Psilocybin modulates social behaviour in male and female mice in a time-dependent manner

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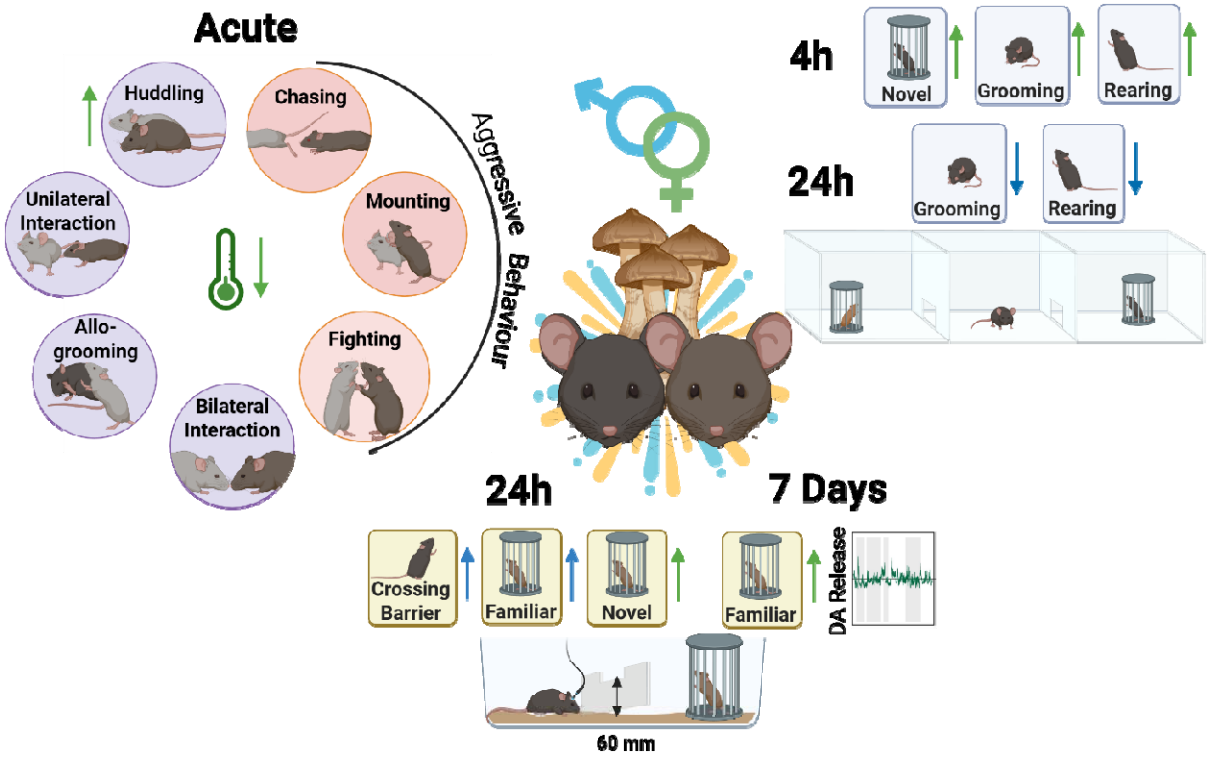
Funding: This work was supported by a National Health and Medical Research Council (NHMRC) of Australia Ideas Grant (GNT2011334) awarded to CJF. SS is supported by a Monash Biomedicine Postgraduate Discovery Scholarship (Mbio).

Disclosures: None

Word count:

Keywords (up to 7): Psychedelics, Psilocybin, Serotonin, Social Behaviour, Sex Differences

Graphical Abstract



Abstract (206 words)

With the resurgence of psychedelic research and the growing interest in their therapeutic potential, there is an urgent need to understand how these compounds act across biological sexes. Despite widespread interest in their use for conditions marked by social impairments, including depression, anxiety, and anorexia nervosa, the influence of sex as a biological variable (SABV) on the prosocial effects of psychedelics remains poorly understood. Indeed, enhanced connectedness, sociability and empathy are common outcomes of psychedelic use and these have shaped human social structures for millennia. Here, we investigated the sex-specific effects of a single dose of psilocybin (1.5 mg/kg) in C57BL/6J mice on various aspects of social behaviours. We show an intriguing connection between huddling behaviour and body temperature acutely elicited by psilocybin that was restricted to females. We also observe temporally distinct patterns of social behaviour alterations in female mice, whereby enhanced preference for social novelty was observed after acute effects subsided (4 h post-administration), which was maintained for ~24 h. Longer-term, the impact of psilocybin was reversed and promoted preference for familiar over novel conspecifics when assessed 7d post-administration, which was associated with prolonged nucleus accumbens dopamine signalling during familiar sniffing. In males, psilocybin reduced stress-related behaviours at 24 h and increased preference for familiar conspecifics, along with blunted novelty-evoked dopamine responses at both 24 h and 7 days post-treatment. Both 5-HT1A and 5-HT2A receptors were involved in modulating these behaviours, though in sex-specific ways. These findings highlight that the prosocial effects of psychedelics are not universal and emphasize the importance of sex-informed approaches in both preclinical research and clinical application.

Introduction

Social behaviour is a fundamental component of human cognition, shaping the structure and stability of societal networks through mechanisms of cooperation, affiliation, and trust (Dunbar, 2018). The ability to form and maintain social bonds is crucial for psychological well-being and adaptive functioning, and social deficits are a hallmark of numerous psychiatric and neurological disorders, including autism spectrum disorder, schizophrenia, depression, and anorexia nervosa (Brotman et al., 2017; Kennedy & Adolphs, 2012). Emerging evidence suggests that psychedelics, particularly psilocybin (the psychoactive alkaloid produced by *Psilocybe* mushrooms), may enhance social cognition, promote prosocial behaviour, and increase social adaptability by modulating key neural circuits involved in social reward and emotional processing (Preller & Vollenweider, 2019; Rodríguez Arce & Winkelman, 2021). The resurgence of psychedelic research has provided compelling insights into their therapeutic potential for a range of psychiatric conditions that include the abovementioned; major depressive disorder, generalised anxiety disorder, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD) and anorexia nervosa (Barber & Aaronson, 2022; Carhart-Harris & Goodwin, 2017; Carhart-Harris et al., 2017; Davis et al., 2020; Davis et al., 2021; Griffiths et al., 2019; Moreno et al., 2006; Peck et al., 2023; Ross et al., 2016). However, despite the central role of social behaviour in human adaptability and mental health, the impact of psychedelics on social cognition and motivation remains a relatively underexplored domain. Current research has largely focused on primary symptom reduction in clinical populations or preclinical models of psychiatric conditions, overlooking the potential for psychedelics to enhance social functioning that may be critical for long-term therapeutic efficacy. Expanding research into the social effects of psychedelics could offer novel insights into their mechanistic actions and refine their clinical applications in disorders characterized by social impairments.

Emerging evidence suggests that psychedelics such as lysergic acid diethylamide (LSD) and psilocybin can enhance social connectivity in humans, potentially by modulating affective processing and social cognition (Forstmann et al., 2020). Psilocybin improved the understanding of negative stimuli (Pokorny et al., 2017), reduced feelings of social exclusion (Preller et al., 2016), and enhanced empathy and prosocial behaviours (Dolder et al., 2016). However, these effects are not consistently observed across all studies assessing post-acute changes in empathy (Rucker et al., 2022). These effects have been observed in both healthy individuals and patients with major depressive disorder (Bhatt & Weissman, 2024; Jungwirth et al., 2024; Pokorny et al., 2017; Preller et al., 2016), supporting a role for psilocybin in fostering interpersonal connectedness (Carhart-Harris et al., 2018). Furthermore, a single dose of psilocybin produced long-lasting improvements in social functioning and mood (Griffiths et al., 2008; Griffiths et al., 2011; McCulloch et al., 2022), suggesting persistent neuroplastic adaptations that contribute to enhanced social engagement (Aleksandrova & Phillips, 2021; van Elk & Yaden, 2022). Healthy volunteers also report elevated

positive mood states and prosocial behaviours under the acute effects of psilocybin (Gabay et al., 2018; Mason et al., 2019; Preller & Vollenweider, 2019), reinforcing its potential as a modulator of affective and social processing.

Preclinical studies investigating the influence of psychedelics on social behaviour in rodents show mixed outcomes. Psilocybin elicits both prosocial and antidepressant-like effects in male rats (Kolasa et al., 2024) and LSD and psilocybin both increase social interaction, preference for social novelty (De Gregorio et al., 2021; Gattuso et al., 2025; Markopoulos et al., 2021; Song et al., 2025) and social reward learning (Nardou et al., 2023) in male mice. However, a recent multi-institutional study investigating the impact of psilocybin on various behavioural aspects in male and female mice failed to replicate effects of psilocybin on social reward learning or preference for novel social stimuli (Lu et al., 2025). Notably, these two studies used both male and female mice without reporting or discussing potential sex-specific differences in sociability. These findings underscore the need for standardised behavioural protocols, reproducibility in psychedelic research, and the inclusion and separate analysis of both sexes to evaluate sex-dependent effects.

