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RESEARCH ARTICLE

A procedure to identify persistent and effort-independent individual differences in preference for heroin over rewarding social interaction

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Background and Purpose: In some individuals, opioid use leads to decreased interest in socially relevant rewards. Recent studies showed that after extended-access heroin self-administration, rats strongly prefer social interaction over single unit-dose heroin infusions. We hypothesized that this strong social preference results from access to a suboptimal heroin dose during testing, and individual differences in heroin versus social choice would emerge if rats were given access to their 'preferred' heroin dose.

Experimental Approach: In Experiment 1, we trained male rats to lever-press for social interaction, followed by heroin self-administration under continuous-access, no-timeout schedule, which promotes burst-patterned heroin taking. We then tested the rats for choice between single-unit heroin dose and 1-min full-contact social interaction, or 5-min heroin-access (sufficient for burst-patterned heroin taking) and 5-min social interaction. In Experiment 2, we extended the 5-min access procedure to female rats and tested heroin versus limited-contact (screen-based) social interaction. We also manipulated response requirements (effort) for heroin.

Key Results: Rats given a single-unit heroin dose during choice testing, strongly preferred social interaction. In rats given 5-min heroin-access, large individual differences in heroin preference emerged. These differences were independent of sex, social-interaction conditions and effort manipulations. High heroin intake and burst-patterned heroin taking during self-administration, and high heroin seeking during abstinence predicted individual differences in heroin preference.

Conclusion and Implications: Access to 'preferred' heroin doses during the choice tests leads to stable and effort-independent individual differences in heroin

Abbreviations: FR, fixed ratio; No-TO, no-timeout; SA, self-administration.

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preference. This procedure provides a platform to study mechanisms of resilience and vulnerability to opioid addiction.

KEYWORDS
addiction, animal models, discrete choice, individual differences, opioids, social

1 | INTRODUCTION

Each year, millions of people use opioids at least once (European Monitoring Centre for Drugs and Drug Addiction, 2023). However, only a subset develops opioid addiction (Anthony & Helzer, 2002; Kreek et al., 2019). Compared to recreational users, individuals who transition to addiction typically engage in harmful drug-taking patterns, such as administering high opioid doses through rapid routes (e.g., intravenous injection) to achieve an 'euphoric rush', experiencing severe withdrawal and facing a high risk of overdose (Darke, 2011; Dole et al., 1966; Griffiths et al., 1994; McAuliffe & Gordon, 1974). These behaviours are often driven by intense craving and a preference for opioids over non-drug rewards, such as social relationships (Bornstein & Pickard, 2020; Heilig et al., 2016; Hogarth, 2020; Zilverstand et al., 2018).

Despite the importance of individual differences in addiction vulnerability, most preclinical research focuses on group-level mechanisms and rarely examines individual variability (Augier et al., 2018; Deroche-Gamonet et al., 2004; Kuhn et al., 2025). This state of affairs may be due to two factors that promote group-level investigation, rather than that of individual differences. First, preclinical self-administration training often yields uniform drug taking behavior across subjects due to 'over-constrained' designs, including experimenter-imposed fixed unit-doses and timeout intervals (Morgan et al., 2009; Roberts et al., 2013; Roberts & Zimmer, 2020). Second, in preclinical drug-versus-non-drug choice procedures, where rats choose between an opioid or psychostimulant unit-dose and a non-drug reward (such as palatable food or social interaction), most rats strongly prefer the non-drug reward (Ahmed, 2010, 2018; Venniro et al., 2020), suggesting that drug preference may not be as pronounced in rats as in humans (American Psychiatric Association, 2013; Heilig et al., 2016; Hogarth, 2020).

