and water, body size did not vary with the housing environment. Despite this, social preferences for both strains were altered, suggesting that preference changes are not solely due to body size differences. Moreover, we did not observe a significant increase in interaction
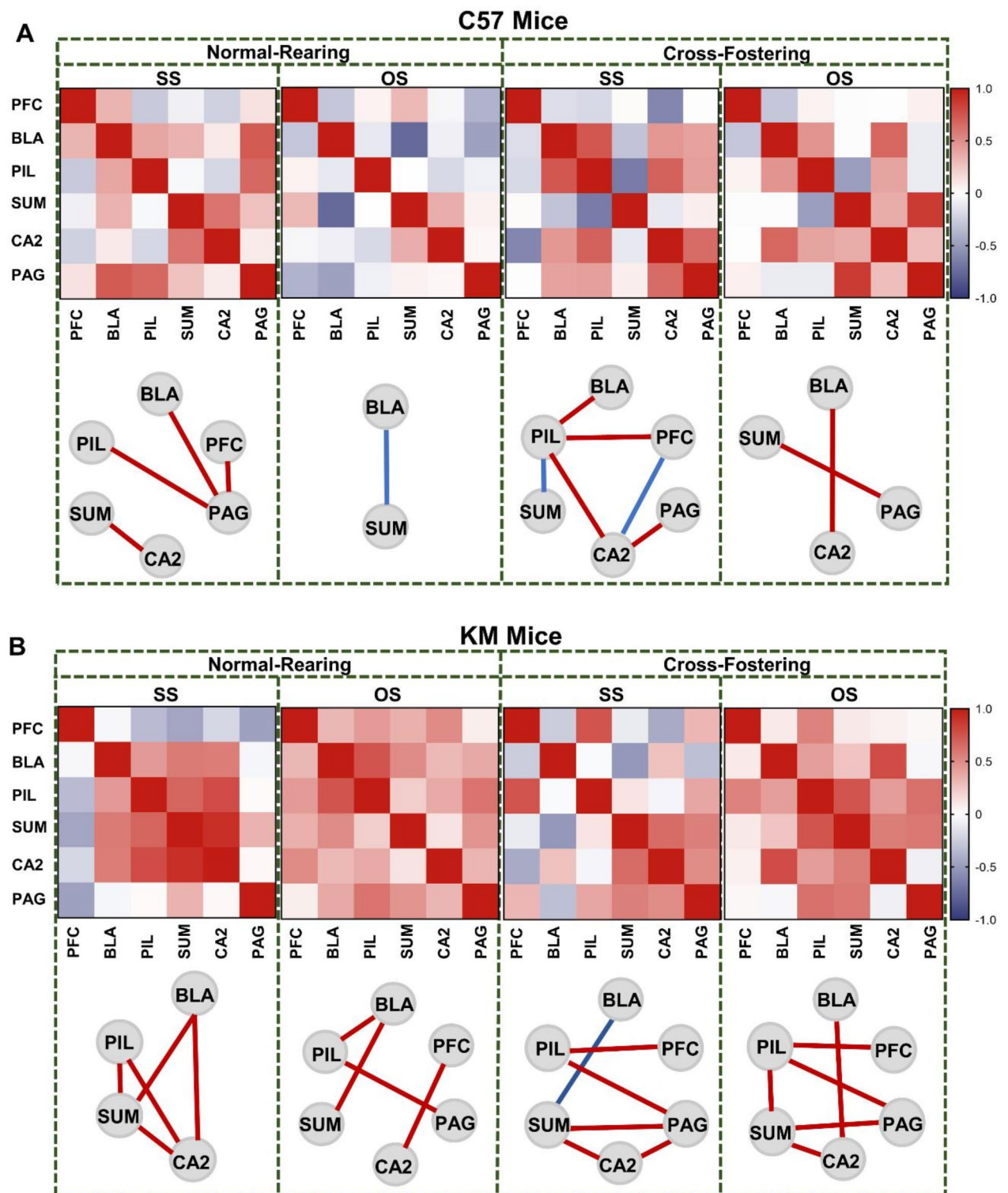
Fig. 7. Interregional correlations patterns visualization in C57 and KM mice. (**A**) Correlation matrices of c-Fos-positive cells across brain regions for C57 mice under Normal-Rearing and Cross-Fostering conditions, when interacting with same-strain and different-strain conspecifics. Connectivity network diagrams of brain regions for C57 mice under Normal-Rearing and Cross-Fostering conditions, illustrating positive correlations ($r > 0.5$) in red and negative correlations ($r < 0.5$) in blue. (**B**) Correlation matrices of c-Fos-positive cells across brain regions for KM mice under Normal-Rearing and Cross-Fostering conditions, when interacting with SS and OS conspecifics. Correlation patterns visualization of brain regions for KM mice under Normal-Rearing and Cross-Fostering conditions, illustrating positive correlations ($r > 0.5$) in red and negative correlations ($r < 0.5$) in blue.
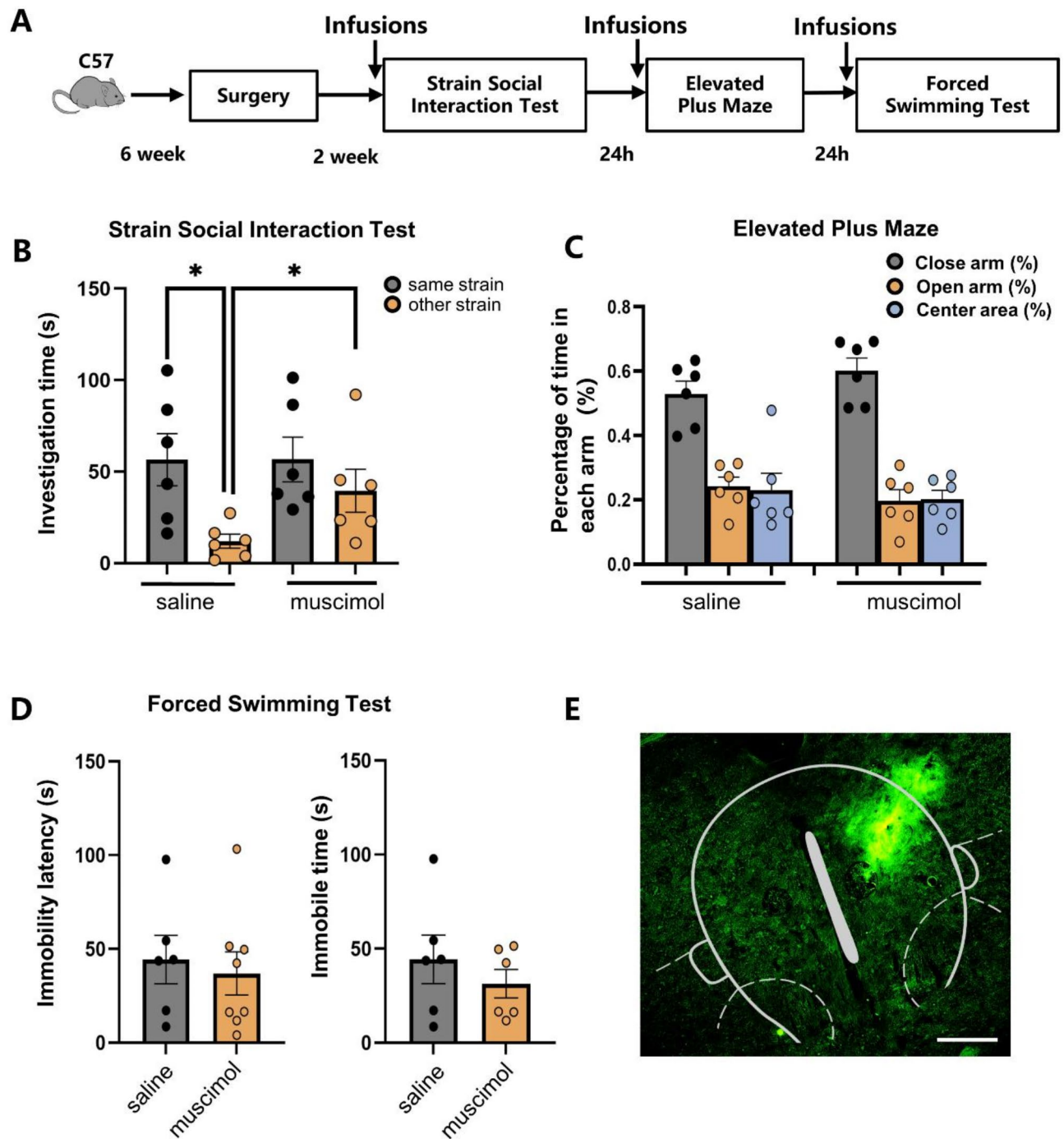