While interest in the behavioural effects of psychedelics has increased in recent years, the underlying neurobiological mechanisms remain inadequately understood. The most commonly reported site of psychedelic effects in both human imaging and animal studies is the medial prefrontal cortex (mPFC), a region crucial for social decision-making and regulation of prosocial behaviours (Duerler et al., 2022; Ko, 2017; Luo, 2018). However, psilocybin also modulates activity within the anterior cingulate cortex (ACC), which is involved in social pain processing (Preller et al., 2016), helping behaviour and empathy (Kim et al., 2014; Yamagishi et al., 2020) and social decision making (Lockwood & Wittmann, 2018), as well as the amygdala, which plays a role in social anxiety and threat perception (Sladky et al., 2013). The modulation of these regions is closely linked to interactions with the mesolimbic dopamine system and the oxytocin producing pathways, both of which are known to regulate pair bonding (Liu & Wang, 2003). For instance, MDMA has been shown to reopen social reward learning windows via the activation of the oxytocin receptors in the nucleus accumbens (Nardou et al., 2019), while LSD and psilocybin modulate connectivity between the default mode network (DMN) and social processing hubs, potentially reducing egocentric bias and increasing social connectedness (Gattuso et al., 2023). Rodent studies further implicate the paraventricular nucleus of the hypothalamus (PVN), a key site of oxytocin production, as a target for psychedelic-induced social facilitation (Effinger et al., 2024). Taken together, these findings suggest that psychedelics reshape social behaviour by engaging cortical and subcortical circuits that regulate social motivation, reward, and emotional salience.

While findings in this field are promising, the majority of psychedelic research in humans and animal models has disproportionately (or often times exclusively) focused on male participants. Such limitations hinder the generalizability of findings, particularly given well-documented sex differences in neurobiology, psychiatric disease prevalence, and pharmacological response profiles

(Bangasser & Cuarenta, 2021; Shadani et al., 2024). For instance, selective serotonin reuptake inhibitors (SSRIs), which target the same serotonergic system that is key for psychedelic actions, exhibit sex-dependent efficacy and side-effect profiles (Kuehner, 2017). Moreover, conditions such as depression, anxiety, PTSD, and eating disorders, many of which are potential targets for psychedelic therapy, are significantly more prevalent in females, underscoring the urgent need for sex-inclusive research (Christiansen & Berke, 2020; Farhane-Medina et al., 2022; Timko et al., 2019). To address this critical gap and align with the National Institutes of Health mandate to integrate sex as a biological variable (SABV) in experimental design (Clayton, 2018), the present study investigated the effects of psilocybin on multiple aspects of social behaviour in both male and female mice and across different time points (from acute to post-acute administration). In addition, we tested the hypothesis that psilocybin binding to specific serotonin (5-HT) receptor subtypes is necessary for eliciting prosocial behaviour and we paired social behaviour tasks with *in vivo* fiber photometry to investigate the neuronal substrates for these effects.

Methods

Animals and housing

All animals were obtained from the Monash Animal Research Platform (MARP; Clayton, VIC, Australia). To examine the direct effects of psilocybin on various aspects of social behaviour, male (n= 182) and female C57Bl/6 mice (n= 145) were utilised, aged ten weeks upon arrival at the laboratory. In all cases, mice were pair-housed in a climate-controlled room (temperature 22-24°C and humidity 30-50%) on a reverse light cycle (lights on at 2000h, off at 0800h) for 7 days before the experiments commenced for acclimation to the housing conditions. Mice had ad libitum access to food and water, except during behavioural testing windows. Each mouse underwent only one experimental condition to avoid potential confounding effects from repeated exposures. Testing was conducted between 1000h and 1700h. Four sex- and age-matched C57Bl/6 mice were used per cohort as novel mice in the 3-chamber and barrier climbing tests and were housed in a separate room under the same conditions as the test mice. All experimental procedures adhered to the Australian Code for the Care and Use of Animals for Scientific Purposes and were approved by the Monash Animal Research Platform Ethics Committee (ERM 30852, ERM 40619).

Drugs

Psilocybin (USONA Institute Investigational Drug Supply Program; Lot# AMS0167) was dissolved in saline and administered at a dose of 1.5 mg/kg. Also dissolved in saline were the MDL100907 (volinanserin; Sigma-Aldrich, CAS 139290-65-6; 0.1 mg/kg) and WAY100635 maleate compounds (Tocris Biosciences, CAS 1092679-51-0; 0.5 mg/kg), antagonists of 5-HTR subtypes 2A and 1A, respectively, were administered 30 minutes prior to psilocybin (PSI) or 0.9% NaCl saline (SAL) control treatment. Each animal received only one combination of PSI or SAL alone, or in

combination with one 5-HTR subtype antagonist. The doses were selected based on previous studies conducted in the lab (Conn et al., 2024). All drugs were delivered intraperitoneally with a 26-gauge needle at an injection volume of 10 ml/kg.

Acute core body temperature and activity monitoring

The UID Temperature Monitoring System (Unified Information Devices, Kenosha, WI, USA) was used to measure digital biomarkers such as temperature and locomotor activity continuously, 24/7, from conscious, unrestrained mice in their home cage environment. This approach reduced stress and ensured reliable data collection through a temperature-sensitive microchip in an undisturbed setting. Upon arrival, pair-housed mice were subcutaneously implanted with a 2.1 mm x 13 mm microchip, which uses RFID technology for animal identification and body temperature monitoring (model UCT-2112; Unified Information Devices, Kenosha, WI, USA), implanted via a specialised injection device. After seven days, mice received a single dose of PSI or SAL and core body temperature was recorded one hour before and up to two hours after injection.

Home-cage social behaviour

To examine the effects of psilocybin on social behaviour in a group setting, pair-housed mice were habituated to the test room for one hour in their home cage. Subsequently, they received either PSI or SAL injections and the duration and frequency of various social behaviours were recorded acutely for 1 h. Manual scoring using Ethovision XT software (Noldus, Netherlands) included bilateral interaction, huddling, unilateral interaction, allogrooming, mounting, fighting and chasing. There were no visible injuries resulting from these interactions.

3-Chamber test

At eleven weeks of age, mice were subjected to the 3-chamber social preference and novelty test, either 4 h or 24 h or 7 d after injection. Mice were placed in the central chamber of an apparatus containing three chambers, each with a wire cage at either end, for a 10-minute habituation period. A 1 min inter-trial interval (ITI) followed, during which the mice were confined to the middle chamber to minimise experimenter interference. Subsequently, an unfamiliar, age- and sex-matched mouse (Novel 1) was placed in a wire cage in one side chamber, while a novel object in a wire cage occupied the opposite chamber. The test mouse was then allowed to explore for 10 min (social preference trial). After another 1 min ITI, a second unfamiliar age- and sex-matched mouse (Novel 2) replaced the novel object, with Novel 1 remaining as the familiar mouse. The test mouse was observed for another 10 min (social novelty trial). Locomotor activity, interaction frequency, time spent in each chamber, and social preference were measured using video tracking and EthoVision XT software. Social preference was calculated using the formula: $(T_s - T_{ns}) / (T_s + T_{ns})$, where T_s is time spent with the novel mouse, and T_{ns} is time spent with the empty cage or familiar mouse. To obtain a comprehensive and integrated assessment of behavioural measures, emotionality-related data were normalised using a Z-score methodology, as previously (Guilloux et

al., 2011). In brief, the behavioural parameters for each mouse were calculated using the following formula: $Sociability\ Z\ Score = (x - \mu)/\sigma$

μ and σ, respectively, correspond to the mean and standard deviation of the SAL-treated (control) group, with x referring to the individual data points for mice in the treatment cohorts. The Sociability Z-Score for each mouse was determined based on both the duration and frequency of chamber entries. The Sociability Z-score quantifies time and frequency spent in the social versus neutral chamber, normalised to the control group, whereas the Sociability Index measures relative time spent interacting with either a novel conspecific, an empty cage, or a familiar mouse. Stereotypic behaviours assessed included grooming, rearing, cage climbing and freezing.

Barrier climbing test

To assess the social motivation of the mice, we adapted a maternal motivation task described in the literature (Kohl et al., 2018; Salais-López et al., 2021; Swart et al., 2021). The behaviour of mice was recorded for a total of 20 min, including habituation to the barrier (10 min), exploration of an unfamiliar age- and sex-matched mouse (5 min) and exploration of a cage mate (5 min). The novel mouse and cage mate were placed in a wire cage on the opposite side of the barrier to the test mouse. The following behavioural components were analysed during the test: latency to the first barrier climb, total number of climbs and latency to sniff the mouse under the wired cup. A barrier climb was defined as the moment when all four paws of the mouse touched the floor on opposite side of the 60 mm transparent barrier. Each instance of the mouse crossing the barrier and placing all four paws on the opposite side was counted as a barrier climb. Sociability Z-score was calculated as described above.

Estrous cycle assessment

Vaginal smears were collected daily at 1000h over a period of 7 d prior to testing and on the test days following the procedures. A volume of 10 µL of saline was flushed into the vagina, transferred onto a glass slide, and examined as stained preparations using hematoxylin and eosin (H&E). The stage of the estrous cycle was determined and classified as proestrus, estrus, or metestrus/diestrus, based on observed ratios of cornified epithelial, nucleated epithelial, and polymorphonuclear leukocytes, as described previously (Nelson et al., 1982).