Our study aimed to develop a human-relevant procedure in which individual differences in drug self-administration and choice naturally emerge when rats choose between heroin and rewarding social interaction. To achieve this goal, we combined a **heroin** self-administration procedure that promotes burst-patterned drug intake (modelling binge-like drug-taking observed in humans) and within which individual differences naturally emerge (D'Ottavio et al., 2025), with a refined version of the drug-versus-social choice procedure (Venniro et al., 2019; Venniro & Shaham, 2020). Our heroin self-administration procedure eliminates timeouts after each drug infusion, resulting in burst-patterned heroin intake (D'Ottavio et al., 2025), mimicking harmful human drug-taking patterns (Darke, 2011; Faupel, 1991; Griffiths et al., 1994; Harding, 1988). In our procedure, rats rapidly self-administered multiple unit doses, followed by periods of inactivity (D'Ottavio et al., 2025). This loading phase leads to high heroin brain concentrations, following the dose chosen by the rats themselves, reflecting their 'preferred' drug dose (Morgan et al., 2009; Roberts & Zimmer, 2020).

We hypothesized that a single unit-dose—a fraction of the rats' 'preferred' dose (Roberts & Zimmer, 2020)—is insufficient in drug-versus-non-drug choice procedures when compared to an alternative highly rewarding social interaction (Venniro et al., 2022, 2018). Therefore, to identify bona fide individual differences, a choice procedure should compare the rats' 'preferred' heroin dose—not a unit-dose—against an alternative incentive. To test this hypothesis, we trained rats for full-contact social self-administration with a peer, followed by heroin self-administration (without timeouts). We then tested them in two heroin-versus-social choice procedures: (1) a standard choice procedure, where rats could access one unit-dose of heroin versus 1 min

What is already known?

- People addicted to heroin seek rapid, high-doses euphoric-rush, while social engagement gradually deteriorates.
- In preclinical drug-versus-nondrug choice procedures, rats prefer rewarding social interaction over opioid and psychostimulant drugs.

What does this study add?

- Access to 'preferred' heroin doses during choice tests leads individual differences in heroin preference.
- High heroin intake, burst-patterned heroin taking and high heroin seeking during abstinence predicted heroin preference.

What is the clinical significance?

- Our model can identify rats preferring heroin over social interaction, reflecting a key addiction trait.
- Our model can be used to identify new treatments targeting social impairments in addiction.

of full-contact social interaction (Venniro et al., 2019, 2018), and (2) a revised choice procedure, where rats could engage in 5 min of heroin self-administration (enabling burst-patterned heroin taking; D'Ottavio et al., 2025) or 5 min of full-contact social interaction. In a second set of experiments, we assessed the generality of the results to females and to the limited-contact fully automated social reward self-administration procedure, where rats interact through a screen (Venniro et al., 2019). Finally, we tested if other addiction-like behaviours in rats, such as high and burst-patterned heroin taking (Ahmed et al., 2000; Belin et al., 2009) and relapse to heroin seeking (Reiner et al., 2019), would predict individual differences in heroin preference.

2 | METHODS

2.1 | Animals

All animal care and experimental procedures carried out at the Santa Lucia Foundation IRCCS in Rome followed the guidelines of the Italian national law (DL 26/2014) on the use of animals for research based on the European Communities Council Directive (2010/63/UE) and approved by the ethics committee of the Italian Ministry of Health and by local Ethical Committee of the Italian Ministry of Health and the local Ethical Committee of the Santa Lucia Foundation. All animal care and experimental procedures carried out at National Institute on Drug Abuse in Baltimore, MD, USA followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf) and approved by the National Institute on Drug Abuse Intramural Research Program Animal Care and Use Committee. Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020).

We used a total of 134 (112 males [52 social partners], 22 females [11 social partners]) Sprague-Dawley rats (Charles River; CD [SD], RRID:RGD_734476) for the study. At the beginning of the experiments, the rats were 5–6 weeks old and weighed between 125–175 g. The rats were pair-housed before the start of social self-administration and individually afterwards. Throughout the experiments, the rats were maintained on a reversed 12-h light/dark cycle (Rome: lights off at 6 AM; NIDA: lights off at 8 AM) with free access to standard laboratory chow and water. Eight rats were excluded due to catheter problems or sickness.

