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Innate anxiety in rats determines vulnerability to social fear conditioning and social fear memory

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ABSTRACT

Social anxiety disorder (SAD) involves excessive fear of social situations and can be induced by social trauma. However, the underlying mechanisms of individual differences in the formation of social fear memory remain unclear. To address this gap, we adapted our mouse social fear conditioning paradigm for rats (rSFC) and compared adult male rats selectively bred for high (HAB) or low (LAB) anxiety-related behaviour and non-selected controls (NAB). HAB and LAB rats are established as a model displaying genetically determined extremes in socio-emotional behaviours. Conditioned HAB, LAB, and NAB rats all developed social fear, but with line-specific persistence. During a social fear discrimination test, HAB and LAB rats expressed social fear 24 h after acquisition, while NAB rats only showed transient social fear (up to 6 h), indicating reduced susceptibility to social trauma. Importantly, only HAB rats showed individual social fear memory, with reduced social investigation of the familiar rat encountered during social fear acquisition (**"known**") versus a novel (**"unknown"**) rat. Blockade of brain V1a receptors after social fear acquisition slightly reduced generalised social fear in HAB rats and abolished their individual social fear memory, whereas central infusion of arginine vasopressin (AVP) modestly reduced generalised social fear in LAB rats. Additionally, rSFC induced a rapid corticosterone response, which was more pronounced in NAB than HAB rats. Blocking glucocorticoid synthesis before social fear acquisition slightly reduced generalised social fear in HAB rats. These findings demonstrate that innate anxiety determines the long-term formation of individual social fear memory, as assessed after a 24-h retention interval, in adult male rats and support rSFC as a useful experimental model to investigate selected neurobiological mechanisms relevant to SAD.

1. Introduction

Social anxiety disorder (SAD) is characterised by persistent and excessive fear and avoidance of social situations (American Psychiatric Association, 2022). With a global prevalence of 17% in young adults, SAD imposes a substantial socio-economic burden (Salari et al., 2024). SAD patients experience significant psychosocial impairments, including reduced productivity, increased academic dropout, and higher suicide risk (Aderka et al., 2012; Ernst et al., 2024; Leichsenring and Leweke, 2017). Over one-third of affected individuals have comorbid psychiatric disorders, including general anxiety disorder (Koyuncu et al., 2022). Current treatments are largely unspecific, often providing only partial remission with high relapse rates (Blanco et al., 2013; Chowdhury and Khandoker, 2023; Stein, 2006; van Dis et al., 2020), highlighting the need for a better understanding of SAD aetiology.

Both genetic and environmental factors contribute to the aetiology of SAD. Heritability estimates range from 15% to 50% (Bas-Hoogendam, 2025; Spence and Rapee, 2016; Stein et al., 2017). Behavioural inhibition (a temperament characterised in early childhood by distress to

Abbreviations: AVP, arginine vasopressin; CFC, cued fear conditioning; Cort, corticosterone; EPM, elevated plus-maze; GC, glucocorticoids; HAB, high anxiety-related behaviour; HPA, hypothalamic-pituitary-adrenal; ICV, intracerebroventricular; IP, intraperitoneal; LAB, low anxiety-related behaviour; NAB, non-selected anxiety-related behaviour; rSFC, rat social fear conditioning; SAD, social anxiety disorder; SFC, social fear conditioning; SFC^{+}, social fear conditioned; SFC^{-}, social fear unconditioned; SFD, social fear discrimination; Veh, vehicle; V1aR-A, V1a receptor antagonist.

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novelty and avoidance of unfamiliar people) has been identified as the strongest temperamental predictor of SAD, with up to 50% of children with behavioural inhibition developing SAD later in life (Clauss and Blackford, 2012; Fox et al., 2021). At the same time, adverse social experiences play a critical role, as more than half of SAD patients report a severe or traumatic social event coinciding with symptom onset (Li et al., 2026; Liu et al., 2025).

From a preclinical perspective, the social fear conditioning (SFC) paradigm in mice has been used to model key features of SAD (Toth et al., 2013, 2012). In this paradigm, a single traumatic social event during social fear acquisition induces generalised social fear that can persist for up to 15 days. During acquisition, social fear conditioned mice (SFC^+) receive punishment upon direct social contact, leading to robust avoidance of same-sex conspecifics, whereas unconditioned (SFC^-) mice are allowed to freely explore the social stimulus. During extinction, repeated exposure to unfamiliar conspecifics gradually reverses social avoidance (Masis-Calvo et al., 2018; Toth et al., 2013). Using this paradigm, we have demonstrated that social fear extinction is facilitated by the neuropeptide oxytocin (Menon et al., 2018; Zoicas et al., 2014) and regulated by non-coding RNAs, including microRNAs and long non-coding RNAs (Bludau et al., 2024; Royer et al., 2022), primarily via mechanisms involving the lateral septum (Menon et al., 2022, 2018).

To examine how genetically determined anxiety-related behaviour influences social fear learning and memory, we adapted the social fear conditioning paradigm for laboratory rats (rSFC). This allowed the use of rats selectively bred for high (HAB) and low (LAB) anxiety-related behaviour, with non-selected (NAB) rats serving as controls (Gryksa et al., 2023).

HAB and LAB rats are a well-established genetic model of extremes in socio-emotional behaviours and, thus, of anxiety- and depression-related disorders (Gryksa et al., 2023; Landgraf and Wigger, 2002). In both males and females, their innate hyper-anxiety is accompanied by a distinct emotional and physiological phenotype including passive stress-coping, anhedonia, and heightened stress susceptibility with exaggerated corticosterone (Cort) responses (Frank et al., 2006; Landgraf et al., 1999). In humans, emotional states strongly influence cognitive functions under healthy and psychopathological conditions, with depression commonly associated with cognitive and memory impairments (Drevets, 2001; Tyng et al., 2017). Consistent with this interaction, adult male HAB rats display enhanced declarative and social memory, outperforming LAB rats in spatial learning and social discrimination abilities (Frank and Landgraf, 2008; Gryksa et al., 2023; Landgraf and Wigger, 2002; Ohl et al., 2001, 2002). Importantly, profound line differences were also found in fear regulation with adult male and female HAB rats showing impaired cued fear extinction (Muigg et al., 2008; Sartori et al., 2011; Slattery et al., 2015). Together, these findings suggest that emotional traits critically shape cognitive processes, including social fear-related memory (McGaugh et al., 1996; Roozendaal et al., 2008; Timmer and Sandi, 2010).

The high anxiety phenotype of male and female HAB rats is associated with elevated hypothalamic expression of arginine vasopressin (AVP), driven by a single nucleotide polymorphism (SNP) in the *Avp* promoter (Gryksa et al., 2023; Murgatroyd et al., 2004). AVP is a key player promoting anxiety, learning, memory, and social discrimination abilities (Engelmann and Landgraf, 1994; Lukas and Neumann, 2013). Consistent with this, enhanced AVP signalling has been shown to contribute to the high trait anxiety and superior social discrimination abilities of male HAB rats (Wigger et al., 2004). Glucocorticoids (GC) also play a critical role in social fear memory and emotional learning (Timmer and Sandi, 2010; Weger et al., 2018), and have been linked to social avoidance in SAD patients (Roelofs et al., 2009). Accordingly, the exaggerated corticosterone (Cort) stress response of HAB rats (Landgraf et al., 1999) may further contribute to their enhanced spatial and social memory under laboratory conditions.

Using the adapted SFC protocol for rats (rSFC), we hypothesised that (i) adult male HAB, LAB and NAB rats differ in their social fear memory and social fear responses at different time points (2 h until 24 h) after social fear conditioning, and (ii) brain AVP signaling and GC differentially contribute to the line-specific social fear memory in SFC^+ HAB, LAB and NAB rats. Thus, HAB and LAB rat lines are not only valuable for studying trait anxiety, but also for investigating how innate anxiety shapes long-term social fear memory, a key feature of human SAD.

2. Materials and methods

2.1. Animals and husbandry

Adult male HAB and LAB Wistar rats (250–500 g) were bred in the animal facility at the University of Regensburg, Germany, and selected based on their performance in the elevated plus-maze (EPM) test at 9 weeks of age (for a detailed description see Gryksa et al., 2023). NAB rats were bred in parallel without behavioural selection. Age- and sex-matched stimulus Wistar rats were obtained from Charles River (Sulzfeld, Germany). Rats were group-housed in standard rat cages until three days before the experiments started, after which they were single-housed in observation cages (40 × 36 × 24 cm). Animals were maintained on a 12-h light/dark cycle (lights on at 7:00 AM) at 22 ± 2 °C with 55 ± 5% humidity with food and water ad libitum. All experimental procedures were approved by the Committee on Animal Health and Care of the local government and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Unterfranken, the ARRIVE guidelines (Percie du Sert et al., 2020), and EU Directive 2010/63 on the protection of animals used for scientific purposes.

2.2. Experimental design

2.2.1. Behavioural characterisation of HAB, LAB and NAB rats

To validate their trait anxiety profiles, HAB and LAB rats were tested on the EPM at 9 weeks of age (see below). Three weeks later, social motivation was assessed using the social preference test (see below). One week thereafter, HAB, LAB and unselected NAB were subjected to the modified rSFC paradigm and subsequently assigned to SFC^+ or SFC^- groups. Both generalised and individual social fear memory were assessed in the social fear discrimination (SFD) test after a 24-h retrieval interval (see below). Afterwards, HAB, LAB and NAB rats were tested for fear learning in a non-social context using the cued fear conditioning (CFC) paradigm (see below). Pain sensitivity was assessed in HAB, LAB, and NAB rats using the Hargreaves' plantar test (see below). In addition, an independent experiment in NAB rats examined social fear acquisition and memory at shorter retrieval intervals (2, 4, or 6 h).