Fig. 8. Effect of muscimol infusion into the PAG on social preference in C57 mice and assessment of anxiety- and depression-like behaviors. (**A**) Experimental timeline for C57 mice, including cannula implantation at 6 weeks of age, a two-week recovery period, and behavioural testing conducted 40 min after drug administration. (**B**) Strain social interaction test comparing social preferences between the saline control group and the muscimol-treated group (Paired t test, saline SS vs. saline OS: $t_5 = 3.468$, $p = 0.0179$; muscimol SS vs. muscimol OS: $t_5 = 1.421$, $p = 0.2144$. Unpaired t test, saline SS vs. muscimol SS: $t_{10} = 0.008162$, $p = 0.9936$; saline OS vs. muscimol SS: $t_{10} = 2.236$, $p = 0.0493$. $n = 6$). (**C**) Elevated plus-maze test assessing anxiety-like behaviour in C57 mice following muscimol administration (Unpaired t test, close arm: $t_{10} = 1.276$, $p = 0.2310$; open arm: $t_{10} = 0.9844$, $p = 0.2310$; center area: $t_{10} = 0.4542$, $p = 0.6594$; $n = 6$). (**D**) Forced swim test evaluating depression-like behavior in C57 mice post-muscimol treatment(Unpaired t test, Latency to immobile time (s), $t_{10} = 0.1979$, $p = 0.8471$; Immobile time(s), $t_{10} = 0.8698$, $p = 0.4048$; $n = 6$). (**E**) Verification of cannula placement and example of drug infusion state (scale bar: 1:200 μm). Data are presented as means ± s.e.m, *$p < 0.05$.