In all the experiments, the investigators were not blinded to the training conditions.

2.2 | Drug treatments

Heroin was donated from the Drug Supply Program of the National Institute on Drug Abuse (NIDA) and dissolved in sterile saline (0.9% NaCl). For drug self-administration training, we used unit-doses of 0.075 mg·kg^{-1} per infusion for heroin hydrochloride (diamorphine), based on previous studies (Airavaara et al., 2011; D'Ottavio et al., 2023; O'Neal et al., 2020).

2.3 | Intravenous surgery

We anaesthetised the rats with **isoflurane** (5% induction, 2%–3% maintenance) and injected them with analgesic (Rome: **Carprofen** [2 mg·kg^{-1}, subcutaneous injection]; NIDA: **ketoprofen** [2.5 mg·kg^{-1} subcutaneous injection]), immediately after the surgery and for the following 5 days to relieve pain and decrease inflammation. We inserted a silastic catheter into the jugular vein as previously described (Caprioli et al., 2015; Caprioli et al., 2007). We placed the distal end of the catheter into the right jugular vein, attached the proximal end of a modified 22-gauge cannula and placed it on the back in the mid-scapular region. We allowed the rats to recover for a minimum of 5–7 days. We flushed the catheters daily with a 0.2-ml sterile saline solution containing **gentamicin** (4.25 mg·ml^{-1}) to test the catheter patency and prevent occlusion during the recovery, training and abstinence phases. If during the training phase the catheter failed the patency test, we catheterized the left jugular vein using the same procedure or eliminated the rat from the study.

2.4 | Experiment 1. Effect of heroin access time on choice for heroin versus social interaction

In Experiment 1 (conducted in Rome, n = 86 males [43 social partners]), we tested the hypothesis that the unit-dosing strategy for heroin was suboptimal in a heroin-versus-social choice procedure and masks individual differences in heroin preference. We compared two choice procedures: (1) a standard procedure where the rats chose between 1 unit-dose of heroin and 1 min of social interaction and (2) a revised procedure where the rats chose between 5 min of heroin self-administration (allowing for burst-patterned heroin taking) and 5 min of social interaction.

2.4.1 | Self-administration chambers

Chambers were equipped with a stainless-steel grid floor, and two operant panels placed on the left and right walls. The left panel was equipped with a house light and the drug-paired active (retractable) lever. Responses on this lever activated the infusion pump and the discrete white light located above the lever. The right panel was equipped with the social partner-paired active (retractable) lever. Responses on this lever activated the three-light located above the lever and determined the opening of the guillotine-style sliding door. The drug was delivered through a modified cannula (Plastics One; Roanoke, VA, USA) connected to a liquid swivel (Instech; Plymouth Meeting, PA, USA) via polyethylene-50 tubing that was protected by a metal spring.

2.4.2 | Social self-administration

The training procedure used here was modelled on that described previously (Venniro et al., 2018). We trained the rats to press a lever to have access to a social partner 2 h·day^{-1} for 6 days. The resident rats were housed with their social partner (cage mate) until 3 days prior to social interaction self-administration, and each resident rat lever pressed for its previously paired partner. The training sessions started with the illumination of the house light, followed 10-s later by the insertion of the social partner-paired lever (that remained inserted throughout the session); responses on this lever resulted in access to the social partner (1 min, full-contact interaction), paired with the illumination of the three-light cue (1 min).

2.4.3 | Heroin self-administration

After the social self-administration, we trained the rats to self-administer heroin 6 h·day^{-1} for 15 days under continuous-access no-timeout conditions. Heroin self-administration training sessions started with the illumination of the house light, followed 10-s later by the insertion of the drug-paired lever. Responses on the active lever (fixed ratio 1 [FR1] schedule) were reinforced by a heroin infusion (over 3 s), paired with the cue light (3 s) (D'Ottavio et al., 2025). After every three consecutive drug self-administration sessions, we tested the rats for drug versus social interaction preference in a discrete choice procedure (see below). We randomly assigned the rats to the two choice conditions described below.