2.2.2. Role of AVP in long-term social fear memory (24-h retention)

HAB rats carry a SNP in the promoter region of the *Avp* gene, resulting in hypothalamic AVP overexpression (Gryksa et al., 2023; Murgatroyd et al., 2004). Given the established role of AVP in social recognition and discrimination, enhanced AVP signalling may contribute to the long-term social discrimination, assessed after a 24-h retention interval, observed in SFC^+ HAB rats. To test this, HAB, LAB, and NAB rats were stereotaxically implanted with guide cannulae targeting the lateral ventricle (see below). After a 3-day recovery period with daily handling, all animals underwent social fear acquisition followed by intracerebroventricular (ICV) infusion of either synthetic AVP (1 ng/5 µl Ringer; NAB and LAB rats), a selective V1a receptor antagonist (V1aR-A; 0.75 µg/5 µl Ringer; HAB rats), or vehicle (Veh; Ringer's solution; in all lines) (see below). Twenty-four hours later, the animals were tested in the SFD test.

2.2.3. Effects of GC on social fear consolidation

To monitor plasma Cort levels across different SFC phases, HAB/SFC^+, HAB/SFC^-, NAB/SFC^+ and NAB/SFC^- rats were implanted with a

jugular vein catheter (see below). In this experiment, LAB rats could not be included due to breeding limitations. After a recovery period of 5 days, a basal blood sample was collected 1 h after attaching the collection line to the rat's catheter followed by repeated blood sampling during social fear acquisition, i.e., at 5, 15 and 60 min following first shock exposure in the SFC$^+$ groups or following the first social approach in the SFC$^-$ groups. SFD was evaluated 6 h after acquisition, and 3 more blood samples were collected 1 h before, and 5 and 15 min after the presentation of the social stimuli.

In addition, given the elevated basal plasma Cort levels observed in HAB rats prior to social fear acquisition, we investigated the involvement of Cort in social fear memory in HAB/SFC$^+$. To this end, rats received an intraperitoneal (IP) injection of either the Cort synthesis inhibitor metyrapone (2-methyl−1,2-di−3-pyridyl−1-propanone; 50 mg/kg; Sigma-Aldrich Chemie GmbH), prepared in a solution of 60% saline and 40% propylene glycol, or Veh 90 min prior to social fear acquisition. The dose was selected based on previous studies (Roozendaal et al., 1996). Twenty-four hours later, animals were tested in the SFD task to assess the effects of Cort synthesis inhibition on generalised social fear and individual social fear memory.

2.3. Behavioural tests

All tests were conducted during the light phase between 08:00 and 15:00. Behaviours were manually scored using JWatcher version 1.0 (available from http://www.jwatcher.ucla.edu), the distance traveled was automatically tracked using Noldus EthoVision (Noldus Information Technology, Germany). Each used behavioural apparatus was cleaned with soapy water between subjects to prevent olfactory cues.

2.3.1. Elevated plus-maze (EPM)

The EPM test was used to measure anxiety-related behaviour (Pellow et al., 1985). The apparatus consisted of a plus-shaped platform with two open arms (50 × 10 cm, with a 0.5 cm rim, 40 lux) and two closed arms (50 × 10 × 40 cm, 5–10 lux), connected by a central zone (100 cm^2) and elevated 80 cm above the ground. At the start of the test, the animal was placed in the central zone facing a closed arm and allowed to explore the apparatus for 5 min. Based on the percentage time spent on the open arms (% open arm time = time in open arms / total arms time × 100), the offspring were classified as HAB (less than 10%) or LAB (more than 45%) rats (Gryksa et al., 2023; Liebsch et al., 1998).

2.3.2. Social preference test

To compare social motivation of rats, we used a modified social preference paradigm (Lukas et al., 2011). Briefly, the rat was placed in a gray arena (80 × 40 × 38 cm, 20 lux) to acclimate for 2 min, before a non-social stimulus (an empty wire-mesh cage) and a social stimulus (a same sex, weight-matched conspecific within a wire-mesh cage) were simultaneously presented at opposite sides of the arena. After 4 min of exploration, the rat was returned to its home cage. A social preference ratio was calculated as: (social exploration time – non-social exploration time) / total exploration time. A positive preference ratio (significantly greater than zero) indicated social preference.

2.3.3. Rat social fear conditioning (rSFC)

The original SFC paradigm developed in mice (Toth et al., 2012) is based on an operant avoidance conditioning protocol, which has been substantially modified for application in rats (see Fig. 1). The adapted rSFC paradigm allows to assess general social fear expression evidenced by a significant reduction in social investigation and increase in freezing behaviour toward both presented social stimuli. Moreover, the rSFC

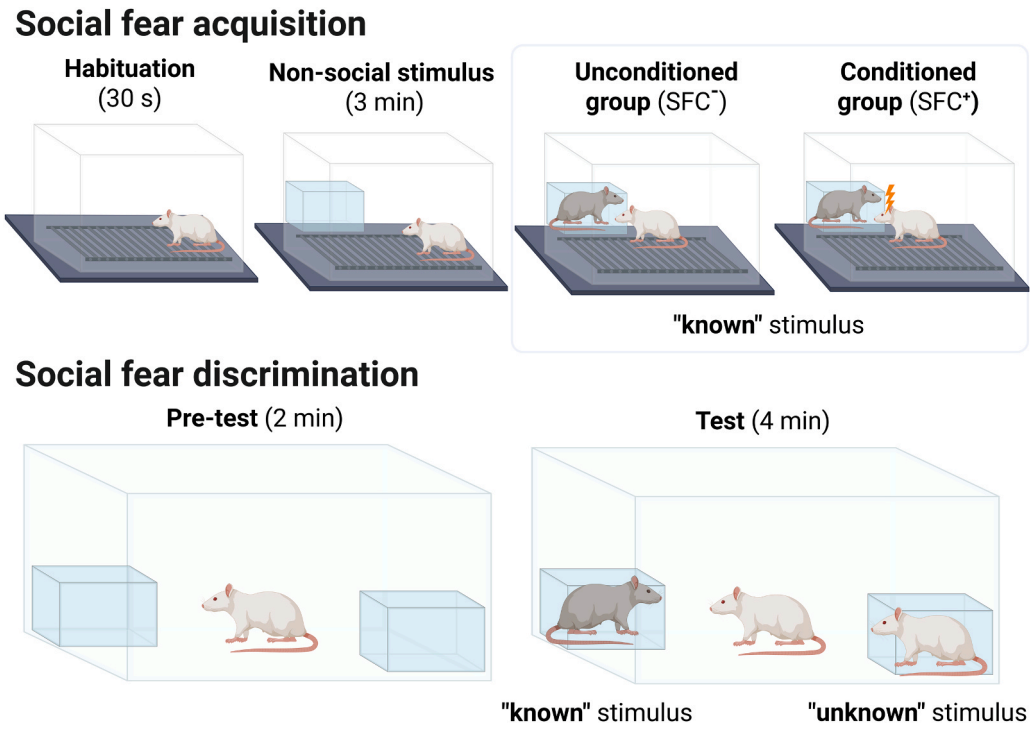

Fig. 1. Social fear conditioning protocol adapted in Wistar rats (rSFC). Social fear acquisition: Following a 30-s habituation in the conditioning chamber, the rat is exposed to a non-social stimulus for 3 min, which is replaced by a social stimulus, i.e., a same-sex, same-age conspecific referred to as the **"known"** rat (grey). Rats are assigned to either unconditioned (SFC$^-$) or conditioned (SFC$^+$) groups: whereas SFC$^-$ rats can freely explore the social stimulus, SFC$^+$ rats receive a mild footshock (1 mA, 2 s) each time they approach and investigate the social stimulus. Rats are then returned to their home cage for a retention interval of 2–24 h, depending on the experiment. Social fear discrimination: Following a 4-min habituation in the arena (pre-test), the empty cages are replaced with two cages, containing either the **"known"** (grey) or an **"unknown"** (white) same-sex, weight-matched conspecific. The time spent investigating the "**known"** and "**unknown"** rats is recorded over 4 min. Created with BioRender.com.

protocol enables the evaluation of individual social fear memory, i.e., the ability to differentiate between the previously encountered **"known"** conspecific and a novel, **"unknown"** conspecific, after defined retrieval intervals between 2 h and 24 h. The 24-h retention interval was used as it is widely adopted in social recognition studies as the operational benchmark for long-term memory (Cum et al., 2024; Pirri et al., 2024; Popik et al., 1991; Rudy and Morledge, 1994).

Social fear acquisition (day 1): One day before social fear acquisition, rats were allowed to habituate to the conditioning chamber (transparent plastic, 45 cm × 45 cm × 22 cm, with a steel grid floor) (TSE System GmbH, Bad Homburg, Germany), enclosed within a wooden box to minimise external disturbances, for 1 min. Prior to social fear acquisition, rats were again allowed to adapt to the chamber for 30 s (Fig. 1A), before a non-social stimulus (an empty wire-mesh cage, 20 × 10 × 9 cm) was placed in one corner of the chamber (Fig. 1B), which was replaced by a social stimulus (a same sex and weight-matched conspecific enclosed in a wire-mesh cage) after 3 min. The stimulus rat was referred to as the "**known"** rat and represents the conditioned stimulus (CS) in the Pavlovian learning paradigm. Animals were assigned to either the unconditioned group (SFC^-) and allowed to freely explore the social stimulus for 3 min (Fig. 1C) or to the conditioned group (SFC^+), which received a mild electric foot shock (unconditioned stimuli, US; 1 mA pulsed current, 2 s; TSE software under manual control) each time they approached and investigated the "**known"** stimulus (Fig. 1D). For SFC^+ rats, the acquisition session ended after 5 min, when repeated CS-US pairing occurred (i.e., social approaches paired with shocks), or 7 min after the first CS-US pairing, when no further social approaches were made. After the session, each animal was returned to its home cage.

