



OPEN Paternal social fear conditioning drives sex-specific behavioral alterations and septal gene regulation

Laura Stangl¹, Anna Bludau¹, Virginie Rappeneau^{1,2} & Rohit Menon^{1,2}✉

The intergenerational transmission of traumatic experiences is increasingly recognized as a contributor to behavioral and neurobiological vulnerability in offspring. However, the inheritance of paternal traumatic social experiences remains poorly understood. Here, we employed the social fear conditioning (SFC) paradigm in mice to investigate the transmission of paternal socially aversive experiences to the next generation. First, we confirmed that social fear acquisition selectively impaired social approach behavior without affecting locomotion or general anxiety-related behavior in adult male mice of the F0 generation. Second, we examined the consequences of F0 paternal social fear conditioning for first-generation (F1) offspring. We found that male but not female F1 offspring of conditioned (SFC⁺) fathers displayed reduced sociability. This behavioral alteration was accompanied by increased cellular activity in the lateral septum, as indicated by elevated cFos expression. Finally, exploratory transcriptomic analyses within the septum revealed that social fear acquisition was associated with an overall upregulation of synaptic plasticity-related genes in SFC⁺ F0 males compared to SFC⁻ F0 controls. Remarkably, this transcriptomic profile was inverted in F1 male offspring of conditioned fathers, who displayed relative downregulation of the same synaptic plasticity-associated genes. Notably, six genes (*Adcy8*, *Cdh2*, *Mapk1*, *Nptx2*, *Plcg1*, and *Srf*) were significantly upregulated in F0 males but downregulated in F1 male offspring. Together, our findings establish the SFC paradigm as a selective and robust approach to study the intergenerational transmission of paternal traumatic social experiences. Moreover, they reveal specific gene expression changes associated with social behavior that may underlie behavioral deficits in the next generation, thereby highlighting molecular targets for future studies.

Keywords Intergenerational transmission, Paternal trauma, Social fear conditioning, Social behavior, Synaptic plasticity, Lateral septum

Abbreviations

<i>Adcy8</i>	adenylyl cyclase type 8
<i>Cdh2</i>	cadherin-2
CS	conditioned stimulus
F0	parental generation
F1	first filial generation (offspring)
LDB	light-dark box
LS	lateral septum
<i>Mapk1</i>	mitogen-activated protein kinase 1 (MAPK 1)
<i>Nptx2</i>	neuronal pentraxin-2
OFT	open-field test
PCR	polymerase chain reaction
<i>Plcg1</i>	phospholipase C gamma 1
PTSD	post-traumatic stress disorder
PVN	paraventricular nucleus of the hypothalamus

¹Department of Behavioural and Molecular Neurobiology, University of Regensburg, Universitaetsstr. 31, 93053 Regensburg, Germany. ²Virginie Rappeneau and Rohit Menon contributed equally to this work. ✉email: rohit.menon@ur.de

SFC	social fear conditioning
SFC ⁺	social fear conditioned
SFC ⁻	social fear unconditioned
Srf	serum response factor
TCT	three-chamber test
US	unconditioned stimulus

Traumatic experiences can exert long-lasting effects beyond the directly exposed individual, influencing behavioral, physiological, and neuronal outcomes in subsequent generations. Human studies suggest that parental trauma, including exposure to war¹, famine^{2,3}, or severe emotional stress⁴ is associated with increased risk for stress-related disorders in descendants. Complementary rodent studies demonstrate that parental stress exposure can alter offspring behavior, stress responsivity, and gene regulation, providing a biological basis for intergenerational inheritance^{5–7}. Collectively, these observations indicate that traumatic experiences leave enduring biological traces that affect offspring phenotypes, even in the absence of direct exposure. However, the specific information transmitted across generations and whether it reflects parental experience rather than generalized stress vulnerability remain unclear.

Importantly, intergenerational stress effects have been demonstrated across a wide range of experimental paradigms, including both early-life stress and adult stress exposure models. On one hand, rodent models focusing on early-life stress paradigms, such as maternal separation combined with unpredictable stress or chronic social instability (for review, see⁸). These approaches have uncovered epigenetic and transcriptional mechanisms underlying stress inheritance, including alterations in offspring anxiety-related behavior, stress hormone regulation, metabolic function, and cognitive performance, often persisting across multiple generations^{9–11}. On the other hand, a substantial body of work demonstrates that stress exposure during adulthood is also sufficient to induce heritable effects. In particular, paternal stress paradigms in adulthood, including chronic variable stress, restraint stress, and chronic social defeat stress, have been shown to produce robust and reproducible alterations in offspring behavior and physiology. These studies report changes in offspring stress responsivity, affective behavior, and gene expression across central and peripheral systems, often linked to alterations in sperm-derived molecular signals of the sires^{6,12–14}. Notably, adult social stress models such as chronic social defeat stress induce enduring changes in emotional and social behavior in offspring, highlighting that socially relevant experiences in adulthood can be biologically transmitted across generations.

However, such approaches broadly affect emotionality and physiology, and may involve parent-offspring interaction, complicating the dissociation between biological and behavioral transmission^{15,16}. Additionally, chronic or early-life stress paradigms typically involve prolonged or repeated stress exposure. Moreover, they lack specificity for discrete behavioral domains, particularly social behavior, which is often disrupted in trauma-related psychiatric conditions^{17–19}. Taken together, there is a need for models that capture discrete, experience-defined social learning events in adulthood. Such approaches allow assessment of whether specific social experiences, rather than generalized stress states, can be transmitted across generations. Hence, it is important to use paradigms that specifically model social trauma to study the transmission of social deficits across generations.

Social fear conditioning (SFC) is a well-established experimental paradigm in mice that selectively induces persistent and robust social avoidance by pairing social stimuli with aversive outcomes^{20,21}. Importantly, SFC induces social avoidance without inducing generalized anxiety-related behavior or locomotor deficits, enabling the study of social domain-specific trauma effects. This specificity allows the investigation of whether intergenerational transmission of trauma preserves discrete behavioral features rather than global emotional dysregulation. At the circuit level, SFC engages the lateral septum (LS), a basal forebrain structure regulating social behavior, emotional processing, and stress responses²². The LS integrates inputs from the hippocampus, amygdala, and hypothalamus, influencing downstream emotional and motivational states²³. Thus, rather than functioning solely as a relay within the social fear network, the LS represents a key integrative node through which contextual and affective information can be translated into behavioral responses.

Beyond circuitry, post-transcriptional regulation in the LS critically modulates social fear learning. In particular, microRNA-, epigenetic-, and long non-coding RNA-dependent mechanisms dynamically shape septal plasticity and contribute to the formation, persistence, and success of social fear extinction^{24–26}. Specifically, septal miR-123-3p is dynamically regulated following SFC and functionally involved in the extinction of social fear²⁴; HDAC1-mediated GABAergic regulation is essential for long-lasting extinction memory²⁵; and Meg3 long non-coding RNA isoforms support successful extinction²⁶, highlighting RNA-based control of septal plasticity. Collectively, these findings indicate that the LS integrates circuit activity with multilayered molecular regulation to shape socio-emotional behavior.

In the present study, we leveraged SFC to investigate the intergenerational transmission of paternal traumatic social experiences. We hypothesized that SFC-induced socially aversive learning in fathers would be transmitted to the F1 offspring, leading to alterations in their social behavior and associated neuronal and molecular signatures. By focusing on paternal exposure and separating fathers from dams early during gestation, we excluded postnatal paternal influences and isolated biological inheritance mechanisms. Combining behavioral assays with cellular activity mapping and exploratory gene expression profiling, we assessed whether paternal socially aversive learning alters offspring social behavior and whether these effects are associated with septal activity and transcriptional changes. Exploratory gene expression profiling identified molecular signatures of paternal trauma and their regulation across generations. Notably, a comparison between the F0 and F1 generations revealed that six genes (*Adcy8*, *Cdh2*, *Mapk1*, *Nptx2*, *Plcg1*, and *Srf*) exhibit opposite patterns of regulation, with increased expression in F0 SFC⁺ fathers and decreased expression in F1 SFC⁺ offspring. Our approach combines a trauma model with behavioral and gene expression analyses to study the biological transmission of discrete traumatic experiences.