2.4.4 | Heroin seeking test

We tested the rats for drug seeking under extinction conditions on abstinence Day 1. The duration of the test sessions was 30 min. The sessions began with the illumination of the house light, followed 10-s later by the insertion of the drug-paired lever; the house light remained on for the duration of the session. Lever presses during the tests resulted in the contingent presentation of the light cue (3 s), previously paired with unit-doses of drug, but no drug infusion was delivered. After the heroin seeking test on Day 1, we brought the rats to their home cages.

2.4.5 | Discrete choice

We conducted the choice procedure using the same parameters (dose of drug and time of access to the social partner per reward and stimuli associated with the two active retractable levers) selected for the training phase. We allowed the rats to choose between the drug-paired and the social partner-paired levers. We tested the rats for 11 days.

1. *Discrete choice '1-minute social access vs 1 unit-dose of heroin'*

Choice sessions lasted for 120 min. Each 120-min choice session was divided into 15 discrete trials that were separated by 8 min. Each trial began with the presentation of the house light followed 10-s later by the insertion of both the social partner-paired and drug-paired levers. Rats then had to select one of two levers. The operant response requirement for the lever's selection was set to two consecutive responses (FR2) to avoid accidental choices. If the rats responded within 3 min, they received the reward corresponding with the selected lever. Reward delivery was signalled by the social partner-paired cue (1 min) or drug-paired cue (20 s), the retraction of both levers and turning off the house light. If the rat failed to respond on either active lever within 3 min, both levers retracted, and the house light was turned off with no reward delivery (Venniro et al., 2018).

2. *Discrete choice '5-minutes social access vs 5-minutes heroin access'*

Choice sessions lasted for 6 h. Each 6-h choice session was divided into six discrete trials that were separated by 55 min. Each trial began with the flashing of the house light for 30 s (a discriminative stimulus that signalled the choice session), followed by the illumination of the houselight and the insertion of both the social partner-paired and drug-paired levers. The operant response requirement for the lever's selection was set to five consecutive responses (FR5). If the rats responded within 3-min, they had access to the selected lever for a total of 5 min under an FR1 schedule. The duration of access to the social partner was matched with the time allocated to heroin access. We chose to match the choice time for heroin and social interaction, because Chow et al. (2022) demonstrated that access time to the social partner has no significant effect on the rewarding effects of social interaction (assessed by choice between different access times and progressive ratio responding). If the rats failed to respond on either active lever within 3 min, both levers were retracted, and the house light was turned off with no reward delivery.

2.4.6 | Discrete choice with increasing access time to a social partner

We tested a subgroup of rats (n = 16) for four consecutive days in a choice procedure with incremental access time to the social partners. To allow the rats to evaluate each option separately before making their subsequent choice, each choice session was preceded by four sampling trials spaced by 55 min. During the sampling, each trial began with the flashing of the house light for 30 s (discriminative stimulus that signalled the session), followed by the illumination of the house light and the insertion of only one of the two levers (heroin-paired, social-paired) in a random order. We counterbalanced the order of lever presentation (i.e., whether heroin- or social-paired lever

was presented first) across rats. During the sampling, the rats were required to complete each trial to advance (Chow & Beckmann, 2021). During the choice (four trials), each trial began with the flashing of the house light for 30 s (discriminative stimulus that signalled the session), followed by the illumination of the house light and the insertion of both the social partner-paired and heroin-paired levers. During sampling and choice, the response requirement was set to 5 (FR5 schedule) consecutive responses. Next, the access time to the social partner was increased across testing days from 1 to 15 min and the access time to heroin was maintained constant at 1 min (see Figure 1).