Social fear discrimination (SFD) test (day 2): To evaluate the level of social fear discrimination abilities, we adapted the established social discrimination paradigm (Engelmann et al., 1995). During a pre-test habituation session, the SFC^+ or SFC^- rat was placed in the centre of an open arena (80 × 40 × 38 cm, opaque plastic walls and floor, 20 lux) containing two empty wire-mesh cages (20 × 10 × 9 cm) positioned at opposite sides of the arena (Fig. 1E). After 2 min, the animal was removed, and the empty cages were replaced by one cage containing the "**known"** stimulus and another one containing an "**unknown"** stimulus rat positioned into opposing corners of the arena (Fig. 1F). During the SFD test, the experimental rat was again placed in the centre of the arena and allowed to freely explore both the "**known"** and "**unknown"** stimulus for 4 min, after which it was returned to its home cage. The entire test session was videotaped. Generalised social fear expression was assessed as total social investigation time (i.e., time spent investigating both the "**known"** as well as "**unknown"** conspecifics). Freezing behaviour was defined as immobility with the animal's head oriented toward either the "**known**" or the "**unknown"** stimulus. Both measures were scored manually. In addition, individual social fear memory was quantified using a discrimination ratio calculated as: (investigation time of the "**unknown"** stimulus − investigation time of the "**known"** stimulus) divided by total investigation time. A positive discrimination ratio significantly different from zero indicates a preference for the **"unknown"** conspecific and thus reflects intact social discrimination and social fear memory.

2.3.4. Cued fear conditioning (CFC)

Cued fear conditioning (CFC) was performed as previously described (Slattery et al., 2015). The time spent freezing was automatically measured by the conditioning chamber software. Briefly, the protocol consisted of three phases:

Cued fear acquisition (day 1): The rat was placed in a conditioning chamber (45 × 45 × 40 cm) with context A (i.e., transparent walls, a grid floor, 50 lux, and lemon-scented soap). After 2 min of adaptation, it was exposed to five CS (80 dB, 8 kHz, 30 s) - US (0.7 mA pulsed current, 2 s) pairings. The CS co-terminated with the US, with a 2-min inter-stimulus interval. The animal was returned to its home cage after the final pairing.

Cued fear extinction (day 2): The rat was placed in context B (a black cage with a smooth floor, neutral scent, 10 lux). After 2 min of adaptation, it received 30 CS presentations without any US, with a 5-sec inter-stimulus interval. It was returned to its home cage after the final CS.

Cued fear recall (day 3): The rat was again placed in context B. After 5 min of adaptation, it was exposed to 5 CS presentations with a 2-min inter-stimulus interval before being returned to the home cage after the final CS.

2.3.5. Hargreaves' plantar test

The Hargreaves' plantar test was used to assess differences in pain perception between the HAB, LAB, and NAB lines (Jochum et al., 2007). Rats were habituated to a Plexiglas box with a glass floor (Ugo Basile, model 7371, Italy) for 5 min. A focused thermal heat stimulus was then applied to the plantar surface of the hind paw, and paw withdrawal latency was recorded. Each hind paw was tested three times, with a 2-min interval between trials. The average latency across all 6 trials per animal was used for analysis.

2.4. Surgical interventions

2.4.1. Stereotactic implantation of guide cannulas and drug infusions

Guide cannulas for ICV administration were implanted stereotaxically under semi-sterile conditions and isoflurane anaesthesia (Baxter Deutschland GmbH, Germany). The ICV guide cannula (21 G, 12 mm) was implanted 2 mm above the right lateral ventricle (relative to bregma: anteroposterior: −1.0 mm, mediolateral: +1.6 mm, and dorsoventral: +2 mm; Paxinos and Watson, 1998). The cannula was secured to the skull using two screws and dental cement (Kallocryl, Speiko-Dr. Speier GmbH, Münster, Germany) and sealed with a stainless-steel stylet. Following surgery, the rats received an IP injection of the analgesic Buprenovet (0.05 mg/kg Buprenorphine, Bayer, Germany). To prevent infection, a subcutaneous injection of enrofloxacin (Baytril; 10 mg/kg, Bayer, Germany) was administered. To minimise postoperative stress, rats were single-housed and handled daily. Behavioural testing commenced 5 days after surgery.

For ICV infusion, a 27 G infusion cannula was inserted into the pre-implanted guide cannula. Rats received either synthetic AVP (1 ng/5 µl Ringer), a selective AVP receptor antagonist (V1aR-A) (0.75 µg/5 µl Ringer) (provided by Prof M. Manning, Toledo, USA) (Manning et al., 2012) or Veh (sterile Ringer solution). Infusions were delivered immediately after social fear acquisition. Doses and timing were selected based on established protocols (Lukas et al., 2011; Veenema et al., 2012). Social fear discrimination was assessed 24 h after conditioning. Cannula placement was verified histologically by postmortem infusion of blue ink followed by Nissl staining. Animals with blocked ICV guide cannulas (n = 8) were excluded from statistical analyses.

2.4.2. Jugular vein catheter implantation and blood sampling

HAB and NAB rats were implanted with a jugular vein catheter as previously described (Neumann et al., 1998). After a 6-day recovery period, and 90 min prior to the first blood sampling, the catheter was connected to a sampling tubing (35-cm PE−10 plastic tubing) attached to a 1-ml disposable plastic syringe filled with heparinised saline (Heparin-Natrium 25,000, Ratiopharm, Germany). Blood samples (0.3 ml) were collected under basal conditions in the home cage prior to social fear acquisition, as well as 5, 15 and 60 min thereafter (see above) and replaced by sterile saline (0.9%; B. Braun Melsungen AG, Germany). Samples were collected into EDTA-coated Eppendorf tubes and centrifuged at 5000 rpm for 10 min at 4°C. Plasma aliquots (10 µl) were stored at −20°C until Cort concentrations were analyzed via ELISA following the manufacturer's protocol (IBL International GmbH).

2.5. Statistical analysis

All statistical analyses were performed using GraphPad Prism (v10.6.1, GraphPad Software, San Diego, USA) and JAMOVI (https://www.jamovi.org). Data were first tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using Brown–Forsythe and Bartlett's tests.

For non-normally distributed data or data failing the homogeneity test, non-parametric tests were applied. Comparisons between two groups were analyzed with the two-tailed Mann-Whitney U-test, while comparisons among more than two groups used the Kruskal–Wallis test followed by Dunn–Bonferroni post hoc analysis when appropriate. For normally distributed data with equal variances, parametric tests were applied. CS–US pairings during social fear acquisition and Hargreaves paw withdrawal latency were analyzed by one-way ANOVA or Welch's ANOVA when homogeneity of variance was violated. Cued fear conditioning data were analyzed by two-way repeated-measures ANOVA with Bonferroni-corrected multiple comparisons. Plasma Cort levels before and after rSFC were analyzed using a mixed-effects model (REML) with time as a within-subject factor and line as a between-subject factor, applying the Geisser–Greenhouse correction for violations of sphericity. For experiments assessing the role of AVP in long-term social fear memory and the effects of GC on social fear consolidation, data were analyzed using two-way ANOVA with SFC (SFC^+ vs. SFC^-) and treatment (drug vs vehicle) as between-subject factors followed by Bonferroni-corrected post hoc tests. In case, ANOVA did not detect significant SFC × treatment interactions, we performed planned separate comparisons between vehicle- and drug-treated SFC^+ groups, i.e. the primary comparison of interest, using unpaired Student's *t*-test. When necessary, data were log-transformed to meet normality assumptions before parametric testing. Preference indices in the social preference test and discrimination ratios in the SFD test were analyzed using one-sample *t*-tests against zero or the Wilcoxon signed-rank test when normality was not met.

All data are presented as means ± SEM or median with minimum/maximum values, and statistical significance was accepted at $p < 0.05$. Effect sizes are reported for all analyses: rank-biserial correlation (r_rb) for Mann–Whitney U tests, Cohen's d for *t*-tests, ε^2 for Kruskal–Wallis tests, and partial eta squared (ηp^2) for ANOVAs and mixed-effects models.

3. Results

3.1. Behavioural characterisation of HAB, LAB and NAB rats

To validate line-specific phenotypes, adult male HAB, LAB, and NAB rats underwent standardised tests of anxiety, social behaviour, fear learning, and pain sensitivity (Fig. 2).

EPM: Significant line differences were observed in the percentage of time spent in the open arms ($H(2) = 37.5$, $p < 0.001$; $\varepsilon^2 = 0.75$) (Fig. 2A). HAB rats spent significantly less time in the open arms than LAB rats ($p < 0.001$), whereas LAB rats spent significantly more time in the open arms than NAB rats ($p < 0.001$), confirming the high- and low-anxiety phenotypes of HAB and LAB rats, respectively. Significant line differences were also found in the number of entries into the closed arms ($H(2) = 25.5$, $p < 0.001$; $\varepsilon^2 = 0.51$) (Fig. 2A), with HAB rats displaying reduced locomotor activity than both NAB and LAB ($p < 0.001$).

Social preference test: No line differences were observed in social investigation time compared with the non-social stimulus ($H(2) = 3.09$, $p = 0.213$; $\varepsilon^2 = 0.08$; Fig. 2B). Although a significant line effect was found for the preference index ($H(2) = 6.35$, $p = 0.042$; $\varepsilon^2 = 0.17$), post hoc analyses revealed no significant pairwise differences (Fig. 2B). All rat lines displayed a significant preference for the social stimulus (HAB: $W = 105$, $p < 0.001$; NAB: $W = 78$, $p < 0.001$; LAB: $W = 67$, $p = 0.005$).

rSFC: During social fear acquisition, no significant line differences were found in the number of CS–US pairings required (Welch's one-way ANOVA: $F(2, 14.1) = 3.46$, $p = 0.060$). Although the effect did not reach significance, HAB rats required fewer pairings than NAB rats (Fig. 2C).

Hargreaves' plantar test: No differences were found between replicates or left/right paws (data not shown); mean paw withdrawal latencies were used. Thermal nociception did not differ between lines (Fig. 2D).