Material and methods

Animals

Male and female CD1 mice were purchased from Charles River (Sulzfeld, Germany) at 8 weeks of age. CD1 mice were used due to their outbred genetic background and well-characterized socio-emotional behavioral profile. Compared to commonly used inbred strains such as C57BL/6, CD1 mice exhibit higher behavioral variability and more stable baseline sociability, which reduces strain-specific ceiling or floor effects in social behavior assays and improves generalizability of findings²⁷. Males were single-housed (16 × 22 × 14 cm) to avoid potential dominance hierarchy effects, while females were group-housed (42 × 22 × 14 cm; 2–4 per cage) until breeding. All mice were maintained in an environmentally controlled vivarium (22 °C, 60% humidity, 12 h light/dark cycle, lights on at 07:00 h) with *ad libitum* access to standard chow and water. All experiments were conducted in accordance with the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines²⁸ and international regulations for the care and use of animals (Directive 2010/63/EU), with ethical approval from the government of Unterfranken.

Experimental design

This study aimed to investigate whether SFC-induced social avoidance in male mice produces paternally transmitted behavioral effects in the next generation. To address this question, three independent cohorts of animals were used (Fig. 1).

Cohort 1: Naïve adult male mice were subjected to the SFC paradigm (see Sect. “[Social fear conditioning \(SFC\)](#)”) and subsequently tested to determine the direct behavioral effects of SFC. Locomotor activity was assessed in the open-field test (OFT), anxiety-related behavior in the light–dark box (LDB) test, and social interaction in the three-chamber test (TCT).

Cohort 2: A separate group of adult male mice (F0) underwent the SFC paradigm and was assigned to either the SFC⁺ or SFC[−] group. Following conditioning, males remained single-housed and undisturbed for 10 days to allow potential germline transmission of somatic changes²⁶. Single housing was applied to all F0 males to avoid social hierarchy effects, but this may have elevated baseline stress levels in both SFC[−] and SFC⁺ groups, potentially blunting the magnitude of group differences. Future studies using group-housed controls or larger cohort designs could address this limitation. Subsequently, males were paired with naïve adult females for 7 days and then females were housed individually until parturition. F1 offspring were reared by their mothers until weaning (3–4 weeks of age), group-housed with same-sex littermates (2–5 animals per cage) until 8 weeks of age and individually housed for 3 days before testing. Weaning occurred between postnatal days 21 and 28 according to standard facility practice, with all littermates weaned on the same day. Weaning age was not systematically recorded across individual pups, but litters from SFC[−] and SFC⁺ fathers were processed identically and in parallel. Any residual variation in weaning age would be randomly distributed between groups and therefore unlikely to bias group comparisons systematically. Anxiety-related behavior and social interaction were assessed using the LDB and TCT, respectively (see Sect. “[Light-dark box test \(LDB\)](#)” and [Three-chamber test](#)). One mouse was randomly selected from each litter for each test as a representative of its respective F0 father. Following behavioral testing, a subset of F1 offspring was perfused 90 min after TCT exposure for immunohistochemical analysis of cFos expression in the LS to determine whether behavioral alterations were associated with changes in neuronal activation following social exposure.

Cohort 3: A third independent cohort underwent the same SFC, breeding, and housing procedures as Cohort 2. In this cohort, F0 males and all male F1 offspring were tested in the TCT. F0 males were sacrificed 90 min after TCT exposure, whereas F1 offspring were sacrificed 90 min after social exposure. Brain tissue was collected for molecular analyses. To maximize phenotypic contrast and enhance sensitivity for detecting transcriptional differences, we selected F1 offspring of SFC[−] fathers showing the highest social preference and offspring of SFC⁺ fathers showing the lowest social preference for molecular analyses. This extreme-group approach is appropriate for exploratory screenings but may overestimate effect sizes and limit generalizability to the full phenotypic range. Therefore, the reported gene expression differences should be considered hypothesis-generating, and validation in unselected cohorts is warranted.

Social fear conditioning (SFC)

The SFC paradigm, based on an operant avoidance conditioning protocol, was adapted from previous studies^{20,29}. Experimental mice were single-housed in observation cages (30 × 25 × 35 cm). Weight- and strain-matched mice served as social stimuli. During acquisition (day 1), conditioned mice (SFC⁺) received an electric foot shock (unconditioned stimulus [US]: 0.7 mA, 1 s, pulsed current) each time they investigated an unfamiliar conspecific placed in a small wire-mesh cage (7 × 7 × 6 cm) (conditioned stimulus [CS]), using a computerized fear-conditioning system (TSE Systems GmbH, Bad Homburg, Germany). Acquisition ended when the mouse avoided the conspecific for 6 min after the first shock; if it approached the conspecific two or more times, shocks were repeated to reinforce social avoidance until 2 min of continuous avoidance was achieved. After the acquisition, mice were then returned to their home cage. Unconditioned controls (SFC[−]) were allowed to freely explore the conspecific for 3 min without punishment.

Behavioral testing

General procedure

All tests were conducted between 08:00 h and 13:00 h to coincide with the animals’ circadian trough of corticosterone levels³⁰. The EthoVision XT video-tracking system (Noldus Information Technology, Wageningen,