2.4.7 | Discrete choice during late abstinence

On day 60 from the beginning of the experiment, we tested the rats for discrete choice after prolonged abstinence. This choice test was preceded by four sampling trials, as described above, to allow rats to evaluate each option separately before making their subsequent choice. After the sampling, we allowed the rats to choose between the social partner-paired and drug-paired levers. During the choice (four trials), each trial began with the flashing of the house light for 30 s (discriminative stimulus that signalled the session), followed by the illumination of the house light and the insertion of both the social partner-paired and heroin-paired levers.

2.5 | Experiment 2. Generality of the effect of heroin access time during choice to female rats and screen-based social interaction

In Experiment 2 (conducted in NIDA, n = 18 males [-9 social partners], n = 22 females [11 social partners]), we tested if the results of Experiment 1 would generalize to females and to the limited-contact, screen-based social interaction procedure (Venniro et al., 2019).

2.5.1 | Self-administration chambers

Chambers were equipped with a stainless-steel grid floor, and two operant panels placed on the left and right walls. The left panel was equipped with a red-light, a white-light, an inactive lever and a drug-paired active (retractable) lever. Responses on this lever activated the infusion pump and the white light located above the lever. The right panel was equipped with a house light, a tone and a social partner-paired active (retractable) lever. Responses on this lever activated the tone located above the lever and determined the opening of the guillotine-style sliding door.

2.5.2 | Social self-administration

The training procedure was as described in Experiment 1, with the key difference that the rats interacted through a screen (screen interaction), as previously described (Venniro et al., 2019). The training sessions started with the illumination of the house light followed 10-s later by the insertion of the social partner-paired lever (that remained inserted throughout the session); responses on this lever resulted in access to the social partner (1 min), paired with the activation of the tone cue (1 min).

2.5.3 | Drug self-administration

The training procedure was as described in Experiment 1. Drug self-administration training sessions started with the illumination of the red-light followed 10 s later by the insertion of the drug-paired lever. Responses on the active lever (FR1 schedule) were reinforced by unit-doses of drug (3 s), paired with a white-light (3 s).

2.5.4 | Drug seeking test

We tested the rats for drug seeking under extinction conditions on abstinence Day 1 as described in Experiment 1. The sessions began with the illumination of the red-light, followed 10-s later by the insertion of the drug-paired lever. Lever presses during the tests resulted in the contingent presentation of the white-light cue (3 s), previously paired with unit-doses of drug, but no drug infusion was delivered.

2.5.5 | Discrete choice

We conducted the choice procedure as described in Experiment 1—Discrete choice '5 vs 5 minute access'. Choice sessions lasted for 6 h. Each 6 h choice session was divided into six discrete trials that were separated by 55 min. We allowed the rats to choose between the drug-paired and the social partner-paired levers. Each trial began with the illumination of the house light and the insertion of both the social partner-paired and drug-paired levers. The operant response requirement for the lever's selection was set to two consecutive responses (FR2 schedule). In Experiment 1, we observed that responding was stable after 7 days of choice; thus, we tested the rats for a total of 7 days.

2.5.6 | Discrete choice with increasing effort required to access heroin

After the third consecutive day of the choice test, we tested the rats in a discrete choice procedure with increasing effort required to access heroin.

Throughout this phase, the response requirement for social reward was 2 (FR2 schedule). Heroin was available on an FR4 schedule during the first session, FR8 in the second session, FR16 from sessions three to five and FR32 from sessions six to nine. We tested the