Surgical and Viral Injection Procedures

Mice were anesthetised with isoflurane (2-3%; Pharmachem, QLD, Australia) and administered meloxicam (5 mg/kg, Boehringer Ingelheim, Germany) subcutaneously prior to surgery. Each animal was then positioned in a stereotaxic frame (Stoelting, IL, USA) on a heating pad maintained at 37.2 °C. Mice we unilaterally injected in the right hemisphere with hsyn-GRAB$_{DA2m}$ (AddGene #140553) for dopamine (DA) recordings targeting the nucleus accumbens (NAc). A volume of 300 nL was injected at a rate of 30 nL/min using a 2 µL Hamilton syringe (Model #7002), and

the needle was left in place for 5 min post-infusion to allow for diffusion. Injection coordinates were as follows: AP: +1.4 mm, ML: ±0.75 mm, DV: -4.2 mm from dura. Optical fibres (RWD, 0.39 NA; length: 4.7 mm; core: 200nm) were implanted 0.1 mm above the injection site (final DV: -4.1 mm) and secured using bonding agent (GBond, Japan) and light cured dental cement (G-aenial Universal Flow, GC Dental).

Fibre photometry

Experiments began at least five weeks post-surgery to allow sufficient time for recovery and viral expression. Recordings were conducted using the RWD R821 fibre photometry system, with 470 nm and 410 nm lasers used for the signal and isosbestic control channels, respectively. Data acquisition was performed using RWD software during the barrier climbing task described above, and behavioural video timestamps were manually aligned to the photometry recordings.

Data analysis

Statistical analyses were performed using GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, CA, USA). A significance threshold of $p < 0.05$ was applied, while $p < 0.10$ was considered indicative of a trend, though not statistically significant. Depending on the data type, number of groups, and comparisons of interest, appropriate statistical methods were used, including two-tailed unpaired t-tests, one-way and two-way analyses of variance (ANOVA) with Sidak's post hoc multiple comparisons and mixed-effects models. Details of all statistical tests can be found in the **Supplementary Materials**.

Results

Psilocybin-induced social thermoregulation in female mice is 5-HTR dependent

To evaluate the acute effects of psilocybin on home-cage social behaviour, we examined huddling in pair-housed female mice. Psilocybin significantly increased total huddling behaviour within the first 60 min post-administration ($p = 0.0391$; **Figure 1A**), with peak effects observed at 25 min ($p = 0.0133$; **Figure 1B**). This coincided with significant reductions in core body temperature beginning at 5 min ($p = 0.0277$) and persisting until 70 min ($p = 0.0073$) post-administration (**Figure 1C**). 5-HT1AR antagonism abolished huddling behaviour regardless of subsequent treatment (saline: $p = 0.0062$; psilocybin: $p < 0.0001$), while 5-HT2AR antagonism selectively reduced huddling in psilocybin-treated mice ($p = 0.0029$; **Figure 1D**). In saline-treated mice 5-HTR antagonism did not affect huddling (**Figure 1E**), however, in psilocybin-treated mice, 5-HT1AR antagonism abolished huddling during its peak at 30 min ($p = 0.0158$; **Figure 1F**). Overall, both 5-HT1AR and 5-HT2AR antagonism were observed to reduce core body temperature in saline-treated control mice, which was more pronounced and longer lasting for mice administered the 5-HT1AR antagonist ($p = 0.0244$), with only two timepoints significantly altered following 5-HT2AR antagonism (45 min: $p =$

0.0258 and 65 min: p = 0.0099; **Figure 1G**). In psilocybin-treated mice, 5-HT1AR antagonism prolonged the reduction in body temperature, whereas 5-HT2AR antagonism led to an acute increase in temperature (p = 0.0398) that was again elevated above psilocybin-only levels between 60-90 min post-administration (85 min: p = 0.0429; **Figure 1H**).

We also assessed other social behaviours and the modulatory role of psilocybin and 5-HTR antagonism. Unilateral interaction was significantly reduced by 5-HT2AR antagonism, but not by 5-HT1AR antagonism, in pair-housed female mice regardless of subsequent treatment (saline: p = 0.0339; psilocybin: p = 0.0328). Neither psilocybin nor 5-HTR antagonism affected aggressive behaviour in female mice (**Supplementary Data 1**). Within the groups pre-treated with 5-HTR antagonists, psilocybin did not significantly alter huddling behaviour. However, it reduced core body temperature in mice treated with 5-HT2AR antagonists at 45 min (p = 0.0297), and in 5-HT1AR-antagonised mice at 75 min (p = 0.0446) and 90 min (p = 0.0478; **Supplementary Data 2**).

Huddling behaviour in psilocybin-treated male mice is modulated by 5-HTR antagonism

Psilocybin alone did not alter huddling behaviour or core body temperature in male mice (**Figure 1I-K**), nor did 5-HTR antagonism in saline-treated mice (**Figure 1L-M, Figure 1O**). However, 5-HT1AR antagonism significantly reduced huddling in psilocybin-treated males (p = 0.0018; **Figure 1L**), which was consistently observed across the 60min monitoring period (p = 0.0399; **Figure 1N**). In combination with psilocybin, both 5-HTR antagonists reduced core body temperature compared to psilocybin alone in male mice (p = 0.0172; **Figure 1P**). Neither psilocybin nor 5-HTR antagonism affected unilateral interactions in male mice. However, both 5-HT1AR and 5-HT2AR antagonism significantly reduced aggressive behaviour, which was primarily exhibited as mounting, followed by other dominance-related behaviours such as chasing, pinning, and fighting. Notably, these anti-aggressive effects were absent in mice that received 5-HTR antagonists in combination with psilocybin (**Supplementary Data 1**). Finally, when comparing mice that received psilocybin or saline together with 5-HTR antagonists, we observed opposing effects of psilocybin compared to antagonist treatment alone (i.e. in saline-treated mice) in the two antagonist groups. For mice that received the 5-HT2AR antagonist, the co-administration of psilocybin increased huddling (p = 0.0194), whereas for mice that received the 5-HT1AR antagonist, the co-administration of psilocybin reduced huddling (p = 0.0327). In contrast, both psilocybin-treated groups of male mice that received antagonists of 5-HTR subtypes exhibited reduced core body temperature compared to their saline-treated counterparts (5-HT1AR: p = 0.0193; 5-HT2AR: p = 0.0096; **Supplementary Data 3**).

Psilocybin modulates novelty-seeking behaviour in female mice through 5-HT1AR and 5-HT2AR mechanisms

We used the 3-chamber test to assess how psilocybin impacts social preference and novelty-seeking behaviour in mice (**Figure 2A**), and observed effects in female mice at 4 h post-

administration that were not related to changes in locomotor activity (**Figure 2B**). Psilocybin enhanced novelty-seeking with a trend toward increased sociability compared to controls (p = 0.097; **Figure 2C**) and a significant preference for the novel mouse (p = 0.0494; **Figure 2D**). Psilocybin also significantly increased grooming behaviour in female mice (p = 0.0443; **Figure 2E**), particularly during the choice phase of the trial (p = 0.0514; **Supplementary Data 4**). Rearing behaviour was not significantly affected by psilocybin (**Figure 2F**). Both 5-HT1AR and 5-HT2AR antagonism attenuated psilocybin-associated sociability under these assay conditions (5-HT1AR: p = 0.023; 5-HT2AR: p = 0.0252), but neither altered sociability in saline-treated controls (**Figure 2G**). This pattern was similarly reflected in direct sociability measures (5-HT1AR: p = 0.0243; 5-HT2AR: p = 0.0007; **Figure 2H**). Notably, 5-HT2AR antagonism blocked psilocybin-induced sociability without affecting locomotor activity, supporting a specific role for 5-HT2AR at the 4 h time point. In contrast, 5-HT1AR antagonism reduced locomotion at at this point (**Supplementary Data 4**), suggesting the suppression of sociability reflects reduced general activity rather than a direct effect on social behaviour. Therefore, the role of 5-HT1AR in mediating psilocybin's prosocial effects should be interpreted with caution. In male mice, neither locomotion nor sociability measures across the duration of the test were affected by psilocybin at this 4 h post-administration timepoint (**Supplementary Data 5**).

Psilocybin attenuates rearing and grooming in male mice at 24 hours post-administration, but does not alter sociability

Motivated by the possibility that the time course of effects of psilocybin on social behaviour may differ between males and females, we conducted the same 3-chamber test in male mice 24h post-administration (**Figure 3A**). We observed no significant changes in locomotor activity induced by psilocybin treatment (**Figure 3B**) or sociability measures (**Figure 3C, D**) However, it significantly reduced grooming (p = 0.0159; **Figure 3E**) and rearing behaviours (p = 0.0033; **Figure 3F**). In contrast to saline-treated females, 5-HT2AR antagonism significantly reduced sociability Z-scores in saline-treated males (p = 0.0168), an effect not observed in psilocybin-treated males that received the same antagonist (**Figure 3G**), which was mirrored in reduced sociability indices (p = 0.0077; **Figure 3H**). Although no significant effects of 5-HTR antagonism was evident in psilocybin-treated mice, pre-treatment with the 5-HT1AR antagonist revealed a trend toward reduced sociability (p = 0.0584; **Figure 3H**). At this 24 h post administration timepoint, 5-HTR antagonism did not affect overall locomotor activity or social preference of male mice (**Supplementary Data 6**) or female mice (**Supplementary Data 7**). Interestingly, 7 d post-administration, psilocybin significantly reduced the sociability Z-score (p = 0.0023) in female mice, reflecting a shift in preference toward familiarity over novelty, without affecting other behavioural measures. In male mice, psilocybin increased grooming behaviour 7 d following treatment (p = 0.0363; **Supplementary Data 8**), opposing the results observed after 4 h.