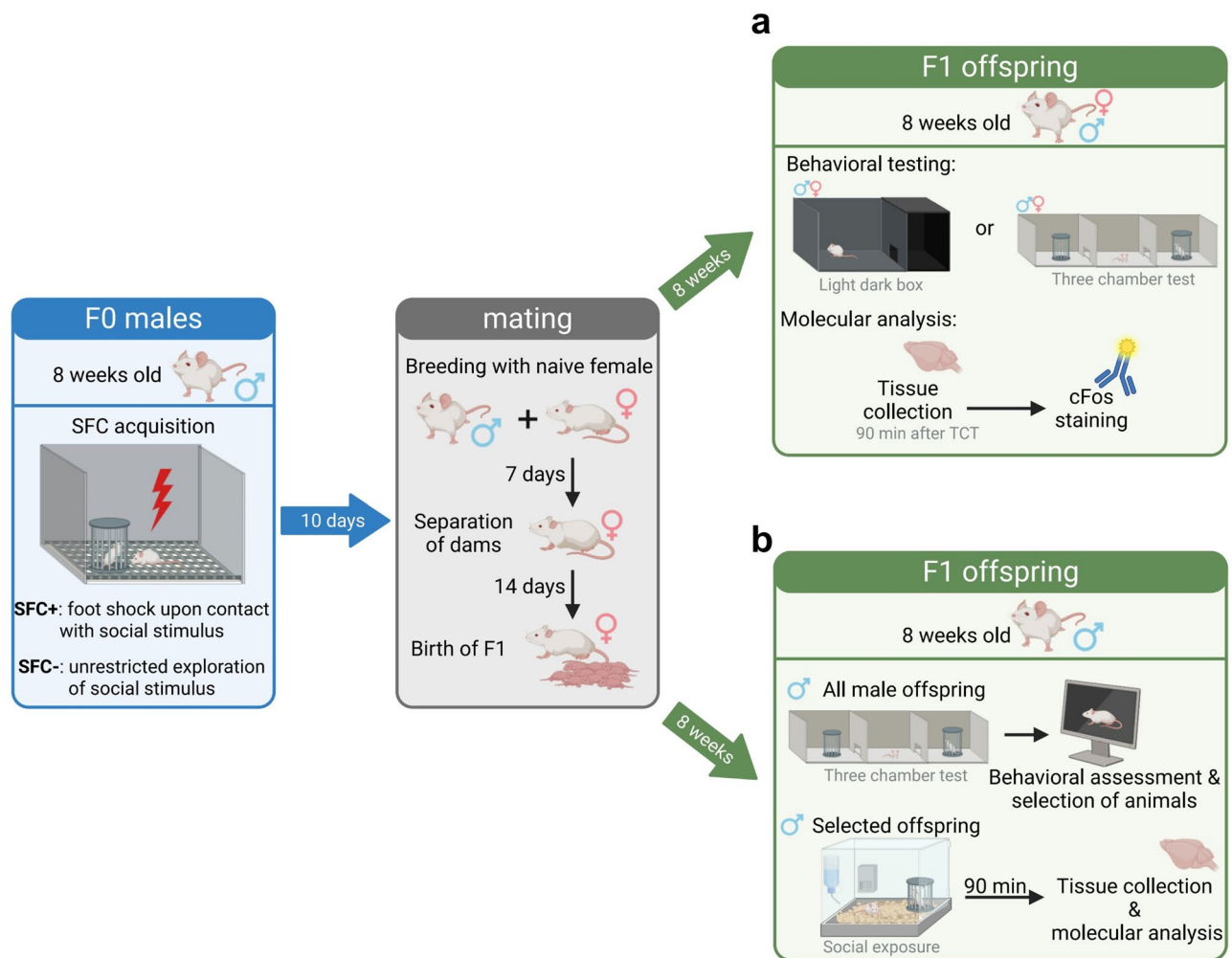


Fig. 1. Experimental design for assessing intergenerational effects of paternal social trauma. Eight-week-old F0 males underwent social fear conditioning (SFC). The protocol consisted of 30 s habituation in the empty chamber, followed by 3 min exposure to a non-social stimulus. Mice in the unconditioned (SFC⁻) group could freely explore a social stimulus for 3 min, whereas mice in the conditioned group (SFC⁺) received a mild foot shock whenever they approached the social stimulus. To investigate the specificity of social fear acquisition, one cohort of animals was tested for general anxiety in the open-field test (OFT) and the light–dark box test (LDB), as well as for sociability in the three-chamber test (TCT). A second cohort of males was bred with naïve females, ten days after conditioning. Dams were separated from the sires 7 days after mating to exclude postnatal paternal influences. F1 offspring were born 14 days later and raised to adulthood. **(a)** At 8 weeks of age, male and female F1 offspring were subjected to behavioral testing, including either the LDB test or TCT. 90 min after TCT exposure, brains were collected and further processed for cFos staining. **(b)** A separate cohort included only male F1 offspring. All animals were tested in the TCT at 8 weeks of age. The behavior was assessed, and behaviorally extreme animals were selected for social exposure. Brains were collected 90 min after for gene expression analyses.

The Netherlands) was used to monitor animal activity during behavioral tests (OFT, LDB, TCT). All stimulus mice used were age- and sex-matched across all tests to ensure consistency of the social stimulus and to avoid confounding effects due to variability in stimulus animal characteristics. The time spent in contact with the social and non-social stimuli in the TCT or the social stimulus during the social exposure was manually scored by a trained observer, blinded to the mouse group, using the Behavioral Observation Research Interactive Software (version 4.1.1)³¹ or JWatcher (1.0) (available from <http://www.jwatcher.ucla.edu>). Contact was defined as the experimental mouse positioning its nose within < 1 cm of the wire mesh cage. This spatial threshold was used consistently across all experiments to standardize scoring and ensure reproducibility. The test apparatuses were cleaned with a lemon-scented soap solution between each mouse.

Open-field test (OFT)

Locomotor activity was assessed in a dimly illuminated open-field arena (40 × 40 × 40 cm, < 15 lx)³². A central square area (20 × 20 cm) was defined as the inner zone. Mice were placed in the outer zone facing the wall and allowed to explore freely for 5 min³³. The total distance travelled was analyzed.

Light-dark box test (LDB)

Anxiety-related behavior was evaluated using the LDB³⁴. The apparatus consisted of a dark compartment (25 × 20 × 21 cm; < 10 lx) and a light compartment (25 × 30 × 21 cm; 330 lx) connected by a small opening (10 × 3.7 × 6 cm). Mice were placed in the dark compartment facing the wall opposite the opening and allowed to explore freely for 5 min³³. The time spent in each compartment was recorded.

Three-chamber test (TCT)

Innate social behavior was assessed in a dimly lit TCT (47.5 × 25 × 18 cm, 15–25 lx)³⁵. Weight- and strain-matched mice served as social stimuli and were placed in small wire-mesh containers (7 × 7 × 6 cm) in the outer chambers, allowing sensory but not physical contact. Experimental mice were acclimatized to the empty apparatus for 5 min, followed by a 5 min sociability session (unfamiliar mouse vs. empty container) and a 5 min social novelty session (familiar mouse vs. novel mouse). Exploration was assessed by measuring direct social contact. The time spent in each chamber was automatically recorded, while the time spent in contact with the social and non-social stimuli was manually scored.

Social exposure

For the social exposure paradigm, mice were transferred from their home cage to a novel observation cage. Animals were allowed to habituate to the observation cage for 5 min prior to the introduction of the age- and sex-matched social stimulus. Social interaction was recorded for 5 min. After the stimulus mouse was removed, the experimental mouse remained in its home cage and was sacrificed 90 min later for molecular analyses.

Immunohistochemistry

Ninety minutes after the social exposure, a subset of F1 mice were deeply anesthetized using xylazine 2% (0.5 ml/kg) and ketamine 10% (1 ml/kg) and intracardially perfused with phosphate-buffered saline (0.01 M PBS, pH 7.4) followed by 4% paraformaldehyde (PFA) in 0.01 M PBS. The brains were removed, immersed overnight in 4% PFA in 0.01 M PBS, and placed in 30% sucrose/0.01 M PBS for cryoprotection. Brains were stored in -20 °C until sectioning. Free-floating coronal brain slices (30 µm) were stained using the standard avidin–biotin–peroxidase method with 3,3'-Diaminobenzidine (DAB) as the chromogen. The primary antibody rabbit anti-cFos was used (1:5000; sc-52 Santa Cruz Biotechnology). Immunostained sections were analyzed using a fluorescent microscope (Leica DM6 B, Leica Microsystems GmbH, Wetzlar, Germany). The planes of the analyzed sections were standardized according to the mouse brain atlas³⁶. The average densities of cFos positive cells in the LS were determined using ImageJ (V 1.53c, National Institutes of Health, USA) by analyzing at least 4 sections and normalizing the results to the counted area.

PCR array

Following the TCT in F0 mice or 90 minutes after social exposure in F1 mice, mice were euthanized with CO₂. Brains were carefully extracted, flash-frozen in 2-methylbutane (Carl-Roth, Karlsruhe, Germany), and stored at -80 °C until further processing for the PCR array analysis. Septal samples were obtained by micro-punching 250 µm coronal sections. Total RNA was extracted using the peqGOLD TriFast™ reagent (VWR, Radnor, USA) according to the manufacturer's instructions. RNA quantity and quality were assessed with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Offenbach, Germany). Complementary DNA (cDNA) was synthesized from 500 ng of total RNA using the RT² First Strand Kit (Qiagen) following the manufacturer's instructions. Given the exploratory nature of this intergenerational study and the absence of prior transcriptional data on paternal social fear transmission using SFC, we prioritized sensitivity to capture modest but potentially biologically relevant expression changes. Therefore, an exploratory gene expression profiling was performed using the RT² Profiler™ PCR Array Mouse Synaptic Plasticity (Cat. No.: 330231, Qiagen) in accordance with the manufacturer's protocol. Expression levels of target mRNAs were normalized against the geometric mean of five housekeeping genes (*Actb*, *B2m*, *Gapdh*, *Gusb*, and *Hsp90ab1*). A comprehensive list of all analyzed genes is provided in Supplementary Tables 2 and 3. Genes with CT values ≥ 30 were excluded from further analysis.

Statistical analysis was conducted using uncorrected p-values for exploratory assessment. To control for multiple comparisons, false discovery rate (FDR) correction was performed using the two-stage Benjamini–Krieger–Yekutieli procedure, and corresponding q-values are reported in the Supplementary Tables 2 and 3. No genes remained significant after FDR correction; therefore, PCR array results are considered exploratory and hypothesis-generating. PCR arrays are commonly used as exploratory screening tools to identify candidate genes for further validation. Genes were classified into three categories: non-significant ($p > 0.08$), trending ($p \leq 0.08$), and significantly differentially expressed ($p \leq 0.05$). A descriptive threshold of $p \leq 0.08$ was used only to indicate trend-level changes and did not represent a criterion for statistical significance.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism (version 10.6.1 for Windows, GraphPad Software, Boston, Massachusetts, USA, <http://www.graphpad.com>), and JAMOV (<https://www.jamovi.org>). Prior to statistical analysis, all datasets were screened for outliers using the Robust Regression and Outlier Removal (ROUT) method implemented in GraphPad Prism³⁷. The false discovery rate (FDR) threshold was set at $Q = 1\%$. Outlier detection was applied consistently across all experimental groups using predefined criteria. Data

points identified as outliers by the ROUT algorithm were excluded from subsequent analyses. No additional data exclusions were performed. All data were tested for normality and equality of error variances using the Shapiro–Wilk test and Bartlett’s test, respectively. Unpaired data were analyzed using the Mann–Whitney U-test. Paired data were analyzed using the Wilcoxon matched-pairs signed-rank test. Gene analysis was done using a student’s t-test. All data in the text are presented as mean $\pm$ SEM or volcano plots. Results are reported as significant at the 0.05 level (two-sided p-values) unless indicated otherwise. Effect sizes are reported for all analyses: rank-biserial correlation (r_{rb}) for Mann–Whitney U and Wilcoxon signed-rank tests. All graphs were generated with GraphPad Prism (version 10.6.1 for Windows, GraphPad Software, Boston, Massachusetts, USA, <http://www.graphpad.com>).