preference for either larger or smaller mice under cross-fostering conditions. Thus, these results indicate that size alone cannot fully explain the observed phenomena. Instead, strain-specific preferences and their plasticity seem to play a more substantial role. Furthermore, we assessed the activity of brain regions in both normally reared and cross-fostered C57 and KM mice. The activity changes in the PAG were consistent with the shifts in social preferences and their modulation by rearing conditions. Therefore, PAG activity appears to be more closely related to strain preferences than to body size preferences. Conversely, the activity in the PIL region of C57 mice remained consistent with the behavioural patterns under normal rearing conditions but was unaffected by cross-fostering. This region is known to encode social information, potentially reflecting the recognition of body size in C57 mice. However, our findings suggest that the PAG is more directly associated with strain preference than with body size preference. This distinction underlines the purpose of our cross-fostering behavioural experiments: to identify brain regions specifically associated with strain-selective social preferences.

Furthermore, body size can influence social interactions, particularly in the context of aggressive behaviours. Numerous studies on aggression typically use smaller, younger mice as targets for aggressive interactions[29,38]. Research has suggested that social behaviour becomes more moderate when CD1 mice of similar age and weight are paired together[39]. To isolate the effect of body size from that of aggression, we selected age-matched C57 and KM mice that differed only in terms of body size. Our free social interaction experiments revealed that interactions between C57 and KM mice were nonaggressive, suggesting that the observed preferences are driven by social contact rather than aggression. However, the short duration of the free interactions may not be sufficient to elicit dominance-related aggressive behaviours[40]. If such interactions are indeed aggressive, modifying these interaction patterns through cross-fostering could offer new insights into the study of emotional valence types of social interactions.

Studies have shown that cross-fostering initially causes stress in young animals, but there is insufficient evidence to conclude that these negative emotions persist long-term[41]. Another study revealed that chronic stress in dams induces anxiety-like behaviours in their offspring. However, cross-fostering these pups to dams without anxiety-like behaviours can alleviate such behaviours in adulthood[42]. It seems that the timing of cross-fostering is correlated with changes in these behaviours[43]. Therefore, in this experiment, we cross-fostered pups before the age of three days, and male pups of different strains were cohoused until adulthood. Previous studies in rats have shown that cross-fostering on the first postnatal day can prevent stress-induced corticosterone secretion, reduce locomotor activity in a novel environment, and improve spatial recognition memory in offspring that are weaned early[44,45]. In this study, we assessed anxiety- and depressive-like behaviours in cross-fostered adult mice. Our results indicated that there were no significant differences in behaviour between cross-fostered and normally reared adult mice. These findings suggest that our cross-fostering strategy did not influence anxiety- or depressive-like behaviours in adulthood. However, because our control group consisted of normally reared mice, we were unable to measure the specific effects of cross-fostering itself on the mice. In other words, it remains to be determined whether cross-fostering with a lactating dam of the SS would affect social behaviours in mice. Future research is needed to address this problem.