Psilocybin differentially modulates social motivation in male and female mice at 24 hours

Sociability in the 3-chamber task is also driven by motivational processes; therefore, we separately assessed the effects of psilocybin on social motivation using a barrier climbing test and observed distinct responses in male and female mice 24 h following administration of saline or psilocybin (with or without antagonism of the 5-HTR subtypes; **Figure 4A**). In females, psilocybin did not alter locomotor activity (**Figure 4B**) or the latency to cross the barrier across different trial phases (**Figure 4C**). However, psilocybin-treated females showed a trend toward increased social motivation, as indicated by a higher sociability Z-score for a novel conspecific (p = 0.0566; **Figure 4D**). The latency to cross the barrier was not affected by 5-HTR antagonism in either treatment group (**Figure 4E**), whereas the elevated sociability induced by psilocybin was blocked by 5-HT2AR but not 5-HT1AR antagonism (p = 0.0694; **Figure 4F**).

In males, psilocybin similarly had no effect on overall locomotor activity (**Figure 4G**), but it significantly increased the latency to cross the barrier when mice were first introduced to the arena (p = 0.0407; **Figure 4H**) and enhanced social preference toward (familiar) cage mates (p = 0.0349; **Figure 4I**). Neither social motivation nor sociability in male mice was influenced by 5-HTR antagonism under control (saline-treated) or psilocybin-treated conditions (**Figure 4J, K**). Additionally, 5-HT receptor antagonism did not affect locomotion or sociability Z-scores in either sex on this task (**Supplementary Data 9**).

Psilocybin prolongs NAc dopamine release in females during initial familiar social interaction at 7 days post administration and reduces immediate dopaminergic response in males

Based on the critical role of the DA system in social motivation and exploratory behaviour, we examined DA dynamics in the NAc during the barrier climbing test using fiber photometry to record $GRAB_{DA}$-mediated fluorescence as a proxy for DA release (**Figure 5A)**. As repeated interactions can attenuate the sensitivity of DA release over time, we focused our analysis on DA release during the first social interaction. Striatal DA was monitored in male and female mice 24 h after administration of either psilocybin or saline, during social exploration involving an empty cage, a familiar or novel mouse (**Figure 5C** for females; **Figure 5G** for males). DA dynamics in the NAc showed sex-and time-dependent effects. In female mice, psilocybin did not significantly affect mean or peak DA release during the first 2 s after sniffing either a familiar or novel mouse (**Figure 5D**). In contrast, male mice treated with psilocybin showed a significant reduction in peak DA release when making initial contact (sniffing) with a novel mouse (p = 0.0386), although overall and immediate average DA release were not significantly altered (**Figure 5H**). At 7 d post-administration, female mice exhibited a prolonged elevation in DA release in the NAc during the first interaction with the familiar mouse, but not the novel one (**Figure 5E**). However, this effect was not reflected in the immediate (0-2 s) mean or peak DA release (**Figure 5F**). In male mice,

psilocybin did not affect total DA release at 7 d (**Figure 5I**) but again led to a significant reduction in immediate peak DA release during the first sniff of the novel mouse (p = 0.0326). In line with the conventional view that novel conspecific interactions are more rewarding that familiar ones, male saline-treated mice showed significantly greater immediate peak DA release when sniffing a novel mouse compared to a familiar one (p = 0.0353; **Figure 5J**), which was not observed following psilocybin treatment.

Discussion

This study demonstrates that psilocybin produces sex- and time-specific effects on social behaviour, mediated by distinct serotonergic and dopaminergic mechanisms. Rather than inducing uniform "prosocial" outcomes, psilocybin modulated behaviour in a context- and sex-dependent manner, even in unstressed, socially housed mice, addressing a key gap in preclinical psychedelic research, where sex differences remain underexplored. While the oxytocin system is often considered central to sociability, its close interaction with the 5-HT system, along with the known prosocial effects of psychedelics, highlights the need to investigate serotonergic contributions. In this study, we examined the acute and delayed effects of a single dose of psilocybin on multiple aspects of sociability in male and female mice and assessed the involvement of 5-HT1AR and 5-HT2AR mediated actions. We also measured DA release in the NAc, a key region within the social reward circuit, to assess neurochemical correlates of social behaviour. Given the established interplay between serotonin and DA in regulating cognition and emotion (McCoy et al., 2025), our findings contribute important insights into the neurobiological substrates underlying psychedelic action.

Acute prosocial behaviour and temperature regulation

Within the acute period following psilocybin administration (60 min), female mice exhibited a pronounced increase in huddling behaviour, initially hypothesised to be driven by reductions in core body temperature, consistent with thermoregulatory social bonding mechanisms (Landen et al., 2024). Interestingly, the reduction in core body temperature observed in our study has previously been reported only at higher doses of psilocybin (3 and 10 mg/kg), but not at lower doses (0.03–1 mg/kg), with no significant sex differences or dose-by-sex interactions reported (McGriff et al., 2025). This apparent discrepancy may arise from methodological differences in how temperature was measured. Using an automated UID system, we continuously monitored temperature changes at 5-min intervals for up to 1 h post-administration, allowing detection of transient fluctuations without disturbing the animals. In contrast, the previous study assessed temperature only once, 30 min after administration, potentially overlooking dynamic thermoregulatory responses occurring within that time window. Crucially, when we examined the involvement of 5-HT receptors in huddling behaviour, the psilocybin-induced increase was abolished by both 5-HT1AR and 5-HT2AR antagonists, this was not recapitulated in temperature measurements, indicating a more

complex interplay between serotonergic signalling and temperature regulation. Specifically, 5-HT1AR antagonism reduced body temperature and suppressed huddling, pointing to its role in tonic thermoregulation and social bonding. In contrast, 5-HT2AR antagonism only reduced temperature in combination with psilocybin and partially disrupted huddling, suggesting it modulates the temporal dynamics of social behaviour, not thermoregulation *per se*. At 4 h, 5-HT2AR antagonism blocked psilocybin-induced sociability without affecting locomotion, whereas 5-HT1AR effects were confounded by reduced activity. Together, these findings suggest a dissociation between hypothermia and social bonding, with 5-HT1AR playing a more central role, in line with previous findings (Tan et al., 2020). Male mice did not exhibit psilocybin-induced huddling or changes in body temperature, consistent with reports that females are more sensitive to the acute behavioural effects of psilocybin (Farinha-Ferreira et al., 2025). Interestingly, both 5-HT1AR and 5-HT2AR antagonists reduced aggression in males, an effect reversed by co-administration of psilocybin. This suggests that psilocybin engages broader serotonergic circuits that override receptor-specific modulation of aggression, contrasting with previous studies in which 5-HT1AR agonists suppressed aggression, and antagonism reversed these effects (Tan et al., 2020). These differing outcomes may reflect compensatory signalling or altered serotonergic tone in the pair-housed animals used in the present study, compared to the socially isolated animals examined in the earlier work.

Sex differences in serotonergic architecture may further explain these divergent effects. Women exhibit higher 5-HT1AR binding potential in multiple brain regions, though binding fluctuates with the menstrual cycle (Jovanovic et al., 2006; Moses-Kolko et al., 2011), and may contribute to increased vulnerability to social anxiety and depression as conditions in which 5-HT1AR is a central target of SSRIs (Lanzenberger et al., 2007; Le Poul et al., 1995; Martin et al., 2020). In contrast, men show higher cortical 5-HT2AR expression (Moses-Kolko et al., 2011). Similar sex differences are observed in rodents whereby female rats exhibit higher 5-HT1AR signalling, while males show greater cortical 5-HT2AR expression (Pitychoutis et al., 2012), potentially explaining the differential involvement of these receptors in social and stereotypic behaviours in our study.

Interestingly, psilocybin-induced hypothermia persisted in both male and female mice, even when 5-HT1AR or 5-HT2AR were antagonised, suggesting that alternative mechanisms are involved. A strong candidate underpinning this effect is the 5-HT7R, which is implicated in thermoregulation and is widely expressed in key regions such as the preoptic area (POA) of the hypothalamus, raphe nuclei, and the parabrachial nucleus, all of which are central to autonomic temperature control (Hedlund et al., 2004; Negoiță et al., 2025). Notably, agonism of 5-HT7R has been shown to induce hypothermia, and the increased serotonergic tone following psilocybin administration may recruit this receptor to mediate the observed temperature decrease. 5-HT7R is also a therapeutic target of several second-generation antipsychotic and antidepressant drugs, further supporting its relevance in neuromodulatory processes (Andressen et al., 2015; Halberstadt &

Geyer, 2011; Okada et al., 2021). Additionally, non-serotonergic systems such as Gamma-Aminobutyric Acid (GABA)-ergic and glutamatergic signalling within the POA have been implicated in thermoregulatory responses and may interact with serotonergic inputs to shape psilocybin's hypothermic effects (Ishiwata et al., 2005; Sengupta et al., 2014).