CFC: During CFC acquisition, freezing behaviour (% time) increased across CS–US pairings in all rat lines, as reflected by a main effect of time ($F(3.59, 118.3) = 26.65$, $p < 0.001$, $\eta p^2 = 0.45$) (Fig. 2E). Post hoc analyses confirmed increased freezing from trial 1 to trials 2–5 in all lines ($p < 0.05$–0.001; detailed pairwise comparisons omitted for clarity). During CFC extinction, freezing differed between rat lines, as indicated by a significant time × line interaction ($F(4.29, 49.4) = 5.540$, $p < 0.001$, $\eta p^2 = 0.33$) (Fig. 2E). LAB and NAB rats showed successful extinction, with decreased freezing levels across CS blocks, whereas HAB rats maintained high freezing, indicating impaired fear extinction. Accordingly, freezing levels were higher in HAB rats compared with LAB and NAB rats during CS blocks 7–10 ($p < 0.05$).

3.2. Social fear memory across rat lines

Generalised social fear (total social investigation time) and individual social fear memory (investigation time of the **"known"** versus the **"unknown"** conspecific) were assessed 24 h after social fear acquisition using the SFD test in HAB, LAB, and NAB rats (Fig. 3).

HAB/SFC^+ rats, compared to HAB/SFC^- rats, showed reduced total social investigation ($U = 11$, $p = 0.002$; r_rb = −0.78) and increased freezing ($U = 13.5$, $p = 0.003$; r_rb = 0.73). Notably, HAB/SFC^+ rats, but not HAB/SFC^- rats, were able to discriminate between the **"known"** and **"unknown"** rats ($W = 24$, $p = 0.047$), indicative of individual social fear memory.

NAB/SFC^+ animals showed increased freezing ($U = 10$, $p = 0.002$; r_rb = 0.75), but no reduction in total social investigation ($U = 25$, $p = 0.190$; r_rb = −0.38) compared with SFC^- rats (Fig. 3B). Also, the discrimination ratio did not differ between the 2 groups and neither NAB/SFC^+ rats nor NAB/SFC^- rats were able to discriminate between the "**known**" and "**unknown**" rats.

LAB/SFC^+ rats exhibited reduced total social investigation ($U = 0$, $p < 0.001$; r_rb = −1.00) and increased freezing ($U = 8.50$, $p = 0.001$; r_rb = 0.81) compared with LAB/SFC^- rats, but the discrimination ratios did not differ. Neither LAB/SFC^+ rats nor LAB/SFC^- rats were able to discriminate between the **"known"** and **"unknown"** rats 24 h after social fear acquisition.

As NAB (and LAB) rats failed to discriminate between the **"known"** and **"unknown"** rats when tested 24 h after social fear acquisition, the SFD test was performed at shorter retrieval intervals of 2, 4, and 6 h in NAB rats (Fig. 4).

Specifically, during social fear acquisition, NAB/SFC^+ rats received on average 2.64 ± 0.264 CS–US pairings overall. Two hours later, NAB/SFC^+ rats showed reduced total social investigation ($U = 10$, $p = 0.006$; r_rb = −0.75) and increased freezing ($U = 4.50$, $p < 0.001$; r_rb = 0.89) compared to NAB/SFC^- controls. Discrimination ratios did not differ between NAB/SFC^+ and NAB/SFC^- rats and only NAB/SFC^- rats were able to discriminate between the **"known"** and **"unknown"** rats ($W = 39$, $p = 0.020$; Fig. 4).

Four hours after social fear acquisition, reduced total social investigation ($U = 12$, $p = 0.011$; r_rb = −0.70) and increased freezing ($U = 12$, $p = 0.008$; r_rb = 0.70 was found in NAB/SFC^+ rats. There were no group differences in the discrimination ratio, and neither NAB/SFC^- nor NAB/SFC^+ rats were able to discriminate between the **"known"** and **"unknown"** rats (Fig. 4).

Also, six hours after social fear acquisition, total social investigation was reduced ($U = 19$, $p = 0.001$; r_rb = −0.82) and freezing increased ($U = 18$, $p < 0.001$; r_rb = 0.82) in NAB/SFC^+ rats, whereas discrimination ratios did not differ between the 2 groups. Only NAB/SFC^- rats retained the ability to discriminate between the **"known"** and **"unknown"** rats ($W = 60$, $p = 0.005$; (Fig. 4).

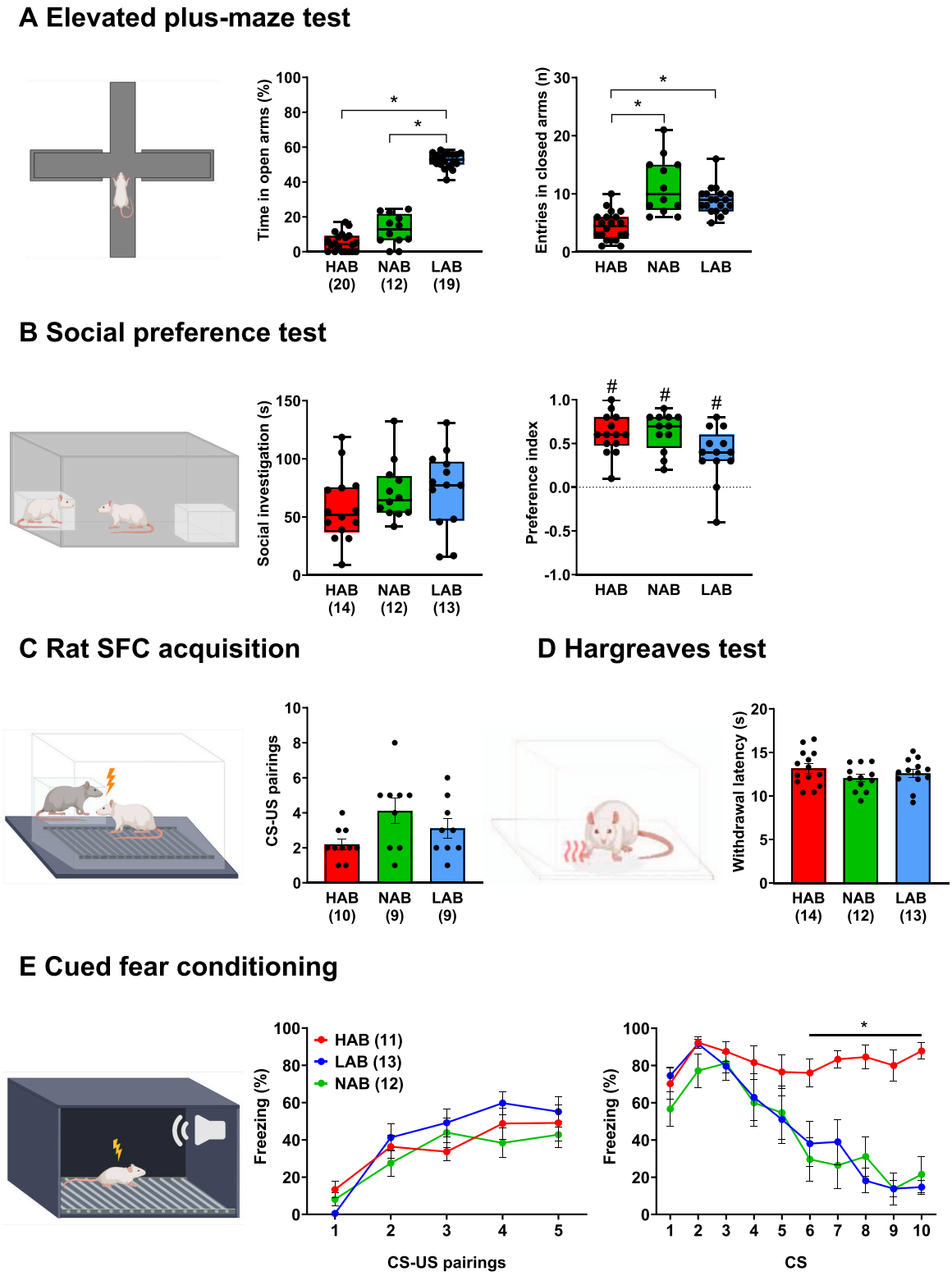

Fig. 2. Behavioural characterisation of male HAB, LAB and NAB rats.(A) Anxiety-related behaviour was assessed in the elevated plus-maze (EPM). Scheme of the apparatus, and percentage of time spent in open arms reflecting anxiety levels and number of entries into closed arms reflecting locomotor activity. * $p < 0.001$ versus NAB (Kruskal–Wallis test followed by Dunn's multiple comparisons). (B) Social preference test: Scheme of the apparatus, social investigation time and preference index, calculated as: (time spent investigating the social stimulus – time with empty cage) divided by total investigation time. Statistical analysis: Kruskal–Wallis test; # $p < 0.001$ versus 0 (Wilcoxon signed-rank test). (C) Rat social fear conditioning (SFC) acquisition: Scheme of the conditioning chamber, and number of conditioned-unconditioned (CS–US) pairings. Statistical analysis: one-way ANOVA. (D) Hargreaves plantar test: Scheme of the apparatus, and mean paw withdrawal latencies. Statistical analysis: one-way ANOVA. (E) Cued fear conditioning: Scheme of chamber, and percentage of freezing during acquisition and extinction. * $p < 0.05$–0.001 versus NAB and LAB (two-way repeated-measures ANOVA with Bonferroni's multiple comparisons test). Created with BioRender.com. Data are shown as box plots (min–max) with individual data points, scatter dot plots with all points overlaid with mean ± SEM, or line graphs showing mean ± SEM. Numbers in parentheses indicate the group size.