In human studies, the social environment can influence an individual's perception of others or groups[46,47]. This social plasticity is advantageous for individuals to integrate into their social environment and promote cooperation among social groups. Is there also postnatal plasticity in strain preference in mice? We utilised cross-fostering to alter the postnatal social environment of mice and investigated whether it could reshape their strain preference. In rodents, cross-fostering has been found to alter many types of social behaviours[48,49]. Additionally, these phenotypic changes can be transmitted nongenetically to offspring[50], highlighting the feasibility of cross-fostering in social behaviour research[51]. Our results revealed that strain preference was altered by cross-fostering in both C57 and KM mice. After cross-fostering with lactating dams from another strain, the strain-based social differentiation behaviours were significantly attenuated. These results suggest that increasing the exposure of C57 and KM mice to different strains in the rearing environment reshaped their strain-dependent social preferences. Notably, the reduction in social interaction differences resulted from an increase in the tendency of the mice to socialise with strains of mice that were not their preferred strains. In human societies, adults often face situations that require integration into other social groups. The social avoidance of individuals outside the group can hinder an individual's social integration. Therefore, investigating social preferences and their plasticity in adult individuals will also have significant research implications. In our study, we changed the rearing environment of the mice within 1–3 days after birth. It would be interesting to further investigate how long the alterations in strain preference last and whether a cross-fostering environment in adults could also cause similar alterations.

To investigate the neural mechanisms behind strain preference, we evaluated the neural activity of different brain regions in mice subjected to interactions with SS or OS under normally reared and cross-fostering conditions. Subsequently, the number of c-Fos$^+$ neurons was measured as an indicator of neural activity. We focused on six brain regions highly correlated with social preference behaviours, namely, the BLA, PFC, PIL, CA2, SUM, and PAG. The results revealed different activity levels of these six brain regions in C57 and KM mice. We performed correlation analyses and constructed network diagrams for each brain region. Our findings revealed that cross-fostering altered the connectivity patterns among brain regions. These findings suggest that cross-fostering may influence the cognitive networks of mice. Generally, the neural activity levels of all six brain regions increased after social interactions, with no difference between the OS and SS groups in the BLA or PFC. Neural activity levels in the PIL, CA2 and SUM regions were heterogeneous between KM and C57 mice.

The changes in neural activity in the PAG were fully consistent with the behavioural changes observed in both KM and C57 mice. The PAG was more active when the mice engaged in social interactions with their favoured strains, which was the SS for C57 mice and the OS for KM mice, and the difference in PAG activity disappeared after cross-fostering, similar to the strain preference behaviour. We then focused on modulating the behavioural phenotypes of C57 mice. Using muscimol to silence the PAG, we preliminarily confirmed the causal relationship of the PAG brain region in regulating strain preference in mice, such that after PAG activity

was inhibited, the strain preference of C57 mice was eliminated. Importantly, the mice did not exhibit deficits in sociability or social novelty or signs of depression or anxiety. These findings highlight the specialised role of the PAG in determining social preferences between strains. Our results suggest that the PAG plays a crucial role in shaping social strategies during selective interactions, suggesting a promising direction for future research. However, for mouse experiments, we could only observe and measure changes in behavioural performance; therefore, the social interaction alterations between cross-fostered mice and normally reared mice might be due to altered perception and recognition of OS mice, or it could be that this perception remains unchanged but that the social strategies or preferences of the mice changed. Both mechanisms are possible. Under our current experimental paradigm, we are unable to distinguish between these two possibilities; therefore, future investigations are needed to address this issue.

Previous research on the PAG has focused predominantly on mouse defensive behaviours, fear-related emotions, and the neural circuits regulating pain[52–54]. Although some studies have confirmed the involvement of the PAG in the regulation of social behaviours, its role in strain recognition or strain preference has not been studied. Projections from neurons in the lateral and ventrolateral periaqueductal grey (l/vlPAG) to paraventricular hypothalamus oxytocinergic neurons can facilitate social behaviours[27]. Additionally, the PAG is involved in the detection and recognition of social odours[55]. Vocalisation recognition is also part of social recognition and aids in mammalian mating and social bonding[56,57]. Therefore, the PAG might regulate the recognition and analysis of sensory information such as odours and vocalisations, thereby affecting strain-dependent social behaviours. Furthermore, the PAG plays a role in determining defensive strategies, including freezing, avoidance, and escape behaviours[58,59].

In conclusion, for the first time, our study clearly demonstrated the existence of strain-dependent social preference behaviours in mice, and we proved that strain preference could be altered by the rearing environment. Additionally, we revealed that the PAG region plays a crucial role in regulating strain preferences. This study fills a knowledge gap in social preference research in mice and provides valuable insights for future social recognition studies.

Data availability
Data is provided within the supplementary information files.

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Author contributions

Author Contributions: Author contributions: Z.X., and T.L. designed research; P.K., T.L., J.L., Y.S., X.Y., C.J., W.M., Q.L., and Z.X. performed research; T.L, and Z.X. contributed new reagents/analytic tools; P.K., and Z.X. analyzed data; Z.X., and P.K. wrote the paper.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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