Social preference and exploration

Consistent with previous reports (Lu et al., 2025; Song et al., 2025), psilocybin did not affect social preference in either sex at 24 h or 7 d post-administration. However, at 4 h post treatment, female mice showed increased grooming and social exploration, behaviours associated with both heightened anxiety and sociability in C57BL/6 mice (An et al., 2011). The prosocial effects were abolished by both 5-HT1AR and 5-HT2AR antagonism, confirming involvement of these key serotonin receptor subtypes. At 24 h, psilocybin reduced grooming and rearing in male mice, behaviours that may reflect anxiolytic effects or reduced behavioural responsiveness. Rearing, an investigatory behaviour, more frequent in males (Sturman et al., 2018) is reduced by psilocin through 5-HT2AR mechanisms (Halberstadt et al., 2013), while grooming is suppressed by SSRIs and 5-HT1AR agonists (Brookshire & Jones, 2009) and high doses of psilocybin or LSD (Brownstien et al., 2025; Bruno et al.; Kyzar et al., 2016). These observations collectively support the idea that the reductions in grooming and rearing may reflect either anxiolytic effects or general behavioural blunting, potentially due to psilocybin dampening stress-related behaviours.

Although earlier findings reported increased sociability in stressed female mice 24 h after psilocybin treatment (Meng et al., 2025), we did not observe similar prosocial effects at this time point in our unstressed, wild-type mice. However, we found robust effects at other time points, suggesting that the absence of stress does not preclude psilocybin's behavioural efficacy. These findings challenge the notion that stress or social deficits are necessary preconditions for psilocybin to exert prosocial effects. While prior work has shown that psilocybin can reverse social impairments in models such as autism spectrum disorder, with no effect in controls (Song et al., 2025), our data indicate that psilocybin can modulate social behaviour in healthy animals in a time- and context-dependent manner.

Additionally, our data support previous evidence that 5-HT1AR plays a key role in mediating the social effects of serotonergic compounds. Specifically, we observed that the 5-HT1AR antagonist WAY-100635 blocked psilocybin-induced prosocial behaviours, consistent with earlier reports showing that the same antagonist prevented MDMA-induced increases in social interaction in male rats (Morley et al., 2005). These findings suggest that psilocybin does not produce fixed behavioural outcomes but instead initiates dynamic processes that unfold over time and differ between sexes. The persistence of effects beyond the acute pharmacological window aligns with clinical reports of long-lasting therapeutic benefits and may reflect underlying yet interrelated mechanisms such as neuroplastic changes, altered social learning, or sustained serotonergic

adaptations. The female-specific shift toward familiar social targets at later time points may be indicative of memory consolidation processes. Additionally, the time-dependent effects of psilocybin on preference towards familiar mice could be influenced by interactions between serotonergic and estrogen signalling pathways (Shadani et al., 2024). Although we did not control for the estrous cycle in all behavioural tests, no significant correlation was observed between estrous stage and sociability in female mice (**Supplementary Data 10**). However, studies in female rats have shown that those in the estrous and proestrous phases (when circulating levels of estradiol and progesterone are highest) were less affected by psilocin and LSD treatment across various behavioural measures compared to male rats (Pálenícek et al., 2010; Tylš et al., 2016).

Dopamine dynamics and social reward salience

NAc DA dynamics revealed a possible mechanistic substrate for sex-specific behavioural responses to psilocybin. In females, psilocybin did not alter acute DA responses but induced a prolonged elevation in DA release during familiar, but not novel, interactions at 7 d post-administration. This may reflect enhanced social memory or encoding of familiar cues, consistent with findings that familiarity plays a larger role in prosocial behaviours in females (Misiołek et al., 2023). The psilocybin-induced increase in NAc DA during familiar interactions may reflect a recalibration of social reward circuits, wherein familiar social experiences are no longer “neutral” or “low-reward” but instead become re-evaluated as uniquely rewarding within a new affective or cognitive context, a hallmark of flexible cognition. The delayed response aligns with neuroplastic adaptations, such as increased spine density in the cortex of female mice post-psilocybin (Shao et al., 2021) and improved cognitive flexibility (Conn et al., 2024; Meshkat et al., 2024). These complex interaction underscores that NAc DA signals multiple dimensions including salience, motivation, valence, prediction error. Psilocybin may recalibrate these behavioural computations in sex-specific ways.

In males, psilocybin reduced peak DA release during novel social encounters at both 24 h and 7 d, aligning with behavioural shifts toward familiar preference and reduced novelty salience. Saline-treated controls displayed the expected novelty-biased DA response (Dai et al., 2022), which was absent in psilocybin-treated males. This suggests that psilocybin dampens novelty-driven dopaminergic activity, potentially reshaping the social reward landscape. Whether this reflects reduced novelty "appeal" or increased reward value of familiar conspecifics remains uncertain. However, since the behavioural task employed for photometry recordings did not require choosing between familiar and novel conspecifics, the latter explanation may be more plausible. Consistent with our observations of sex differences in the effects of psilocybin, a recent study also reported sex-specific responses, with psilocybin eliciting stronger behavioural effects and calcium transients in the paraventricular nucleus of female mice compared to males (Cook et al., 2025).

Some limitations warrant consideration: First, we only tested a single psilocybin dose, which limits conclusions about dose-response or cumulative effects. Second, our study focused on only two receptor subtypes (5-HT1AR and 5-HT2AR), while others like the 5-HT7R, 5-HT1BR and 5-HT2CR, which also regulate thermoregulation and social behaviour, should be explored in future work (Negoiță et al., 2025; Wu et al., 2021). Additionally, we observed that 5-HT1AR antagonism reduced locomotor activity in female mice at the 4 h time point. This may reflect a sex-specific sensitivity to the antagonist dose, which complicates interpretation of the associated sociability findings, as the doses were based on prior studies conducted in a different experimental context. While this potential confound should be acknowledged, the observed effects across other behavioural and pharmacological measures still offer preliminary insights into receptor-specific contributions that warrant further investigation. Third, although we discuss serotonergic-dopaminergic interactions, we did not assess the oxytocin system, which plays a central role in social recognition and bonding (Raam et al., 2017). Finally, while Fiber Photometry is a powerful tool for monitoring DA dynamics, it captures only population-level changes and does not quantify absolute concentrations or resolve fine-scale spatial activity. Moreover, these recordings do not clarify whether psilocybin's effects arise from direct modulation of DA neuron activity in the midbrain (e.g. Ventral Tegmental Area or substantia nigra) or from local changes in the NAc, potentially mediated by 5-HT2AR signalling (Alex & Pehek, 2007; Yan, 2000).

Despite these limitations, this study is the first, to our knowledge, to assess acute and delayed effects of psilocybin on multiple dimensions of social behaviour and DA release in male and female mice. Importantly, the use of a single-dose paradigm offers a clearer interpretation of psilocybin's effects, avoiding potential confounds from repeated administration and more accurately reflecting current therapeutic contexts. Additionally, the choice to focus on 5-HT1AR and 5-HT2AR, two of the most well-characterised 5-HT receptors in terms of behavioural effects, strengthens the interpretability of our findings. We demonstrate that psilocybin modulates social behaviour and core body temperature through sexually dimorphic and temporally dynamic mechanisms, mediated by 5-HT1AR and 5-HT2AR signalling and NAc dopaminergic pathways. While 5-HT2AR has historically dominated psychedelic research, our results highlight the need to broaden this focus toward other neurochemical systems. These findings challenge the assumption of universal prosocial effects and underscore the critical importance of incorporating SABV in both preclinical models and therapeutic development to ensure efficacy and relevance across sexes. Future studies should investigate broader receptor systems, hormonal influences, and circuit-level mechanisms to further elucidate the complex, sex-specific neurobiology underlying psychedelic modulation of social cognition and behaviour.