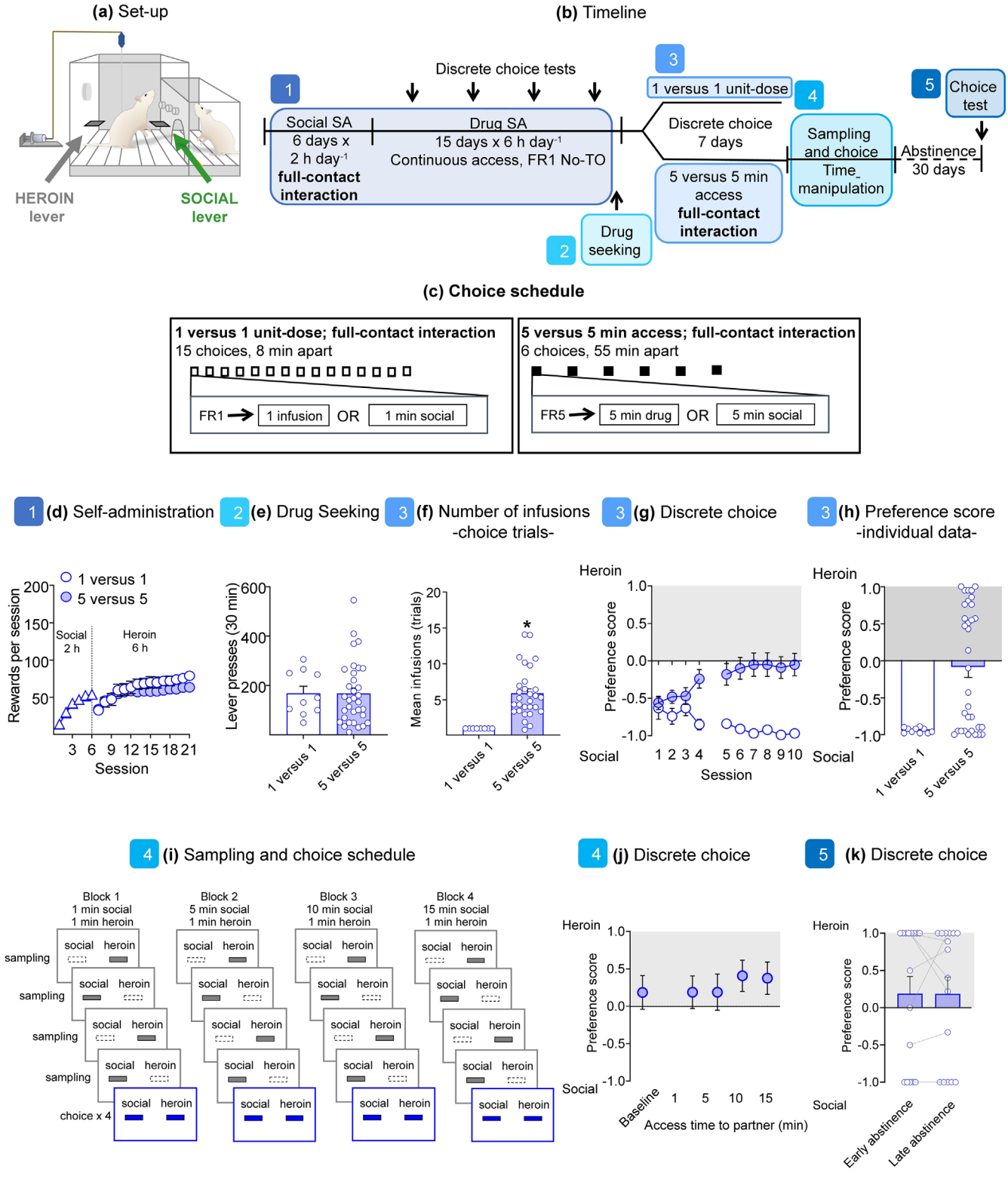

FIGURE 1 Legend on next page.

rats on FR16 and FR32 schedules over several days to determine if the heroin-preferring rats would shift their preference to social interaction because of the higher effort required to obtain heroin.