Results

Social fear conditioning does not alter locomotion or general anxiety-related behavior in F0

To test the specificity of the induced social fear, naïve male mice underwent social fear acquisition and were subsequently tested for locomotion, anxiety, and social behavior (Fig. 2a). During acquisition, SFC⁺ mice received on average 2.1 ± 0.193 CS-US pairings contingent on approaches to the social stimulus, whereas the SFC⁻ group freely interacted with the conspecific (Fig. 2b). Total distance travelled in the OFT did not differ between groups ($U = 49.5$, $p = 0.203$; $r_{rb} = -0.313$) (Fig. 2c), indicating intact locomotor activity. Similarly, no group differences were observed in the percentage of time spent in the light compartment during the LDB test ($U = 55$, $p = 0.347$; $r_{rb} = -0.236$) (Fig. 2d), suggesting that SFC did not induce general anxiety-related behavior.

In the TCT (Fig. 2e), SFC⁻ mice displayed intact sociability, spending significantly more time interacting with the social stimulus than with the non-social cage ($W = 60$, $p = 0.005$; $r_{rb} = -0.909$). In contrast, SFC⁺ mice showed a significant avoidance of the social stimulus, spending less time interacting with the conspecific than with the non-social object ($W = 55$, $p = 0.002$; $r_{rb} = 1.000$). During the social novelty phase (Fig. 2f), SFC⁻ mice showed a clear preference for the novel conspecific ($W = 76$, $p = 0.001$; $r_{rb} = -0.974$), indicative of intact social novelty recognition. SFC⁺ mice did not preferentially explore the novel conspecific ($W = -1$, $p > 0.999$; $r_{rb} = 0.067$) and exhibited near-zero social investigation overall, consistent with the general social avoidance phenotype induced by SFC (Fig. 2f). Together, these results indicate that SFC acquisition selectively impairs social approach and preference, without affecting locomotion or baseline anxiety-related behavior.

Paternal social fear conditioning selectively alters sociability in male offspring

To investigate potential intergenerational effects of SFC acquisition, male mice subjected to SFC (F0) were bred with naïve females (Fig. 3a). Behavioral and cellular outcomes were assessed in their offspring (F1) using the LDB to measure general anxiety-related behavior and the TCT for social behavior, followed by tissue collection (Fig. 3c). During acquisition, SFC⁺ mice received on average 1.9 ± 0.170 CS-US pairings contingent on approaches to the social stimulus (Fig. 3b).

In the F1 generation, no significant differences were observed between offspring of SFC⁻ and SFC⁺ fathers in time spent in the light compartment of the LDB for either males ($U = 24$, $p = 0.694$; $r_{rb} = -0.143$) or females ($U = 20$, $p = 0.620$; $r_{rb} = -0.184$) (Fig. 3d), indicating that paternal SFC did not affect general anxiety-related behavior. In the TCT, both male and female offspring of SFC⁻ fathers spent significantly more time in the compartment containing the social stimulus compared with the non-social compartment (males: $W = 85$, $p = 0.001$; $r_{rb} = -0.934$; females: $W = 83$, $p = 0.007$; $r_{rb} = -0.790$) (Fig. 3e) and showed a clear preference for the social over the non-social stimulus when time in direct contact was measured (males: $W = 89$, $p < 0.001$; $r_{rb} = -0.978$; females: $W = 83$, $p = 0.007$; $r_{rb} = -0.790$) (Fig. 3f). In contrast, offspring of SFC⁺ fathers exhibited reduced sociability, as indicated by the absence of a significant preference between compartments (males: $W = 42$, $p = 0.280$; $r_{rb} = -0.309$; females: $W = 49$, $p = 0.094$; $r_{rb} = -0.538$) (Fig. 3e). This reduction was more pronounced in males than females, as female F1 offspring of SFC⁺ fathers retained a preference for time in contact with the social stimulus (males: $W = 70$, $p = 0.074$; $r_{rb} = -0.515$; females: $W = 65$, $p = 0.022$; $r_{rb} = -0.714$) (Fig. 3f). However, social novelty preference was only observable in the SFC⁺ females, but not in any other group (Supplementary Fig. 1). These findings suggest that paternal SFC selectively impairs social behavior in offspring in a sex-dependent manner.

To determine whether these behavioral changes were accompanied by altered cellular activation following social exposure, cFos expression was quantified in the LS 90 min after the behavioral testing. Male offspring of SFC⁺ fathers exhibited a significant increase in the cFos positive cells compared to SFC⁻ controls ($U = 8$, $p = 0.043$; $r_{rb} = 0.667$), whereas female offspring showed no significant differences ($U = 15$, $p = 0.259$; $r_{rb} = 0.388$) (Fig. 3g, h). Together, these results indicate that paternal SFC induces long-lasting, sex-specific changes in both social behavior and LS cellular activation in the next generation.

SFC induces persistent changes in synaptic plasticity

Based on the differential LS activation in F1 offspring of SFC⁻ and SFC⁺ fathers (Fig. 3h), we examined whether SFC influences differential synaptic plasticity in the septum of F1 mice. For this, exploratory gene expression analyses were performed in F0 males after TCT and in F1 male offspring following social exposure (Fig. 4a, b).

During SFC acquisition, F0 SFC⁺ mice received on average 1.8 ± 0.112 CS-US pairings (Fig. 4c). Consistent with the previous experiments (Fig. 3), SFC⁻ fathers by trend spent more time interacting with the social versus non-social stimulus, although this difference did not reach significance ($W = 48$, $p = 0.064$; $r_{rb} = 0.615$; Fig. 4d). By contrast, SFC⁺ fathers exhibited significant social avoidance, indicated by spending less time with the conspecific than the non-social object ($W = 64$, $p = 0.002$; $r_{rb} = -0.970$; Fig. 4d). 8 out of the original 12 animals per group were selected for the PCR array based on RNA quality. A total of 84 genes were analyzed, and differential expressions were assessed based on p-values alone, with no fold-change cutoff applied. Exploratory differential gene expression analysis identified a limited set of genes that were differentially regulated after the TCT (Fig. 4g). Genes were categorized into three groups: not significant ($p \geq 0.08$), trend ($p \leq 0.08$), and significant ($p \leq 0.05$). A