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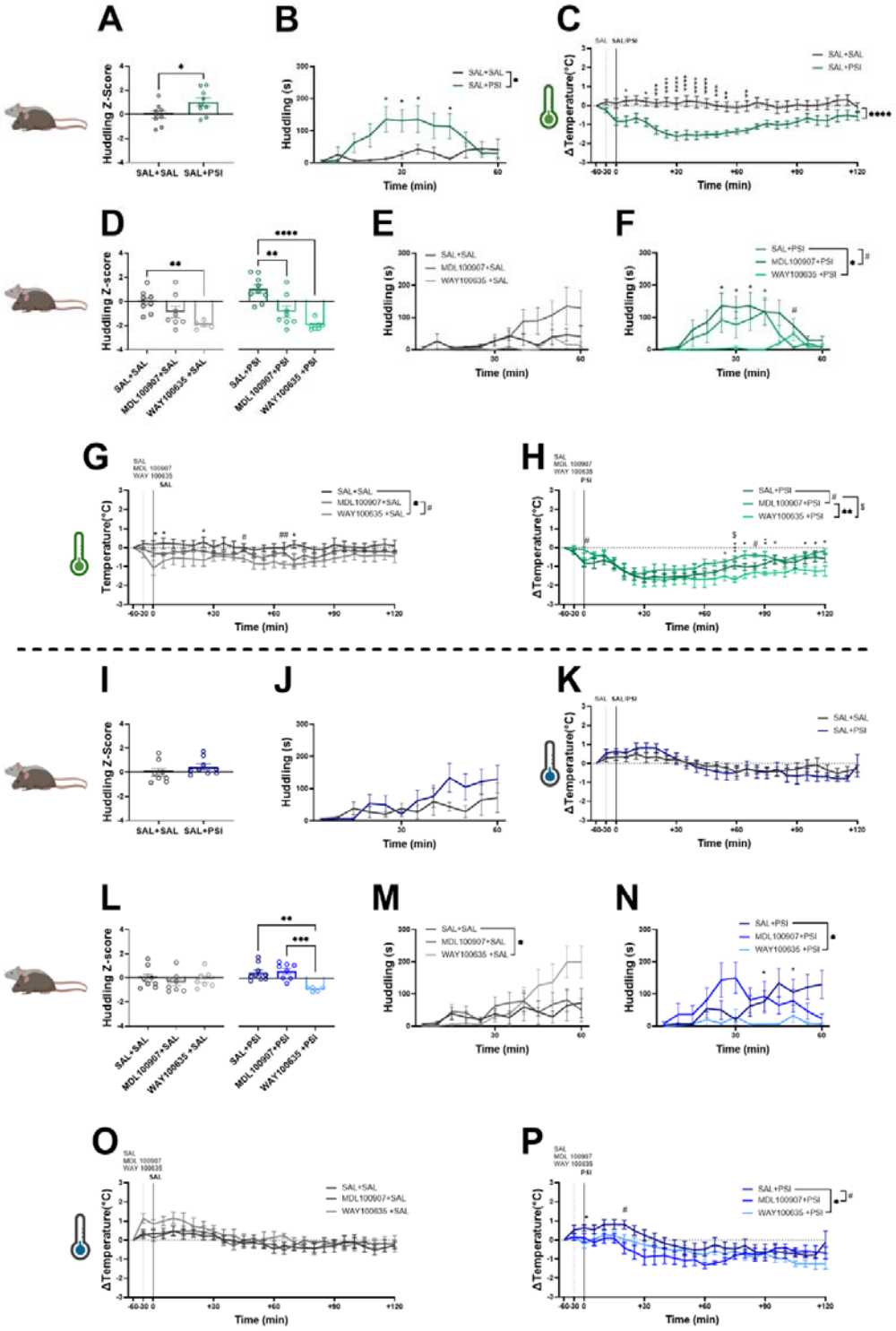

Figure 1. Psilocybin increases huddling in females but not males, while 5-HT1A and 5-HT2A receptor antagonism modulate huddling and temperature in a sex-specific manner. (A) Psilocybin significantly increased huddling behaviour compared to the controls (p = 0.0391) with **(B)** the huddling behaviour peaking at 25 min (p = 0.0133), 30 min (p = 0.0155) and 45 min (p = 0.0311) post-administration. **(C)** Psilocybin significantly reduced core body temperature in a time-dependent manner between 5-70 minutes post-administration (F (1,160) = 16.1, p = 0.001). **(D)** Among SAL-treated mice, 5-HT1AR antagonism reduced huddling behaviour (p = 0.0062) and in PSI-treated mice, both

5-HT1AR (p < 0.0001) and 5-HT2AR (p = 0.0029) antagonists reduced huddling behaviour. **(E)** 5-HTR antagonism did not significantly affect huddling behaviour over the 60 minutes observation period in SAL-treated mice however, **(F)** in PSI-treated mice, 5-HT1AR antagonism reduced huddling behaviour at 25 min (p = 0.0228), 30 min (p = 0.0158), 35 min (p = 0.0275) and 40 min (p = 0.0428) while 5-HT2AR reduced huddling behaviour only at 50 min (p = 0.0419) compared to PSI-treated group. **(G)** In SAL-treated mice, 5-HT1AR antagonism lowered core body temperature at 0 min (p = 0.0206), 5 min (p = 0.0398), 25 min (p = 0.0257), 65 min (p = 0.027) and 70 min (p = 0.0112) compared to SAL-treated group and it produced a stronger temperature reduction than 5-HT2AR antagonist at 45 min (p = 0.0258) and 65 min (p = 0.0099). **(H)** In PSI-treated mice, 5-HT1AR reduced the core body temperature at 75 min (p = 0.021), while 5-HT2AR increased temperature at 0 min (p = 0.0398) and 85 min (p = 0.0429) compared to PSI-treated group. Additionally, 5-HT1AR antagonism led to significantly lower body temperature than 5-HT2AR antagonism between 75-120 minutes (Time x Treatment interaction: F (2,22) = 2.868, p = 0.0152). **(I)** In male mice, total huddling behaviour was not significantly affected by psilocybin and **(J)** no time-dependent differences in huddling behaviour were observed over 60 minutes. **(K)** Core body temperature was not significantly altered by psilocybin. **(L)** In SAL-treated male mice, 5-HTR antagonism did not significantly affect the huddling behaviour. However, in PSI-treated mice, 5-HT1AR antagonism significantly reduced huddling behaviour compared to PSI only group (p = 0.0018) and compared to the 5-HT2AR antagonism (p = 0.0009). **(M)** No significant differences in huddling behaviour were observed over 60 minutes among saline-treated groups. **(N)** In PSI-treated mice, 5-HT1AR antagonism reduced huddling behaviour at 45 min (p = 0.0496) and 55 min (p = 0.0489). **(O)** Core body temperature did not differ significantly among saline-treated groups over 60 minutes. **(P)** In PSI-treated mice, 5-HT1AR reduced core body temperature at 0 min (p = 0.0289) and 5-HT2AR antagonism also lowered core body temperature at 20 min (p = 0.0165). Female mice (A-H), male mice (I-P)**,** psilocybin (PSI), saline (SAL), 5-HT2AR antagonist (MDL100907), 5-HT1AR antagonist (WAY100635). Data are presented as mean ± SEM. Statistical analyses were performed using unpaired t-test, mixed-effects models, one-way ANOVA or two-way ANOVA with Šidák post hoc tests. *p< 0.05, $^{\#}p$< 0.05, $^{\$}p$< 0.05, **$p$< 0.01, $^{\#\#}p$< 0.01, ***p< 0.001, ****p< 0.0001.