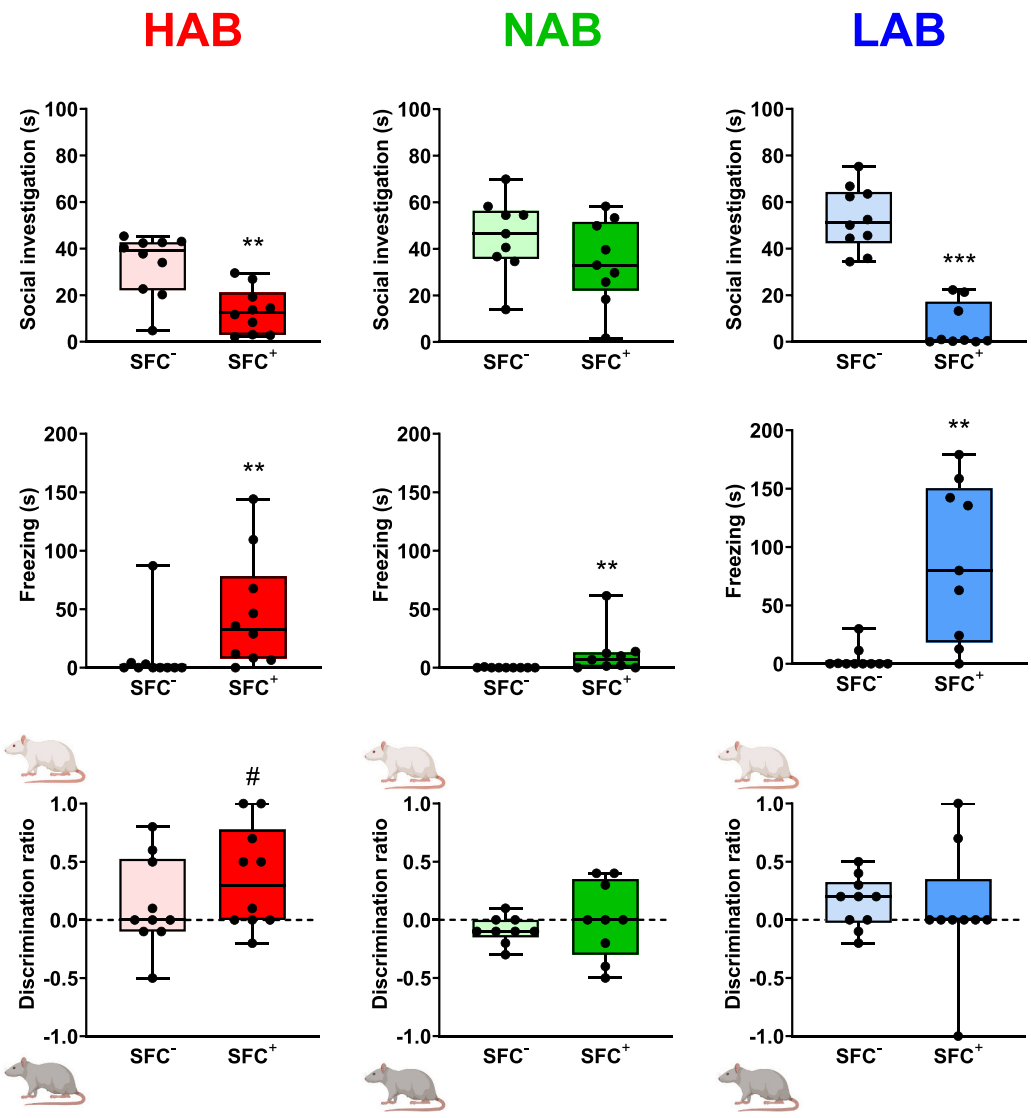

Fig. 3. Behavioural responses of male HAB, NAB, and LAB rats during the social fear discrimination test performed 24 h after social fear acquisition. Total social investigation time (i.e. time of investigating the **"known"** [grey] and **"unknown"** [white] conspecifics), percentage freezing, and discrimination ratio ([investigation time of **"unknown"** stimulus − investigation time of **"known"** stimulus] divided by total investigation time) are shown for conditioned (SFC^+) and unconditioned (SFC^-) rats. Data are shown as box plots (min–max) with individual data points. Number of rats in each group: 9–10. ** $p < 0.01$. *** $p < 0.001$ versus SFC^- (Mann-Whitney *U* test), # $p < 0.05$ versus 0 (Wilcoxon signed-rank test).

3.3. Role of AVP signaling in social fear memory across rat lines

To test whether increased AVP signaling in HAB rats (Murgatroyd et al., 2004) is responsible for their high levels of social fear and social discrimination ability seen after 24 h, a selective V1aR-A receptor antagonist (or Veh) was infused ICV following social fear acquisition in HAB, LAB, and NAB rats (Fig. 5).

Prior ICV infusions (V1aR-A, AVP, or vehicle) was associated with differences in the number of CS–US pairings during acquisition (two-way ANOVA: significant SFC × condition interaction, $F(2,61) = 10.2$, $p < 0.001$, $\eta p^2 = 0.250$), with V1aR-A–assigned HAB rats requiring fewer pairings than AVP-treated NAB and LAB rats (data not shown).

During the SFD test conducted 24 h later, two-way ANOVA revealed significant main effects of SFC on both social investigation ($F(1,37) = 23.1$, $p < 0.001$, $\eta p^2 = 0.371$) and freezing ($F(1,41) = 4.35$, $p = 0.043$, $\eta p^2 = 0.116$) in HAB rats (replicating earlier findings, see Fig. 3). To test the *a priori* hypothesis that V1aR antagonism modulates social behaviour in SFC^+ animals, a planned separate comparison using Student's *t*-test between the SFC^+ groups was performed. This analysis showed that V1aR-A-treated HAB/SFC^+ rats exhibit higher social investigation levels than Veh-treated HAB/SFC^+ controls ($t(27) = 2.33$, $p = 0.027$, $d = -0.668$). Although discrimination ratios did not differ between treatments, one-sample *t*-tests showed that Veh-treated HAB/SFC^+ rats were able to discriminate between the **"known"** and **"unknown"** conspecifics ($t(13) = 2.22$, $p = 0.045$, $d = -0{,}135$), whereas V1aR-A-treated HAB/SFC^+ rats were not, suggesting that AVP–V1a receptor signalling contributes to social fear memory.

AVP was administered to NAB and LAB rats to test the hypothesis whether enhancing central AVP signaling improves social fear discrimination 24 h after social fear acquisition, i.e. in SFC^+ rats. In NAB rats, two-way ANOVA revealed no main effect of SFC or treatment on social investigation. For freezing behaviour, a main effect of SFC was observed ($F(1,30) = 7.12$, $p = 0.012$, $\eta p^2 = 0.192$), replicating findings of Fig. 3. Thus, separate comparison revealed reduced freezing in AVP-treated NAB/SFC^+ compared with Veh-treated NAB/SFC^+ rats ($t(18) = 2.19$, $p = 0.043$, $d = -0.709$; Student's *t*-test). Although discrimination ratios did not differ between treatments, only AVP-treated NAB/SFC^- rats were able to discriminate between the **"known"** and **"unknown"**

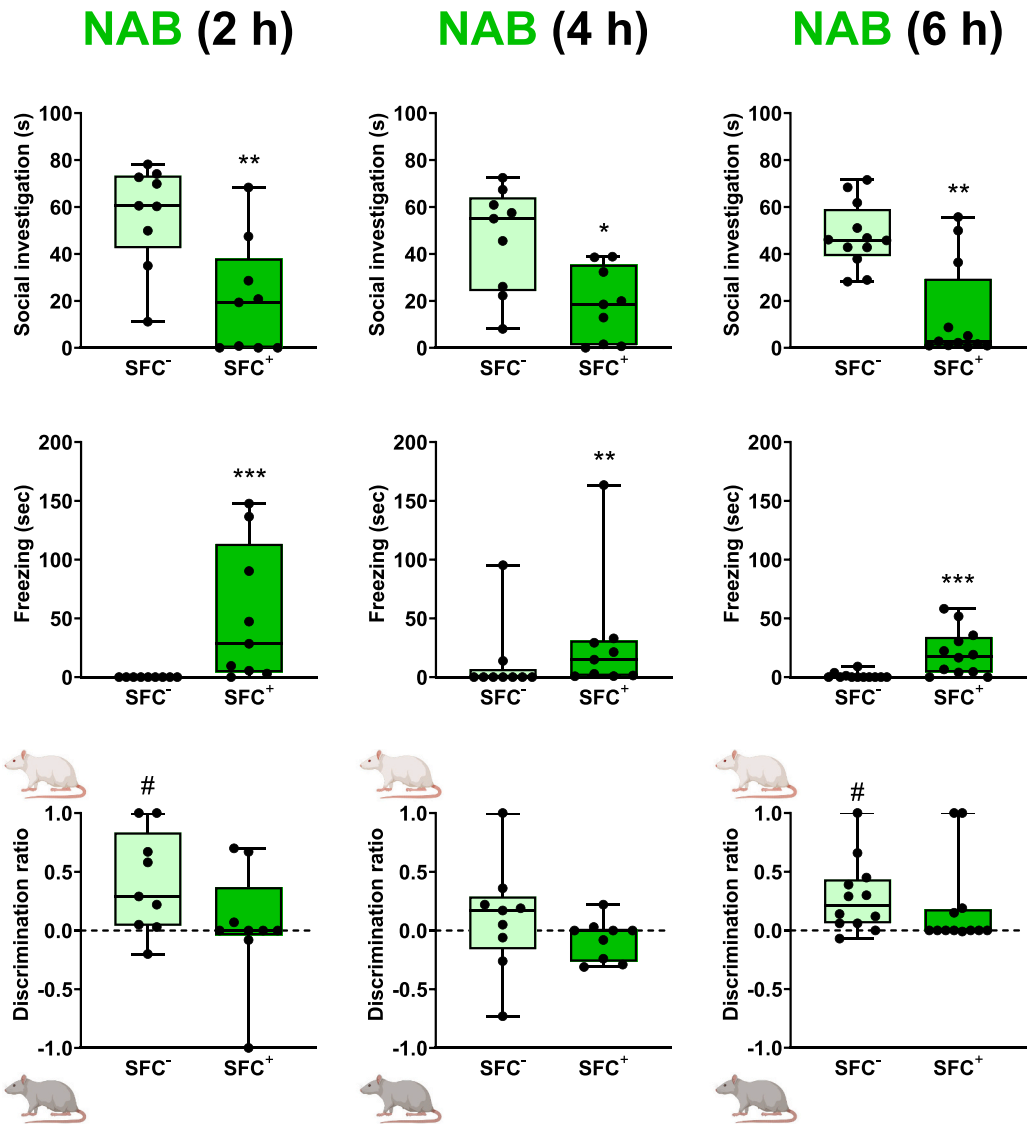

Fig. 4. Behavioural responses in NAB rats during the social fear discrimination test performed 2, 4, or 6 h after social fear acquisition. Total social investigation time reflecting generalised social fear, percentage freezing, and discrimination ratio ([investigation time of **"unknown"** stimulus − investigation time of **"known"** stimulus] divided by total investigation time) are shown for conditioned (SFC^+) and unconditioned (SFC^-) NAB rats. Data are shown as box plots (min–max) with individual data points. Number of rats in each group: 9–12. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus SFC^- (Mann-Whitney *U* test), # $p < 0.05$ versus 0 (Wilcoxon signed-rank test).

conspecifics (one-sample *t*-test: $t(7) = 2.76$, $p = 0.028$, d= 0,976).