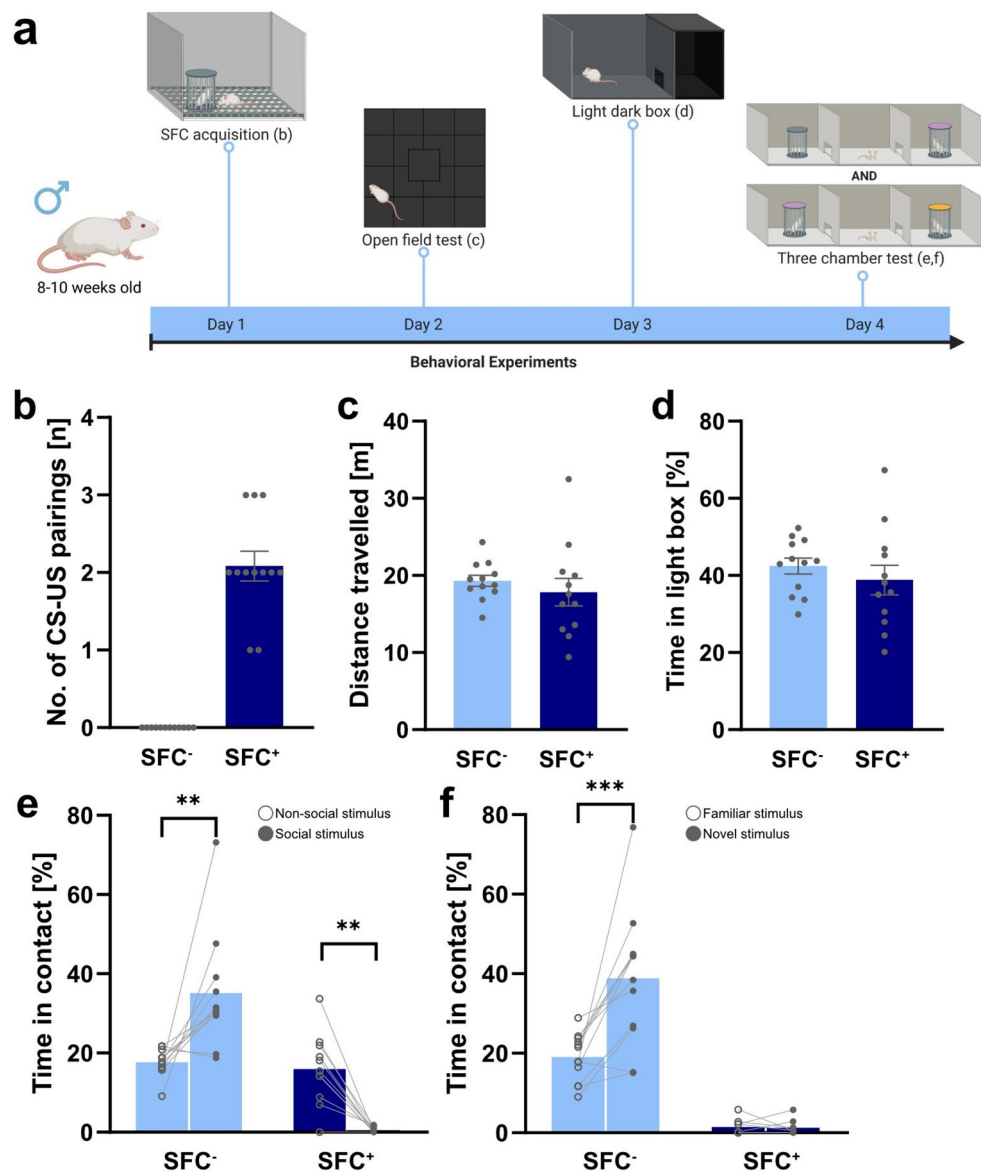


Fig. 2. Effects of social fear conditioning on locomotion, general anxiety, and social behavior. **(a)** Schematic timeline of social fear conditioning (SFC) and subsequent behavioral testing. Male mice (8–10 weeks old) underwent social fear conditioning (SFC acquisition; day 1), followed by the open-field test (OFT; day 2), lightdark box test (LDB; day 3), and three-chamber test (TCT) assessing sociability and social novelty (day 4). **(b)** Number of CS-US pairings delivered during SFC acquisition in unconditioned (SFC⁻) and conditioned (SFC⁺) animals. Sample size: 12/group. **(c)** Total distance travelled in the OFT. Sample size: 12/group. **(d)** Time spent in the light compartment of the LDB (%). Sample size: 11–12/group (1 outlier excluded). **(e)** Time spent in contact with a non-social versus a social stimulus (%) in the three-chamber test. Sample size: 11/group (2 outliers excluded). **(f)** Time spent in contact with a familiar versus novel stimulus (%) in the three-chamber test. Sample size: 9–12/group (3 outliers excluded). Bars represent mean ± SEM, dots represent individual animals. Paired comparisons within groups are shown with lines. Statistics: Between-group comparisons (SFC⁻ vs. SFC⁺) were performed using the Mann-Whitney U test (c,d). Within-group comparisons were analyzed using the Wilcoxon matched-pairs signed-rank test (e, f). ** $p \leq 0.01$, *** $p \leq 0.001$.

complete list of PCR array genes, including fold changes, p-values, and q-values, is provided in Supplementary Table 2. Within the F0 generation, comparison of SFC⁺ versus SFC⁻ animals revealed 20 septal genes with p-values ≤ 0.08 , of which all but one (*Arc*) were upregulated in SFC⁺ animals (Fig. 4l). Among these, 15 genes met the significance threshold ($p \leq 0.05$), and all were significantly upregulated in SFC⁺ relative to SFC⁻ animals (Fig. 4j).

Consistent with our previous findings, male F1 offspring of SFC⁻ fathers spent more time interacting with the social versus non-social stimulus ($W = 78$, $p < 0.001$; $r_{\text{fb}} = -1.000$), whereas offspring of SFC⁺ animals showed a lack of social preference ($W = 48$, $p = 0.061$; $r_{\text{fb}} = 0.6154$) (Fig. 4e). F1 offspring were selected according to their

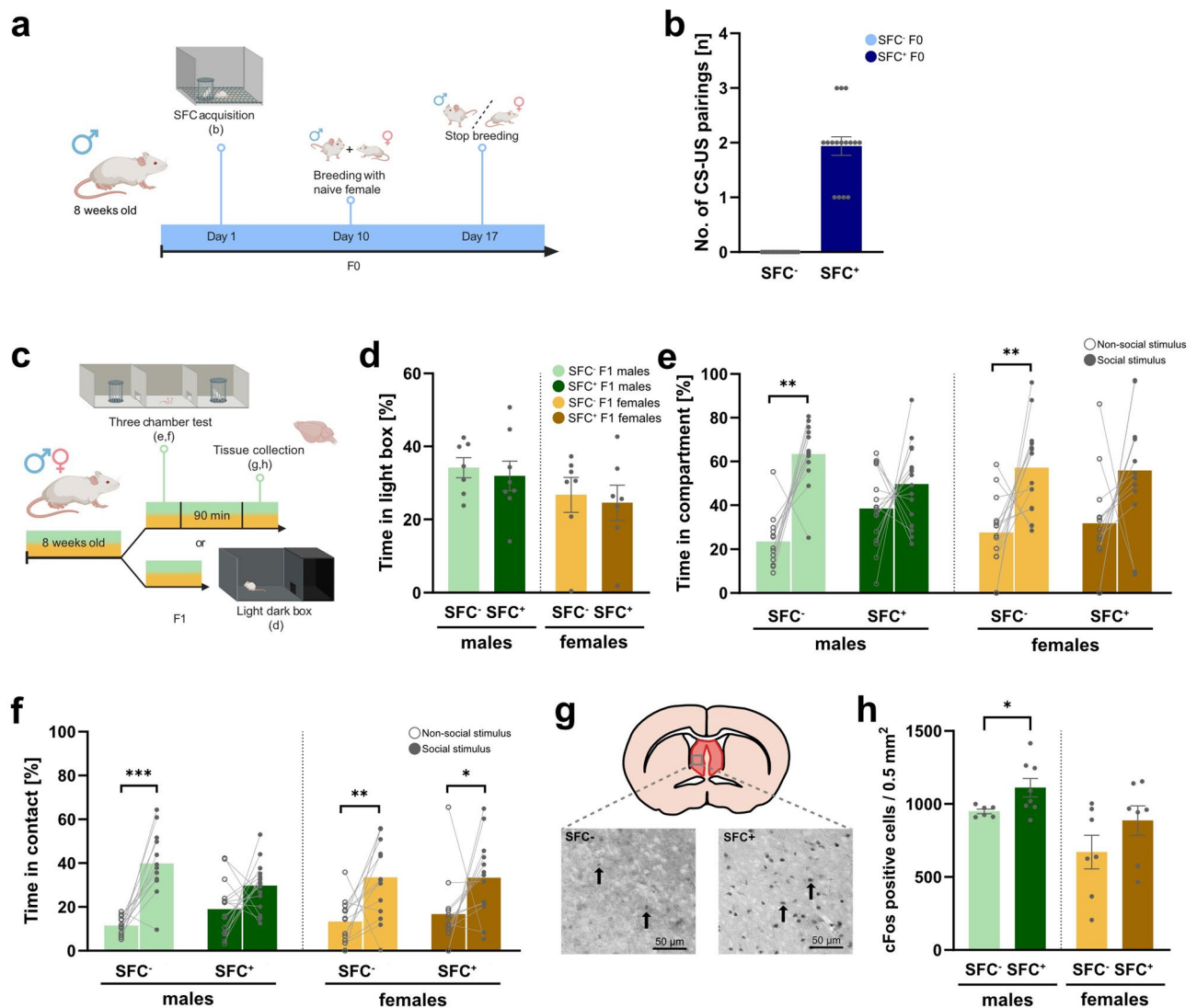


Fig. 3. Sex-specific behavioral and cellular effects of SFC across generations. **(a)** Experimental design for the F0 generation. Male mice underwent social fear acquisition at 8 weeks of age (day 1), were subsequently bred with naive females (day 10), and breeding was terminated on day 17. **(b)** Number of CS-US pairings delivered during SFC acquisition in unconditioned (SFC⁻) and conditioned (SFC⁺) F0 males. Sample size: 16/group. **(c)** Experimental timeline for the F1 generation. Male and female offspring were tested at 8 weeks of age using either the three-chamber test (TCT) followed by tissue collection 90 min later, or the light-dark box (LDB) test. Sample size: 7–8/group. **(d)** Time spent in the light compartment of the LDB (%) in male and female offspring of SFC⁻ and SFC⁺ fathers (F1 generation). Sample size: 6–8/group. **(e)** Time spent in compartment containing a non-social versus a social stimulus (%) in the TCT in F1 males and females. Sample size: 14–16/group (4 outliers excluded). **(f)** Time spent in contact with a non-social versus a social stimulus (%) in the TCT in F1 males and females. Sample size: 14–16/group (4 outliers excluded). **(g)** Schematic representation of the lateral septum and representative images of DAB staining (arrows indicate cFos positive cells). **(h)** Number of cFos positive cells per 0.5 mm² in male and female offspring of SFC⁻ and SFC⁺ fathers (F1 generation) 90 min after TCT. Statistics: Between-group comparisons (SFC⁻ vs. SFC⁺) were performed using the Mann-Whitney U test (d, h). Within-group comparisons were analyzed using the Wilcoxon matched-pairs signed-rank test (e, f). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