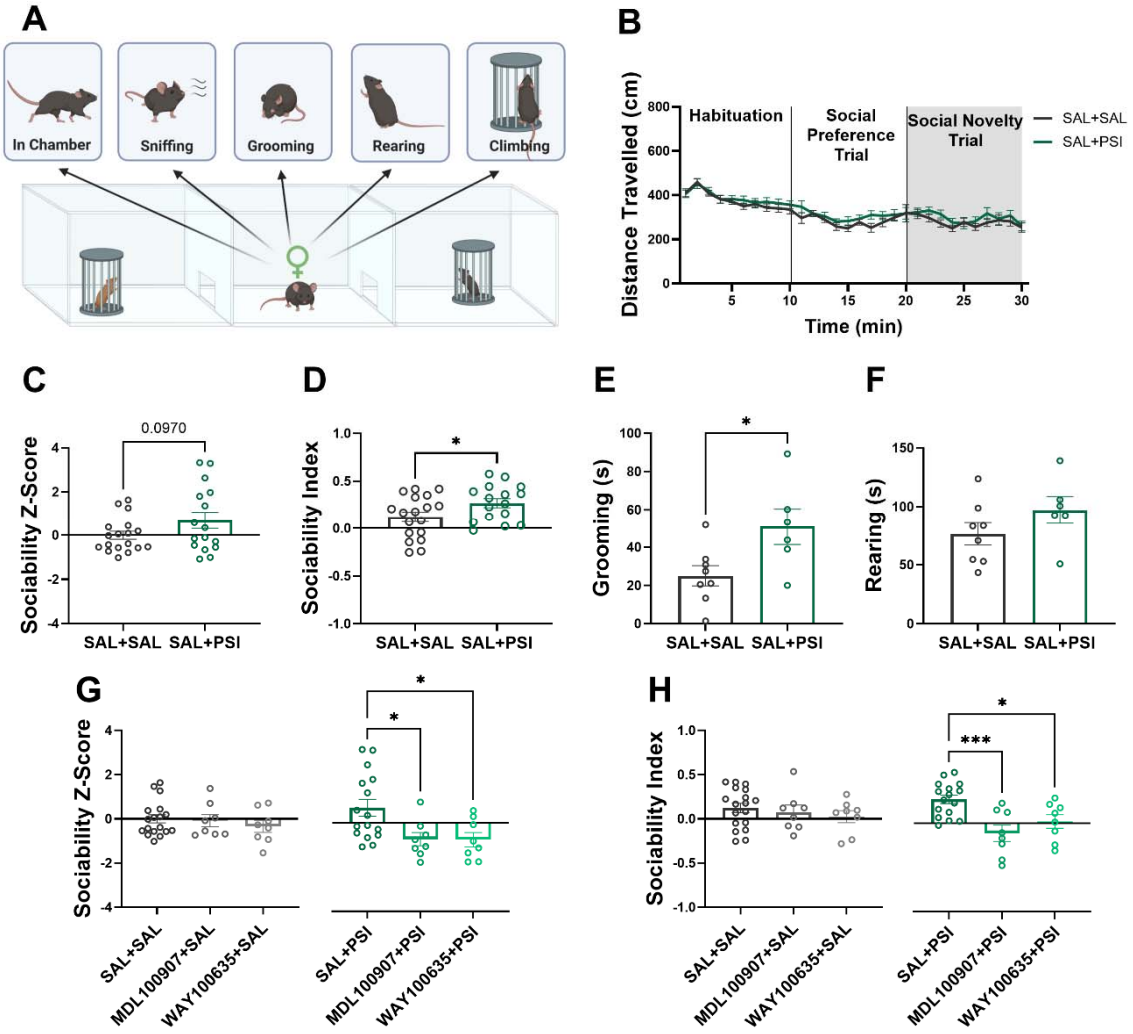

Figure 2. Psilocybin enhances sociability in female mice 4 hours after administration, and these effects are blocked by 5-HT receptor antagonism. (A) Schematic of the social novelty trial and the behaviours scored. **(B)** Psilocybin did not alter locomotor activity of mice across the duration of the experiment. **(C)** No significant differences were observed in the total sociability Z-score between the groups (p = 0.097). **(D)** PSI-treated mice displayed significantly higher sociability compared to controls (p = 0.0494). **(E)** Psilocybin increased grooming behaviour (p = 0.0443) but **(F)** did not affect the rearing behaviour in these mice. **(G)** In SAL-treated mice, 5-HTR antagonism did not affect sociability. In contrast, in PSI-treated mice, both 5-HT1AR (p = 0.023) and 5-HT2AR (p = 0.0252) antagonism significantly reduced sociability Z-score. **(H)** Similarly, 5-HTR antagonism did not affect sociability in SAL-treated mice but reduced sociability in PSI-treated mice with both 5-HT1AR (p = 0.0243) and 5-HT2AR (p = 0.0007) antagonism. Psilocybin (PSI), saline (SAL), 5-HT2AR antagonist (MDL100907), 5-HT1AR antagonist (WAY100635). Data are presented as mean ± SEM. Statistical analyses were performed using unpaired t-test, one-way ANOVA or two-way ANOVA with Šidák post hoc tests. *p< 0.05, ***p< 0.001.

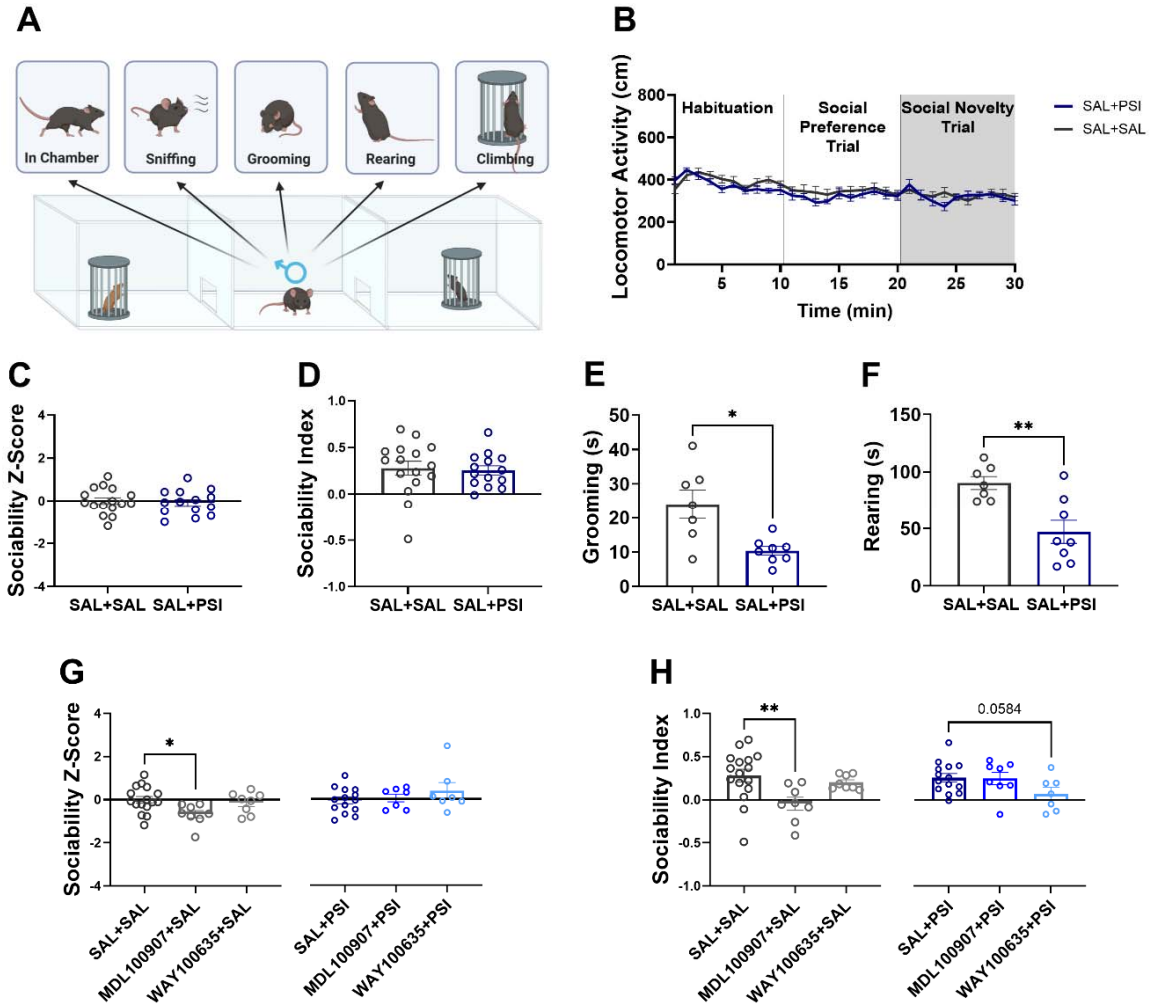

Figure 3. Psilocybin does not affect sociability but reduces grooming and rearing behaviour in male mice 24 hours after administration. (A) Schematic of the social novelty trial and the behaviours scored. **(B)** No effects of psilocybin were seen in locomotor activity of mice across the duration of the experiment. **(C,D)** Psilocybin did not affect the sociability of mice. **(E)** Psilocybin significantly increased grooming behaviour (p = 0.0159) and it **(F)** significantly reduced rearing behaviour (p = 0.0033). **(G)** In SAL-treated mice, 5-HT2AR antagonism reduced sociability Z-score (p = 0.0168). No effects of 5-HTR antagonism were observed in PSI-treated mice. **(H)** Similarly, 5-HT2AR antagonism reduced sociability in SAL-treated mice (p = 0.0077), while 5-HT1AR antagonism in PSI-treated mice showed a trend toward reduced sociability (p = 0.0584). Psilocybin (PSI), saline (SAL), 5-HT2AR antagonist (MDL100907), 5-HT1AR antagonist (WAY100635). Data are presented as mean ± SEM. Statistical analyses were performed using unpaired t-test, one-way ANOVA or two-way ANOVA with Šidák post hoc tests. *p< 0.05, **p< 0.01.