In LAB rats, a significant SFC × treatment interaction was observed for social investigation ($F(1,36) = 5.30$, $p = 0.027$, $\eta p^2 = 0.486$). Post hoc analyses revealed that Veh-treated LAB/SFC^+ rats displayed reduced social investigation compared with Veh-treated LAB/SFC^- rats ($p < 0.001$), replicating previous findings (Fig. 3). AVP treatment restored social investigation in LAB/SFC^+ rats to levels comparable to LAB/SFC^- controls (AVP-treated vs Veh-treated LAB/SFC^+: $p < 0.05$). For freezing behaviour, two-way ANOVA revealed a main effect of SFC ($F(1,35) = 11.4$, $p = 0.002$, $\eta p^2 = 0.245$). Accordingly, separate comparison between the two SFC^+ groups showed that AVP treatment reduced freezing in LAB/SFC^+ rats compared with Veh-treated LAB/SFC^+ rats ($t(18) = 2.49$, $p = 0.023$, d= 1.346; Student's *t*-test). No significant effects were detected for discrimination indices, and none of the LAB groups showed the ability to discriminate between the **"known"** and **"unknown"** conspecifics during the SFD test.

3.4. Role of GC in social fear memory across rat lines

3.4.1. Plasma Cort responses to social fear acquisition

Basal Cort and Cort responses during acquisition were measured across in HAB and NAB rats (Fig. 6A).

Basal plasma Cort: Basal Cort levels measured in the home cage differed significantly between rat lines (two-way ANOVA: main effect of line, $F(1,24) = 18.0$, $p < 0.001$, $\eta p^2 = 0.523$; Fig. 6A). Planned separate comparisons showed higher Cort levels in HAB than NAB rats in both SFC^- ($t(9) = 2.80$, $p = 0.021$, d= 1.70) and SFC^+ ($t(15) = 4.04$, $p = 0.001$, d= 1.54; Student's *t*-test) animals. No significant differences were detected between SFC^+ and SFC^- groups within either rat line.

Cort responses to social fear acquisition: During social fear acquisition, HAB/SFC^+ and NAB/SFC^+ rats received a similar number of CS–US pairings (HAB/SFC^+: 2.00 ± 0.38, n = 7; NAB/SFC^+: 2.56 ± 0.29, n = 9). Plasma Cort levels showed a significant time × line interaction ($F(6.34,55.0) = 10.6$, $p < 0.001$; $\eta p^2 = 0.55$), reflecting line-dependent responses during rSFC (Fig. 6A). HAB/SFC^+ rats had elevated Cort at 5 ($p = 0.025$) and 15 min ($p = 0.027$), returning to baseline by 60 min,

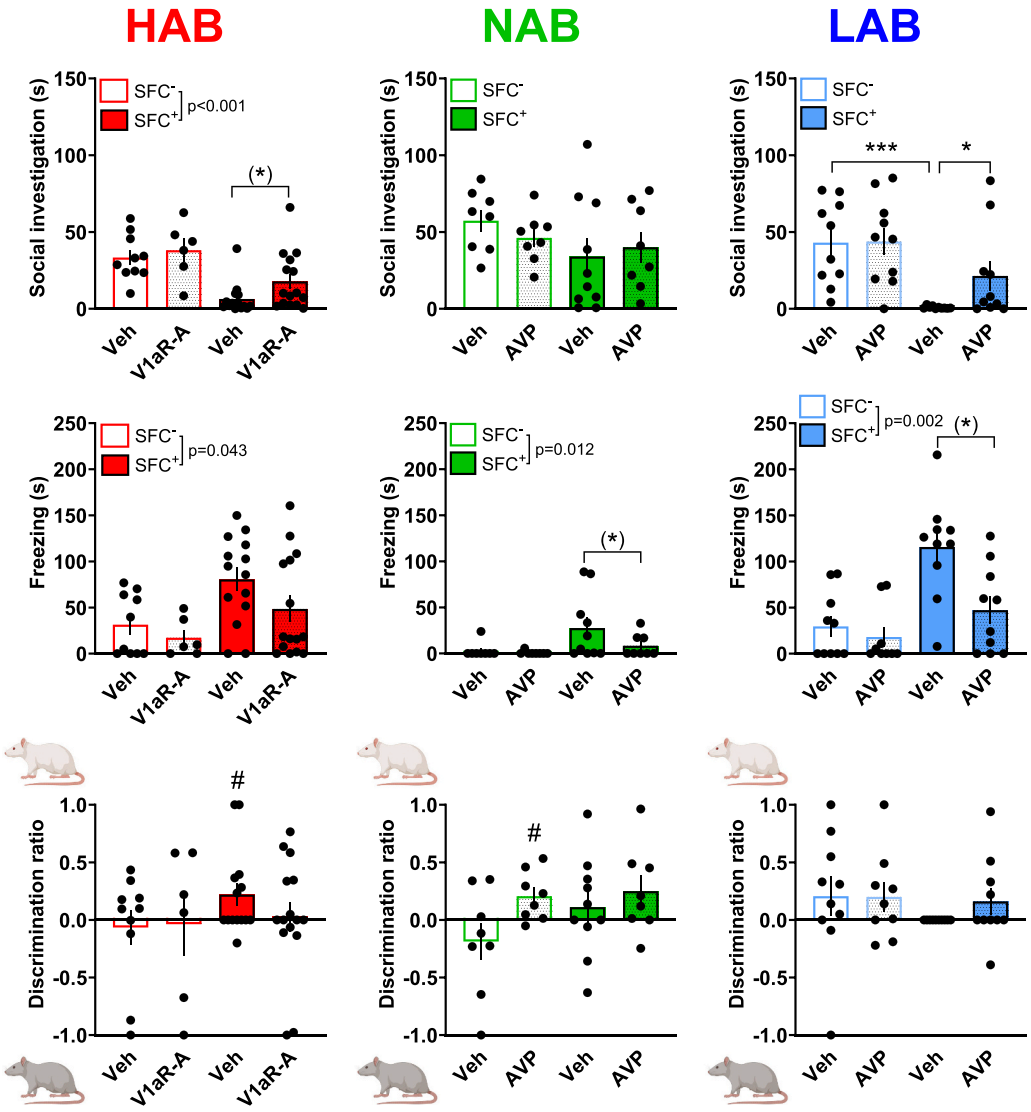

Fig. 5. Effects of intracerebroventricular (ICV) arginine vasopressin (AVP) or V1a receptor antagonist on behavioural responses in male HAB, NAB, and LAB rats during social fear discrimination. The social fear discrimination test was conducted 24 h after social fear acquisition in HAB, NAB and LAB rats. Rats received an ICV infusion of AVP (1 ng/5 μl), a V1a receptor antagonist (V1aR-A, 0.75 μg/5 μl), or vehicle (Veh) following social fear acquisition. Total social investigation time, percentage freezing, and the discrimination ratio ([investigation time of the **"unknown"** stimulus − investigation time of the **"known"** stimulus] divided by total investigation time) are shown for conditioned (SFC^+) and unconditioned (SFC^-) rats. Data are shown as scatter dot plots with all points overlaid with mean ± SEM. Number of rats in each group: 6–15. Exact p values (main effect of SFC, two-way ANOVA); * $p < 0.05$, ** $p < 0.001$ versus respective Veh (Bonferroni-corrected post hoc comparisons); (*) $p < 0.05$ versus Veh in SFC^+ animals (planned separate comparison using Student's *t*-test); # $p < 0.05$ versus 0 (one-sample *t*-test).

whereas HAB/SFC^- rats did not change. NAB rats showed increased Cort at 5 and 15 min in both SFC^- (+5 min: $p = 0.015$; +15 min: $p = 0.004$) and SFC^+ (+5 min: $p = 0.007$; +15 min: $p = 0.010$) groups, normalising by 60 min. At 5 min, between-group comparisons revealed higher Cort levels in NAB versus HAB rats (SFC^-: $p = 0.022$; SFC^+: $p = 0.010$), and in HAB/SFC^+ versus HAB/SFC^- ($p = 0.018$). At 15 min, these differences persisted: NAB rats showed higher Cort than HAB rats (SFC^-: $p = 0.009$; SFC^+: $p = 0.012$), and HAB/SFC^+ remained elevated compared to HAB/SFC^- ($p = 0.022$).

3.4.2. Effects of suppression of Cort synthesis prior to social fear acquisition on social fear memory in HAB and LAB rats

To examine the differential role of Cort in generalised social fear expression and individual social fear memory of HAB and LAB rats at a 24-h retrieval interval, Cort synthesis was blocked with metyrapone (50 mg/kg, IP) 90 min before social fear acquisition (Fig. 6B, C). NAB rats were not included, because they do not exhibit sustained social fear at this time point. CS–US pairings did not differ across groups (data not shown).

In HAB rats (Fig. 6B), there were significant main effects of SFC ($F(1,27) = 7.92$, $p = 0.009$, $\eta p^2 = 0.227$) on social investigation, but no significant SFC × treatment interaction. Separate comparison of the two HAB/SFC^+ groups showed that MET treatment significantly increased social investigation ($t(15) = 2.81$, $p = 0.013$ versus Veh, $d = -1.363$; Student's *t*-test). For freezing behaviour, we identified a significant main effect of SFC ($F(1,25) = 11.5$, $p = 0.002$, $\eta p^2 = 0.313$), but separate comparison of the two SFC^+ animals revealed no significant effect of MET treatment. Also, no group differences were observed for discrimination ratios. One-sample *t*-tests indicated that only Veh-treated HAB/SFC^- rats discriminated between the **"known"** and **"unknown"** conspecifics ($t(5) = 3.06$, $p = 0.028$, $d = 1.250$), whereas all other groups failed to show discrimination.