behavior during the TCT (i.e., F1 offspring of SFC⁻ mice showing the highest social preference and of SFC⁺ mice showing the lowest social preference; see Supplementary Table 1 for a complete list of individual behavior) and sacrificed 90 min following social exposure (Fig. 4f). A complete list of PCR array genes, including fold changes, p-values, and q-values, is provided in Supplementary Table 3. In the F1 generation, comparison of offspring from SFC⁺ versus SFC⁻ fathers identified 16 genes with p-values ≤ 0.08 , of which all but one (*Egr4*) were downregulated in offspring of SFC⁺ mice (Fig. 4h). Of these, 10 genes reached statistical significance ($p \leq 0.05$), and all were significantly downregulated in SFC⁺ relative to SFC⁻ F1 animals (Fig. 4k,l). Notably, when comparing the F0 and F1 generations, six genes (*Adcy8*, *Cdh2*, *Mapk1*, *Nptx2*, *Plcg1*, and *Srf*) display opposite regulation, being

upregulated in F0 SFC⁺ mice and downregulated in F1 SFC⁺ mice (Fig. 4g-i) (see Table 1). However, *Mapk1* and *Srf* only exhibited a trend ($p_{F0}(Mapk1) = 0,066$; $p_{F1}(Mapk1) = 0,069$; $p_{F0}(Srf) = 0,053$) and are therefore not displayed in the corresponding volcano plots of the distinct generations (Fig. 4g, h).

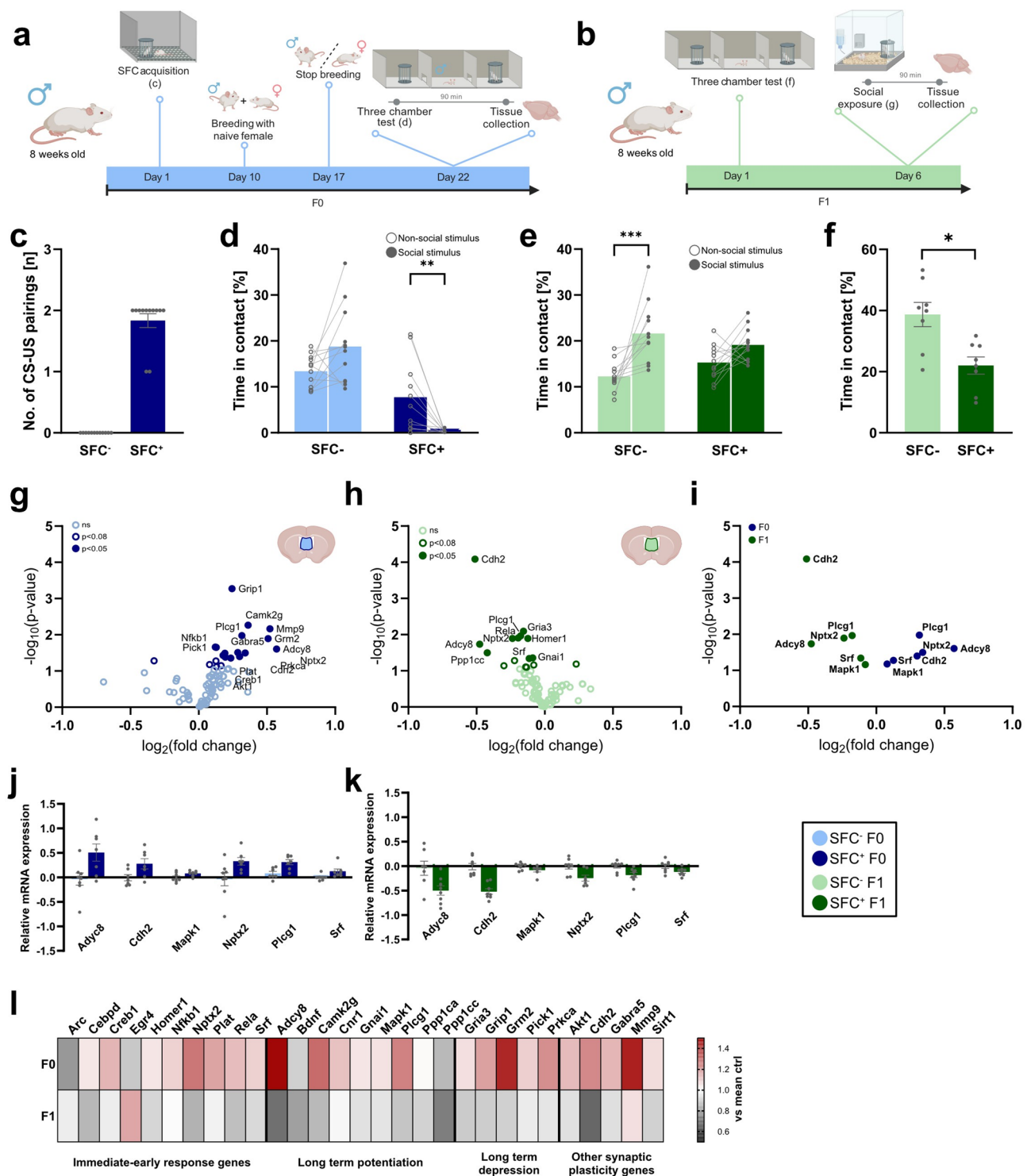
These findings highlight that SFC acquisition induces specific and persistent molecular changes that are transmitted to the next generation but manifest in an opposing regulatory direction.

Discussion

The present study demonstrates that paternal traumatic social experiences in adult male mice can influence social behavior, LS activity, and an exploratory analysis of synaptic plasticity-related gene expression in male offspring. Using SFC as an experimental tool, we first confirmed that it induces robust social avoidance in F0 males without altering general anxiety-related behavior. We then showed that male, but not female, F1 offspring of SFC⁺ fathers exhibit reduced sociability concomitant with enhanced LS activity. From a strictly behavioral perspective, the TCT used in the F1 generation primarily captures reduced social approach and social contact, rather than directly quantifying “social fear” in the offspring. Alternative interpretations such as altered social motivation, diminished social investigation, or heightened social vigilance therefore remain plausible and are consistent with the observed reduction in sociability. In light of these considerations, we interpret our findings as transmission of a vulnerability to social avoidance or social behavior deficits following paternal social fear conditioning, rather than direct inheritance of a discrete social fear memory. At the molecular level, we identified a bidirectional transcription signature: septal synaptic plasticity-related genes were upregulated in F0 males post-SFC but downregulated in male F1 offspring, with six genes (*Adcy8*, *Cdh2*, *Mapk1*, *Nptx2*, *Plcg1*, and *Srf*) showing opposite regulation across generations. Together, these findings establish SFC as a model for studying the intergenerational transmission of paternal trauma, demonstrating that discrete behavioral experience, rather than generalized stress exposure, can shape offspring brain function in a domain-selective manner. The results further implicate the septum, especially the LS, as a key neural substrate linking inherited molecular changes to altered social behavior in male offspring. Importantly, most paternal stress models rely on chronic or multimodal stressors that induce broad physiological and behavioral alterations. It has remained unclear whether a discrete, behaviorally defined experience, such as socially aversive learning, can be biologically transmitted across generations. By isolating a specific social fear memory, the present study addresses this conceptual gap and demonstrates that adverse social experiences can shape offspring disposition.