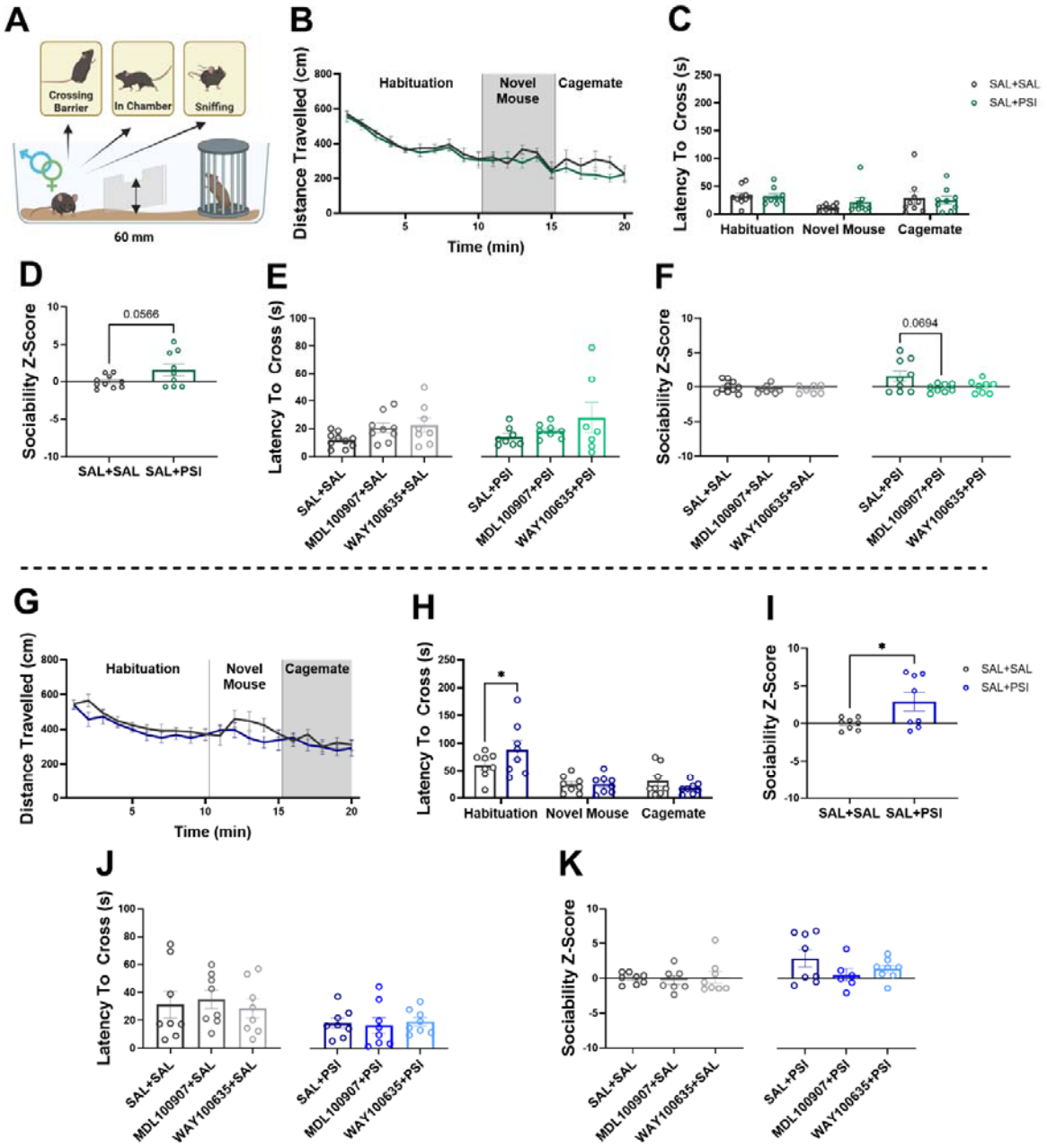

Figure 4. Sex-specific motivational shifts in social preference emerge 24 hours after psilocybin administration. (A) Schematic representation of the barrier climbing test and the behavioural measures assessed. **(B)** Locomotor activity and **(C)** the latency to cross the barrier across the trial was not affected by psilocybin in female mice. **(D)** Psilocybin increased the trend towards increased preference towards novelty in female mice (p = 0.0566). **(E)** 5-HTR antagonism did not significantly alter barrier crossing latency in female mice and **(F)** their sociability Z-score when exploring the novel conspecific however, in PSI-treated mice, 5-HT2AR antagonism showed a trend toward reducing sociability (p = 0.0694). **(G)** In male mice, no effects of psilocybin were seen in locomotor activity across the duration of the experiment. **(H)** Psilocybin increased latency to cross the barrier during the habituation phase (p = 0.0407). **(I)** PSI-treated male mice exhibited significantly higher sociability Z-score toward their cage mates compared to the controls (p = 0.0349). **(J)** 5-HTR antagonism did not significantly affect latency to cross or **(K)** sociability of male mice when exploring their cage mates. Female mice (A-E), male mice (F-J)**,** psilocybin (PSI), saline (SAL), 5-HT2AR antagonist (MDL100907), 5-HT1AR

antagonist (WAY100635). Data are presented as mean ± SEM. Statistical analyses were performed using unpaired t-test, one-way ANOVA or two-way ANOVA with Šidák post hoc tests. *$p < 0.05$.

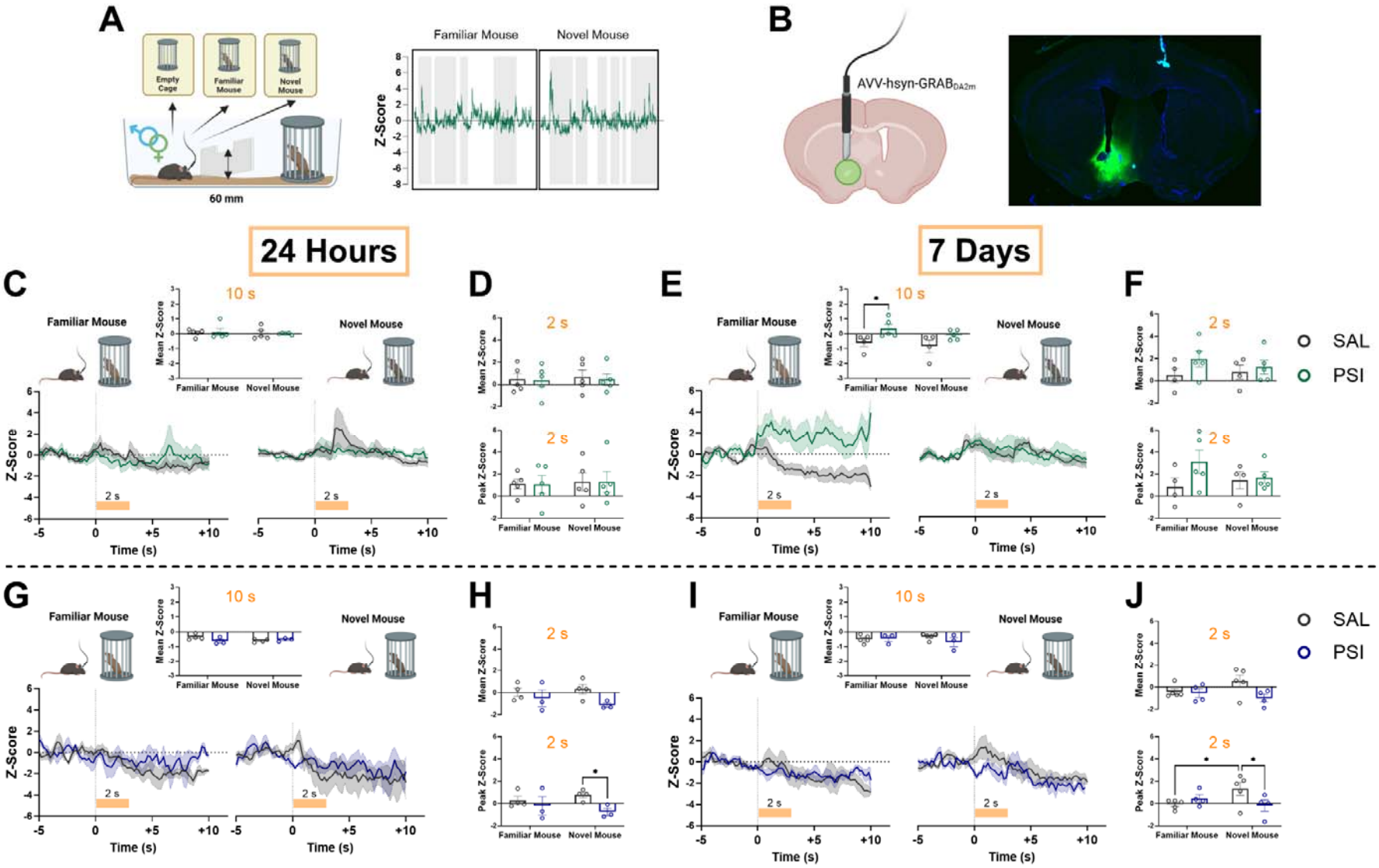
A
Empty Cage
Familiar Mouse
Novel Mouse
60 mm
Familiar Mouse
Novel Mouse
Z-Score
B
AVV-hsyn-GRAB$_{DA2m}$
24 Hours
7 Days
C
D
E
F
G
H
I
J
Familiar Mouse
Novel Mouse
10 s
2 s
Mean Z-Score
Peak Z-Score
Z-Score
Time (s)
SAL
PSI

Figure 5. Psilocybin enhances NAc dopamine (DA) release in female mice when sniffing a familiar conspecific at 7 days post-administration and suppresses novelty-evoked dopamine in males at 24 hours and 7 days during the barrier climbing test. (A) Schematic of the experimental design and the average $GRAB_{DA2m}$ signal across the full trial when the mouse was in the social zone (shaded area) versus the non-social zone. **(B)** Representative Fiber placement and virus injection in the NAc. **(C)** Representative $GRAB_{DA2m}$ traces from the first interaction with a familiar or a novel mouse at 24 hours post-administration of PSI or SAL control in female mice. Inset: no significant effect of PSI on DA release were observed during the first interaction with either mouse. **(D)** At 24 hours in females, PSI did not alter DA release within the first 2 seconds of sniffing either mouse. **(E)** At 7 days post-administration, representative traces show prolonged DA release in response to familiar (but not novel) sniffing in PSI-treated female mice. Inset: PSI significantly increased DA release within the first 10 seconds of familiar sniffing (p = 0.0317), **(F)** but early DA levels (0-2 s) remained unchanged. **(G)** $GRAB_{DA2m}$ traces recorded in male mice during the first sniff of a familiar or novel conspecific, 24 hours after PSI or SAL treatment. Inset: No significant effects of PSI on DA release were observed within the first 10 seconds of the interaction. **(H)** At 24 hours, PSI significantly reduced immediate (0–2 s) DA release during novel sniffing in males (p = 0.0386), with no effect on familiar sniffing. **(I)** Representative $GRAB_{DA2m}$ signal recordings from male mice during their first interaction of either a familiar or a novel conspecific, 7 days post-administration of PSI or SAL. Inset: No significant differences in total DA release within 10 seconds post-interaction. **(J)** At 7 days, PSI significantly reduced immediate novelty-evoked DA release in males (p = 0.0326); SAL-treated males showed greater DA release to novel vs familiar sniffing (p = 0.0353). Female mice (C-F), male mice (G-J)**,** psilocybin (PSI), saline (SAL) and data are presented as mean ± SEM. Statistical analyses were performed using unpaired t-test, one-way ANOVA or two-way ANOVA with Fisher's LSD post hoc tests. *p< 0.05.