In LAB rats (Fig. 6C), MET-treated LAB/SFC^+ animals exhibited significantly increased social investigation compared with vehicle-

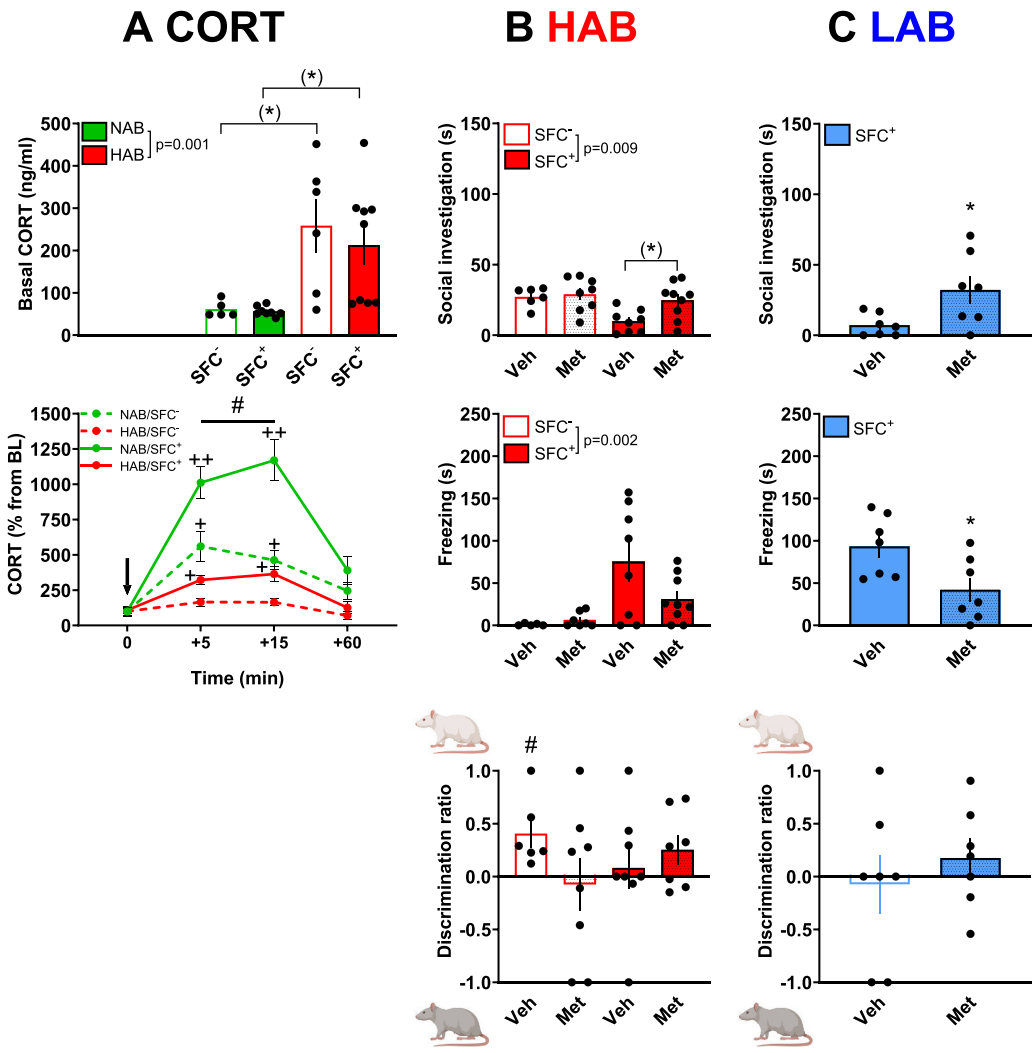

Fig. 6. Corticosterone responses during social fear acquisition and effects of its synthesis inhibition on behavioural responses in male HAB, NAB, and LAB rats during social fear discrimination. (A) Basal corticosterone (Cort) levels in the home cage. ** $p < 0.01$ main effect of Line, two-way ANOVA; (*) $p < 0.05$ versus SFC^- (planned separate comparison using Student's *t*-test). Cort response to social fear acquisition (SFC) at 0, 5, 15, and 60 min after the first social approach during social fear acquisition, expressed relative to baseline (BL; 100%). # $p < 0.05$ NAB vs. HAB irrespective of SFC condition (mixed-effects model); + $p < 0.05$, + + $p < 0.01$ vs. 0 min. (B, C) Effects of metyrapone (MET, 50 mg/kg, IP) or vehicle (Veh) administered 90 min before social fear acquisition on social investigation, freezing (%), and discrimination ratio measured 24 h after social fear acquisition. Data are shown for conditioned (SFC^+) and unconditioned (SFC^-) HAB rats as well as SFC^+ LAB rats. Data are shown as scatter dot plots with all points overlaid with mean ± SEM or line graphs showing mean ± SEM. Number of rats per group: 5–8. Exact p values (main effect of SFC, two-way ANOVA); * $p < 0.05$ versus Veh (unpaired Student's *t*-test); (*) $p < 0.05$ versus Veh in SFC^+ animals (planned separate comparison using Student's *t*-test); # *$p < 0.05$ versus 0 (one-sample *t*-test).

treated LAB/SFC^+ rats (unpaired Student's *t*-test (12)= 2.45, p = 0.031, d= −1.309), along with reduced freezing behaviour (unpaired Student's *t*-test: t(12) = 2.62, p = 0.023, d= 1.398). No treatment effects were detected for discrimination ratios, and none of the groups showed significant discrimination from zero, indicating an absence of discrimination between the **"known"** and **"unknown"** conspecifics during the SFD test.

4. Discussion

This study aimed at studying social fear as the main symptom of SAD in relation to innate, genetically-driven anxiety-related behaviour and general stress susceptibility, i.e., in adult male HAB and LAB rats (Gryksa et al., 2023). Based on our established mouse SFC model (Masis-Calvo et al., 2018; Toth et al., 2013) (Fig. 1) we have, therefore, introduced the rSFC paradigm. Here, we show that trait anxiety contributes to specific aspects of social fear expression, particularly influencing the magnitude and persistence of individual social fear memory. These effects of trait anxiety are modest and occur without affecting baseline sociability, nociception, or the initial acquisition of fear. Remarkably, high trait anxiety was associated with individual social fear memory lasting 24 h, which was found to be dependent on the genetically driven high activity of the AVP system.

5. The adapted rSFC paradigm and social fear processing in HAB LAB and NAB rats

The rSFC protocol differs substantially from that of the SFC paradigm performed in mice and required several methodological modifications. Social fear acquisition (day 1) could be performed in a comparable way in mice and rats, although rats generally required a higher number of CS-US pairings (3 ± 2 shocks, 2 s) and at a higher shock intensity (1 mA) compared to mice (2–5 shocks, 1 s, 0.7 mA) (Toth et al., 2012) to avoid further contact with the conspecific during conditioning. In contrast to mice, which were not pre-habituated, rats were pre-habituated to the conditioning chamber one day before acquisition. Although such

pre-exposure might potentially reduce associative learning (Camats Perna and Engelmann, 2017), particularly in NAB rats (Fig. 3), all rat lines developed social fear (Figs. 3, 4).

In contrast to acquisition, the social fear extinction training performed in mice had to be substantially adapted for rats and was replaced by the SFD test. In mice, social fear extinction is performed in the home cage 24 h after acquisition with consecutive exposure to six different, unknown conspecifics. Here, SFC⁻ mice consistently display high social motivation and sustained social investigation throughout the sessions, i.e. during consecutive exposure to six unknown conspecifics. In contrast, our pilot studies indicated that rats are generally less motorically active and display reduced social motivation, resulting in low levels of social investigation driven largely by passive behaviour during testing in the home cage. Accordingly, SFC⁻ rats showed consistently low social investigation, remaining predominantly inactive and behaviourally passive throughout the session. This species-specific pattern was observed regardless of testing during the light or dark phase and was independent of the presence of home-cage bedding (data not shown). Therefore, social fear expression in SFC⁺ rats was assessed only during a single, simultaneous exposure to both the **"known"** and **"unknown"** conspecific (SFC test) at different time points following acquisition (2, 4, 6 or 24 h). The SFD test was performed in a novel arena, which provided sufficient space to maintain distance between stimuli and thereby facilitated the assessment of individual social fear memory by comparing investigation times directed toward the **"known"** versus an **"unknown"** conspecific.

Using the rSFC paradigm, we were able to observe marked differences in the expression of generalised social fear expression and individual social fear memory between adult male HAB, LAB and NAB lines (Fig. 3). Whereas HAB/SFC⁺ and LAB/SFC⁺ rats showed generalised social fear, characterised by reduced total social investigation and increased freezing 24 h after acquisition, NAB/SFC⁺ rats displayed generalised social fear only for up to 6 h post-acquisition. This may reflect weaker social recognition memory assessed after a delay of 24 h in NAB rats, potentially due to reduced engagement of molecular mechanisms that support persistent social fear memory in the more susceptible HAB and LAB rat lines.