A central strength of this study is the validation of the SFC paradigm as a model for social trauma. Consistent with previous reports^{20,21}, SFC induced robust social avoidance without affecting general anxiety-related behavior (Fig. 2c, d). In contrast to chronic stress or early-life adversity paradigms, which often produce broad changes in anxiety-related behavior³⁸, stress responsivity³⁹, and metabolic parameters⁴⁰, SFC exhibits domain specificity. This distinction is critical as it permits interpretation of its intergenerational effect within a defined behavioral domain rather than confounding it with generalized stress phenotypes. By inducing social avoidance, a core feature of social anxiety disorder and other trauma-related disorders, such as post-traumatic stress disorder (PTSD) or major depression⁴¹, and excluding generalized anxiety alterations, the present findings strengthen the conclusion that the transmitted phenotype reflects specific encoding of socially aversive experience. Intergenerational transmission of behavior can occur through both germline-dependent biological mechanisms and social or parental behavioral pathways^{42–44}. The present study was structured specifically to allow for germline-mediated mechanisms of inheritance and minimize the possibility of behavioral transmission of the social-avoidance phenotype. Fathers were separated from dams at gestation day 7, eliminating postnatal paternal care and behavioral transmission. However, while this design eliminates postnatal paternal interactions, it cannot fully exclude the possibility that maternal behavior or dam-mediated effects contributed to the observed offspring phenotypes. Dams were housed and bred under standard conditions with sires from different experimental groups, and subtle differences in maternal care or prenatal environment may therefore have influenced offspring outcomes. Thus, although the design strongly supports a paternal origin of the observed effects, maternal behavioral transmission cannot be entirely ruled out. Nonetheless, early paternal separation and the absence of father–offspring interaction substantially reduce the likelihood of direct behavioral transmission, thereby strengthening the inference of a primarily germline-mediated mechanism. Similarly, single-housing of dams prior to parturition itself can alter maternal stress physiology, maternal care behavior, and offspring neurodevelopment. Thus, we cannot fully exclude the possibility that maternal isolation contributed to the observed F1 phenotypes. However, because dams were assigned to SFC[−] or SFC⁺ sires in a balanced manner, any effects of maternal single housing would be distributed equally across experimental groups and are unlikely to account for the specific group differences reported here. Nevertheless, future studies using group-housed dams or cross-fostering designs could help dissociate maternal isolation effects from paternal germline transmission.

Importantly, social trauma differs from other forms of stress in arising from interactions with conspecifics and primarily engaging neural circuits specialized for social information processing, including components of the social brain network⁴⁵ and mesolimbic reward pathways⁴⁶, whereas non-social traumatic learning is more commonly associated with classical threat processing circuits⁴⁷. Therefore, future studies directly comparing social versus non-social fear conditioning would be valuable to determine whether the observed inheritance pattern is unique to socially aversive learning or reflects a broader principle of fear-memory transmission. Notably, the intergenerational effect was predominantly observed in male offspring (Fig. 3f). Sex-specific transmission has been reported in several paternal stress models (for review, see⁴⁸), suggesting that inherited vulnerability interacts with sex-dependent developmental trajectories. However, because the estrous stage was not monitored in female offspring, cycle-dependent variation in fear learning or fear expression may have influenced the apparent resilience of females, as previously shown in studies demonstrating estrous stage-dependent effects on fear learning and expression^{49,50}.



However, several non-mutually exclusive mechanisms may contribute to this male-specific phenotype. First, sex chromosomes and early embryonic gene expression programs differ fundamentally between male and female embryos, which can influence how inherited epigenetic information is interpreted^{51,52}. For example, paternal germline signals such as sperm-derived small RNAs or DNA methylation changes may interact with sex-specific transcriptional networks or X-linked gene dosage, leading to differential downstream effects in male versus female offspring^{53,54}. Second, sex-dependent epigenetic reprogramming during early development may differentially buffer or amplify inherited signals, resulting in divergent transcriptional outcomes across sexes⁵⁵. Third, hormonal influences during critical developmental windows, particularly the perinatal testosterone surge in males, may shape circuit maturation in a manner that renders male offspring more susceptible to inherited perturbations⁵⁶.

At a circuit level, several brain regions, including the LS, exhibit sexual dimorphism that could underlie the observed sex differences in behavior. Recent evidence demonstrates sex differences in astrocyte density,

◀**Fig. 4.** SFC induces persistent and intergenerational alterations in social behavior and gene expression in the septum. **(a)** Experimental design for the F0 generation. Male mice underwent social fear acquisition at 8 weeks of age (day 1), were subsequently bred with naïve females (day 10), and breeding was terminated on day 17. All F0 males were tested in the three-chamber test (TCT) on day 22 with subsequent termination 90 min later. **(b)** Experimental timeline for the F1 generation. Male offspring were tested at 8 weeks of age in the TCT (day 1), followed by social exposure and tissue collection 90 min later (day 6). **(c)** Number of CS-US pairings during SFC acquisition in unconditioned (SFC⁻) and conditioned (SFC⁺) F0 males. Sample size: 12/group. **(d)** Time spent in contact with a non-social and social stimulus (%) in the TCT in F0 SFC⁻ and SFC⁺ males. Sample size: 11–12/group (1 outlier excluded). **(e)** Time spent in contact with a non-social and social stimulus (%) in the TCT in F1 offspring from SFC⁻ and SFC⁺ fathers. One data point represents the average of all male offspring of one father. The number of litters and number of male offspring per father are provided in Supplementary Table 1. Sample size: 12/group. **(f)** Time spent in contact with a social stimulus (%) during the social exposure in F1 offspring from SFC⁻ and SFC⁺ fathers. One data point represents the individual animals later used in the molecular analysis. Sample size: 8/group. **(g)** Volcano plot showing differentially expressed genes in the septum following TCT in F0 mice. The x-axis represents log₂ fold change, and the y-axis represents log₁₀(p-value); genes with $p \leq 0.05$ are highlighted. Sample size: 8/group. **(h)** Volcano plot showing differentially expressed genes in the septum following social exposure in F1 offspring. Sample size: 8/group. **(i)** Comparison of differentially expressed genes in the septum of F0 and F1, where all genes are upregulated in F0 and downregulated in F1. Mapk1 and Srf did not meet the predefined threshold for differential expression in all individual comparisons ($p_{F0}(\text{Mapk1}) = 0.066$; $p_{F1}(\text{Mapk1}) = 0.069$; $p_{F0}(\text{Srf}) = 0.053$) and are therefore not displayed in all volcano plots (see Supplementary Tables 2 and 3). **(j)** Quantitative validation of differentially expressed gene expression in F0 males. Log₂ transformed relative mRNA expression levels of Adcy8, Cdh2, Mapk1, Nptx2, Plcg1 and Srf in the septum are shown. Sample size: 8/group. **(k)** Log₂ transformed relative differential gene expression in F1 males of the 6 genes in the septum shows an increase compared to SFC⁻ F1 control. Sample size: 8/group. **(l)** Heatmap showing relative mRNA expression levels of genes in the septum that were altered in F0 and/or F1. Gene expression values are shown relative to the respective SFC⁻ control group within each generation. Red indicates increased expression, black indicates decreased expression. Data are represented as mean change versus mean control of each gene. The heatmap was created in GraphPad Prism version 10.6.1 for Windows, GraphPad Software, Boston, Massachusetts, USA, www.graphpad.com. Statistics: Within-group comparisons were analyzed using the Wilcoxon matched-pairs signed-rank test (d, e). Between-group comparisons (SFC⁻ vs. SFC⁺) were performed using the Mann-Whitney U test (f). Gene analysis was done using a student's t-test (g, h). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Gene	Full name	NCBI gene ID	Functional category
<i>Adcy8</i>	Adenylate cyclase 8	11514	Long-term potentiation
<i>Cdh2</i>	Cadherin 2	12558	Long-term potentiation
<i>Mapk1</i>	Mitogen-activated protein kinase 1	26413	Long-term depression
<i>Nptx2</i>	Neuronal pentraxin 2	53324	Immediate-early response genes
<i>Plcg1</i>	Phospholipase C, gamma 1	18803	Long-term potentiation
<i>Srf</i>	Serum response factor	20807	Immediate-early response genes

Table 1. Overview of synaptic plasticity-related genes exhibiting opposing regulation across generations. The table includes the gene symbol, full gene name, NCBI gene ID, and functional category based on established roles in synaptic plasticity and activity-dependent transcription. Functional categories are based on the gene groupings provided by the manufacturer of the PCR Array (Qiagen RT² Profiler™ PCR Array PAMM-126Z).

morphology, and oxytocin-receptor expression within the caudal lateral septum, as well as sex-dependent astrocytic responses following SFC⁵⁷. This anatomical and cellular dimorphism provides a plausible substrate for male-specific vulnerability, suggesting that inherited molecular signals may interact with pre-existing sex-dependent circuit architecture. In this framework, paternal experience-dependent signals may bias the developmental tuning of septal circuits, but their functional consequences become manifest only within a permissive, sex-specific neurobiological context. Such sex specificity is notable given well-documented sex differences in trauma-related psychiatric disorders in humans⁵⁸, suggesting that inherited vulnerability may be filtered through sex-dependent circuit architecture⁵⁹, sex-dependent epigenetic programming during embryogenesis⁶⁰ and hormonal programming⁶¹. From an evolutionary perspective, the intergenerational transmission of socially aversive learning may represent an adaptive mechanism that prepares offspring for anticipated adverse environments. However, in modern contexts, such recalibration may manifest as vulnerability to social withdrawal and psychopathology. While the present data do not allow a direct mechanistic dissection, these considerations allow us to speculate that the male-specific phenotype likely emerges from an interaction between inherited germline signals and sex-dependent developmental programs at molecular, cellular, and circuit levels. Future studies combining germline profiling with sex-specific developmental and circuit analyses will be required to disentangle these mechanisms and ascertain if our behavioral observations represent an adaptive response or are simply a non-adaptive evolutionary byproduct.

A key finding of this study is that altered social behavior in male F1 offspring is accompanied by an increased LS activity in response to stress, indicated by an increase in cFos positive neurons 90 min after behavioral testing (Fig. 3h). The LS is critically involved in the regulation of social behavior⁶², stress integration⁶³ and emotional processing⁶⁴. Importantly, LS activity has been directly implicated in SFC extinction, suggesting that the LS might form a central node for adaptive processing of socially aversive information^{21,25,57,62,65}. The convergence of behavioral, activity, and molecular changes supports circuit-level reprogramming, suggesting inherited molecular alterations bias LS activity and alter social information processing in F1 offspring. However, LS hyperactivity does not necessarily imply maladaptive dysfunction. It may reflect compensatory engagement or heightened vigilance in social contexts. However, causal manipulation of LS activity in F1 offspring will be essential to determine whether septal hyperactivity drives, counteracts, or merely correlates with the behavioral phenotype.

While the present study focused on the LS as a primary node associated with the behavioral phenotype, social stress processing is mediated by a broader distributed network. Key components include the amygdala and the ventral hippocampus, both of which are critically involved in emotional learning and social threat evaluation^{66,67}. Previous studies demonstrate that single traumatic experiences, such as predator stress or cued fear conditioning, can induce long-lasting activity and plasticity changes within these regions and their downstream projections^{68–70}. Given the extensive reciprocal connectivity among the LS, the amygdala, and the ventral hippocampus, as well as the known involvement of their circuitry in social novelty^{32,71,72}, it is plausible that paternal socially aversive learning induces distributed circuit-level adaptations rather than septum-restricted changes. In this framework, inherited molecular signals may bias the developmental tuning of the broader social stress circuitry, altering how social cues are evaluated and integrated across limbic networks in the offspring. Future studies combining circuit-level activity mapping and causal manipulations across these interconnected regions will be necessary to determine how intergenerationally transmitted signals reshape network dynamics underlying social behavior.

At the molecular level, we observed a bidirectional regulation of genes involved in synaptic plasticity. In SFC⁺ F0 males, several genes involved in activity-dependent signaling were upregulated (Fig. 4g), consistent with experience-driven plasticity following socially aversive learning⁷³. In contrast, F1 offspring of SFC⁺ fathers exhibited overall downregulation of these pathways (Fig. 4h). Six genes (*Adcy8*, *Cdh2*, *Mapk1*, *Nptx2*, *Plcg1*, and *Srf*) were regulated in opposite directions across generations (Fig. 4i). These genes converge on intracellular signaling cascades essential for synaptic plasticity, including cAMP signaling⁷⁴, ERK/MAPK pathways⁷⁵, calcium-dependent signaling^{74,76}, synaptic adhesion⁷⁷, and activity-dependent transcription⁷⁸. Importantly, this comparison is not fully matched across experimental contexts. F0 males were sacrificed following the TCT, whereas F1 males were exposed to a standardized social stimulus prior to tissue collection to capture a defined post-social interaction state. This design was necessary because the relevant behavioral state in F1 animals could not be determined a priori without first exposing them to a social context. Consequently, the observed transcriptional differences may reflect both generational effects and differences in immediate social experience rather than strictly bidirectional changes between F0 and F1 conditions. Having said that, the opposing directionality across generations is particularly noteworthy. The LS is predominantly GABAergic²³, and the observed upregulation of plasticity-associated genes in F0 SFC⁺ males likely reflects activity-dependent strengthening of inhibitory transmission rather than classical excitatory LTP. However, the reduced expression of these genes in the F1 SFC⁺ males suggests that these mice cannot appropriately downregulate their inhibitory output in response to safe social cues. This maladaptive transcriptional profile likely contributes to the persistence of social avoidance in F1 offspring, distinguishing it from the acute changes in plasticity observed in F0 fathers. Thus, rather than simply replicating the paternal molecular state, the offspring exhibit an inverted transcriptional profile, suggesting that inherited information may trigger maladaptive developmental responses. Taken together, this suggests that intergenerational transmission involves active developmental recalibration. Such recalibration may arise through dysregulated compensatory homeostatic mechanisms adjusting synaptic excitability in response to inherited germline cues^{79,80}.

Alternatively, epigenetic modifications in sperm may bias early embryonic transcription toward repression of pathways activated in the paternal brain⁸¹. However, these mechanisms are not mutually exclusive. Mechanistically, several studies implicate sperm small RNAs and epigenetic modifications in paternal transmission of stress effects. Alterations in sperm microRNA content can reprogram offspring stress axis regulation⁶, and sperm microRNA injection experiments demonstrate causal behavioral transmission⁴⁰. Beyond small RNAs, stress exposure can also induce DNA methylation changes⁸², histone modifications⁶⁴, and chromatin accessibility⁸³, some of which may escape fertilization reprogramming⁸⁴. These inherited marks could influence zygotic gene regulation during early embryogenesis or interact with activity-dependent circuit maturation during postnatal development and puberty^{85,86}. Defining the developmental stage at which septal circuitry diverges will be essential for mechanistic clarification. Although we did not directly assess germline epigenetic alterations, the fact that F1 offspring exhibit transcriptional changes in the absence of direct social experience is consistent with the broader literature showing that paternal stress can be transmitted via epigenetic mechanisms such as sperm small RNAs or DNA methylation^{53,87}. Thus, our findings align with, but do not directly demonstrate, an epigenetic mode of inheritance. Conceptually, the present paradigm extends the framework introduced by Dias and Ressler⁴³, who demonstrated transgenerational inheritance of odor fear conditioning. By showing domain-selective transmission of socially aversive learning following a single conditioning session, our findings broaden the scope of experience-dependent inheritance beyond sensory fear paradigms. Taken together, the absence of direct germline epigenetic analysis means that the transmission mechanism remains speculative, and non-epigenetic mechanisms, such as indirect maternal effects, cannot be excluded.

The present study has several limitations. First, the LS activity was assessed correlatively. Causal manipulations will be required to determine whether LS hyperactivity drives the behavioral phenotype. Second, the

exploratory gene expression analyses were limited to selected candidate genes and did not capture genome-wide transcriptional changes. Third, germline epigenetic modifications were not directly examined, and therefore, the transmission mechanism remains speculative.

In summary, we demonstrate that paternal social trauma can be transmitted to offspring in a sex-dependent manner, coinciding with altered septal activity and bidirectional regulation of synaptic plasticity-related genes. The reversal of transcriptional signatures across generations suggests active developmental recalibration rather than passive inheritance. By leveraging SFC as a targeted behavioral model and eliminating father-offspring interaction, this study provides a refined framework for investigating the biological transmission of paternal trauma and identifies candidate molecular and circuit substrates for future mechanistic investigation.

Data availability

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

Received: 26 March 2026; Accepted: 9 September 2026

Published online: 21 September 2026

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Acknowledgements

We want to thank Martina Fuchs and Thomas Stepper for their excellent assistance.

Author contributions

Project conception: LS, AB, RM; Methodology: LS, AB, VR, RM; anatomic analysis: LS, RM; Molecular analysis: AB; Behavior analysis: LS, VR, RM; Writing: LS, VR, RM; Illustrations: LS; Project administration and supervision: VR, RM.

Funding

Open Access funding enabled and organized by Projekt DEAL. This work was supported by the German Research Foundation (DFG) grant (ME 5731/2 to RM) and the DFG Graduate School (GRK-2174 to RM, VR).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-026-71393-6>.

Correspondence and requests for materials should be addressed to R.M.

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