Regarding individual social fear memory, HAB rats were able to discriminate between the **"known"** and an **"unknown"** conspecific, selectively avoiding the **"known"** one, up to 24 h after acquisition. Consistent with social recognition literature showing that rats typically retain individual social memory only for a shorter period (Engelmann et al., 1995; Kogan et al., 2000; Popik et al., 1991; Squires et al., 2006), this prolonged 24-h persistence in HAB rats likely reflects strengthened consolidation of social aversive memory, and resembles the enduring social threat memories observed in SAD patients (American Psychiatric Association, 2022; Seinsche et al., 2023). In contrast, LAB rats failed to discriminate between the **"known"** and an **"unknown"** rat after 24 h, indicating impaired social cognitive processing rather than deficient social fear acquisition. Interestingly, NAB rats exhibited short-term social fear, and failed to consolidate general and individual social fear memory (Figs. 3, 4). NAB rats represent the unselected Wistar population and have previously been characterised as displaying intermediate, non-extreme anxiety and social behaviour phenotypes across behavioural assays (Gryksa et al., 2023). Consistent with this, NAB rats were expected to display intermediate social fear expression and memory persistence relative to HAB and LAB lines. In contrast, long-lasting social discrimination abilities have recently been described in naive HAB rats, which can discriminate between a familiar and a novel juvenile even after an inter-exposure interval of up to 120 min, whereas NAB rats typically show shorter retention (Frank and Landgraf, 2008; Landgraf and Wigger, 2002; Zoicas et al., 2020). These differences appeared independent of olfactory or visual cue processing (Frank and Landgraf, 2008). One key factor contributing to differences in social fear memory could also be behavioural inhibition, a temperament characterised by heightened sensitivity to novelty and avoidance of unfamiliar situations. Behavioural inhibition is a well-established risk factor for SAD, and reflects stable, biologically based differences in anxiety and fear processing across both humans and animal models (Clauss and Blackford, 2012; Fox et al., 2021; Spence and Rapee, 2016). HAB rats exhibit strong avoidance, reduced social approach, and persistent social fear, independent of sensory cues (Gryksa et al., 2023), paralleling human behavioural inhibition. These traits are associated with enhanced retention of emotional and social information (Camats Perna and Engelmann, 2017; Kalisch et al., 2006), highlighting innate anxiety as a key determinant of persistent social fear across species.

Furthermore, we confirmed the differences in fear processing depending on trait anxiety in the CFC paradigm. HAB rats showed impaired extinction of cued fear, as reflected by persistently high freezing levels throughout extinction training, whereas LAB and NAB rats showed normal fear extinction (Fig. 2; Muigg et al., 2008; Slattery et al., 2015). These findings indicate that high trait anxiety predisposes rats to persistent and extinction-resistant fear, highlighting their translational relevance for studying mechanisms underlying both social and non-social fear memory.

Importantly, the observed differences in social fear memory between rat breeding lines were not attributable to innate sociability and baseline social motivation (Fig. 2), as rats of all lines displayed intact social preference in the SPT, consistent with previous findings in male HAB and LAB rats (Schmidtner et al., 2019; Zoicas et al., 2020). Likewise, initial fear acquisition during rSFC did not differ between lines (Fig. 2), indicating that the divergence in long-term memory (24-h retention) likely reflects differences in memory consolidation or retrieval. Line-dependent pain sensitivity is unlikely to account for the observed differences, as paw withdrawal latencies in the Hargreaves test were comparable across lines (Fig. 2). Although HAB rats have previously been reported to exhibit hypoalgesia with higher thermal pain thresholds (Jochum et al., 2007), this was not evident under our conditions. Consistent with this, HAB/SFC⁺ rats did not require increased CS–US pairings during social fear acquisition compared to the other lines (Fig. 2).

6. Line-dependent modulation of social fear by AVP–V1a signalling

The brain AVP system facilitates both anxiety-related behaviour (Wigger et al., 2004) and social discrimination abilities (Engelmann and Landgraf, 1994). Due to a point mutation in the promoter of the *Avp* gene (Gryksa et al., 2023; Murgatroyd et al., 2004; Wigger et al., 2004), increased hypothalamic AVP expression and release has been identified in adult (~10 weeks old) male and female HAB rats to be causal for their hyper-anxiety level. Here, we show that the high activity of the brain AVP system contributes to long-term social fear memory (24-h retention) in HAB rats (Fig. 5). Specifically, V1a receptor antagonism following social fear acquisition prevented the formation of both generalised social fear and individual social fear memory assessed after a 24-h delay. In contrast, in LAB/SFC⁺ rats, increasing central AVP availability via synthetic AVP did not restore individual social fear memory 24 h after acquisition. Interestingly, in NAB/SFC⁻ rats (which did not receive foot shocks), AVP modestly enhanced social discrimination abilities in line with a positive effect of brain AVP on social memory (Engelmann and Landgraf, 1994). This suggests that AVP facilitates individual social memory both under safe but also aversive conditions. However, it has to be mentioned that some of these effects of modulation of AVP signaling were only revealed by separate statistical analyses of respective SFC + groups treated with either vehicle or the respective drug.

7. GC permit, but do not drive, social fear consolidation

As key elements of HPA axis functions, GC are essential modulators of both social and non-social memory (de Quervain et al., 1998; Soeter

and Kindt, 2013). Here, we show that GC play a permissive — but not sufficient — role in social fear consolidation in adult males (Fig. 6). HAB rats exhibited higher basal Cort levels but a smaller stress-induced response during rSFC than expected based on their anxiety phenotype (Landgraf et al., 1999). In line with previous work showing that pre-training inhibition of GC synthesis impairs fear memory retrieval and extinction in stress-exposed animals (Blundell et al., 2011; Careaga et al., 2015; Clay et al., 2011; Roozendaal et al., 1996), blocking Cort synthesis with metyrapone prior to social fear acquisition prevented the consolidation of long-term social fear (24-h retention) in HAB rats. These results demonstrate that, across models, endogenous GC signalling is necessary for the formation and retention of fear memory, linking the vulnerability of HAB rats to broader mechanisms of stress-dependent memory modulation.

In contrast, NAB rats displayed lower basal Cort, but a pronounced stress-induced increase during rSFC, yet failed to form long-term social fear (24-h retention). This dissociation indicates that stress-induced GC elevation alone is insufficient to drive persistent social fear memory. Instead, effective consolidation appears to require the engagement of additional mechanisms, which may be enhanced in stress-susceptible HAB and LAB rats. Together, our findings suggest that high trait anxiety interacts with GC signalling to permit fear consolidation, consistent with a vulnerability-stress framework (de Quervain et al., 1998; Soeter and Kindt, 2013). Unfortunately, LAB rats could not be included in the blood sampling experiment due to breeding limitations. However, they were included in the metyrapone experiment, and previous work indicates lower HPA axis responses to non-social stressors (Landgraf et al., 1999) and exaggerated responses to social stressors (Frank et al., 2006; Veenema et al., 2007) suggesting that social situations may be experienced as particularly stressful in low-anxiety animals. This raises the possibility that GC inhibition could differentially affect social fear memory formation in this phenotype, an important question for future investigation. Thus, while GC are necessary (as shown by metyrapone blockade in HAB rats), they are not sufficient to drive persistent social fear memory in the absence of a vulnerable trait background.

8. Translational relevance and limitations

From a translational perspective, the rSFC paradigm generally mimics selected components of SAD, such as social fear. The line-specific behavioural phenotypes described in our study mirror clinical heterogeneity in SAD patients, who vary in fear intensity, generalisation, and memory persistence (Stein and Stein, 2008), with genetic underpinnings as one likely mechanism. Although 24-h retention is widely used as a proxy for long-term social memory in rodents, operational definitions vary across frameworks, with social recognition studies typically classifying 24 h or more as long term memory (Cum et al., 2024; Pirri et al., 2024). In other behavioural domains, 24 h is also used as a marker of consolidated memory (Brambilla et al., 1997; Rudy and Morledge, 1994), although some studies consider 24–48 h as a recent memory window (Jacques et al., 2019; Sheynikhovich et al., 2022). Importantly, in trauma- and stress-related animal models, behavioural endpoints are often assessed at longer delays ($\geq$1 week), reflecting remote memory processes (Singewald and Holmes, 2019).

The robust and long-term individual social fear memory observed in male HAB rats resembles the precise, enduring social trauma memories reported in SAD, whereas LAB rats rather model generalised social fear. These phenotypes likely arise from differential engagement of circuits involving the extended amygdala, hippocampus, and medial prefrontal cortex, which are known to be modulated by AVP and GC, and show altered activation in HAB rats during stress (Frank et al., 2006; Muigg et al., 2008; Salomé et al., 2004). Thus, the adapted rSFC performed in HAB and LAB rats is an excellent model to further investigate, how trait anxiety shapes specific aspects of social fear processing, as it captures distinct neuroendocrine and behavioural dimensions relevant to SAD, Thus, its strong construct validity for anxiety-like phenotypes and dysregulated stress responsiveness make HAB and LAB rats a valuable translational tool for studying mechanisms of maladaptive social fear and stress vulnerability (Gryksa et al., 2023).

A limitation of our study is the exclusive use of male rats, as sex-dependent mechanisms are relevant for anxiety disorders (Bangasser and Cuarenta, 2021), and sex differences have been described in fear learning, extinction, HPA axis regulation, and social behaviour in rodents (Gruene et al., 2015; Maren et al., 1994; Shansky, 2014). However, the present study mainly aimed to establish the rSFC paradigm and to study the impact of trait anxiety on social fear memory. Future studies should determine, whether trait anxiety differentially modulates social fear processing across sexes.

9. Conclusion

Using a novel rSFC paradigm we demonstrate that innate anxiety critically determines whether social threat is encoded as a persistent, individual social fear memory. Thus, the HAB rat line models a high-risk phenotype characterised by AVP- and GC-dependent consolidation of discriminative social fear. In contrast, LAB rats exhibit generalised social fear accompanied by social cognitive deficits, while NAB controls reflect a resilient phenotype. Thus, the combination of rSFC and rats with genetically driven differences in socio-emotional traits provides a powerful approach to investigate the mechanisms underlying individual susceptibility to social trauma-induced social fear.

Ethics declaration

This study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This study was approved by the Regierung von Unterfranken, Würzburg.

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CRediT authorship contribution statement

MMC: Investigation, Formal analysis, Data curation, Writing (parts of original draft). **VR:** Writing (parts of original draft, review & editing), Formal analysis, Visualization. **IDN:** Original project design, Conceptualization, Writing (parts of original draft, review & editing), Supervision, Funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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