2.6 | Data and statistical analysis

All the data and statistical analysis comply with the reccommendations on experimental design and analysis in pharmacology (Curtis et al., 2022). Data were analysed with the statistical programs SPSS (SPSS, Version 30; RRID:SCR_002865), GraphPad Prism (Version 10; RRID:SCR_002798) and XLSTAT (RRID:SCR_016299). Before any analysis, we tested the data for sphericity and homogeneity of variance when appropriate. When the sphericity assumption was not met, we adjusted the degrees of freedom using the Greenhouse–Geisser correction. Outliers were included in the data analysis and presentation. The level of probability (*P*), for determining group differences, was set at $P < 0.05$. Significant main effects and interactions ($P < 0.05$) were followed with post hoc tests (Fisher's PLSD), which were conducted only if the *F* values in the analyses achieved the appropriate level of statistical significance (see Table S1 for a complete reporting of the statistical analyses).

2.6.1 | Experiment 1

For the training phase, we analysed the drug self-administration data using the between-subject factor of choice condition (1 vs. 1, 5 vs. 5) and the within-subjects factor of session. For the drug-seeking test, we analysed the number of active-lever presses using the between-subjects factor of choice condition (1 vs. 1, 5 vs. 5). For the choice tests, we calculated the preference (Preference Score) in the choice tests by normalizing the indifference level between social and drug choices at 0 using the following formula: preference score = (1 − [% timeout lever choices/50%]) (Lenoir et al., 2007). Then, we analysed the preference score across sessions using the between-subject factor of choice condition (1 vs. 1, 5 vs. 5) and the within-subjects factor of session. We also analysed average preference score during the last 3 days of choice using the between-subject factor of choice condition (1 vs. 1, 5 vs. 5). Additionally, we compared the number of infusions during choice trials between the two choice procedures (during the last 3 days of choice training after heroin self-administration) using the between-subjects factor of choice condition (1 vs. 1, 5 vs. 5).

For the discrete choice test of the 5 versus 5 group with incremental time of access to a social partner, we analysed preference using the within-subjects factor of time (1, 5, 10, 15 min). Lastly, for the discrete choice test of the 5 versus 5 group after abstinence, we compared preference before (during the last 3 days of choice training after heroin self-administration) and after abstinence by analysing the preference using the within-subjects factor of Abstinence Day (Before Abstinence and After Abstinence).

2.6.2 | Experiment 2

For the training phase, we analysed drug intake for infusions using the between-subjects factor of sex (male and female) and the within-subjects factor of session. For the drug-seeking test, we analysed the number of active-lever presses using the between-subjects factor of sex. Responding on the inactive lever was very low (less than two presses during testing), and there were no group differences; thus, we did not include inactive lever in the analyses of the data of Experiment 2. In the choice procedure, we calculated the Preference Score during the 3 days of choice training that followed heroin self-administration. We then analysed the preference score across sessions using the between-subjects factor of sex and the within-subjects factor of session. Additionally, we compared the number of infusions during choice trials using the between-subjects factor of sex. We also analysed the choice data using the between-subjects factor of sex and the within-subjects factor of FR requirement (2 [last FR2 session], 4, 8, 16 [mean of three sessions], 32 [mean of four sessions]).

2.6.3 | Cluster analysis of addiction vulnerability and resilience

We used cluster analysis to examine individual differences in 'addiction vulnerability' in the rats tested under the 5 versus 5 choice condition in Experiments 1 and 2. Our goal was to determine if individual differences in drug intake and drug seeking would predict heroin preference during the choice tests. To classify the rats, we used *z* scores across the following variables: (1) total intake during heroin self-

FIGURE 1 Experiment 1. Effect of heroin access time on choice for heroin versus social interaction. (a) Experimental setup. (b) Experimental timeline. (c) Choice schedule. (d) Social and drug self-administration. Mean ± SEM number of social and drug rewards per session. (e) Drug-seeking test. Individual data of number of lever presses on the active lever during the 30-min extinction tests. (f) Number of infusions during choice trials. Individual data of mean number of infusions during choice trials. (G) Discrete choice. Mean ± SEM number of preference score. Score of −1 indicates a preference for social interaction and score of 1 indicates a preference for heroin. (h) Discrete choice. Individual data of mean of preference score during the last three sessions of social choice. ('1 versus 1 choice': n = 10; '5 versus 5 choice': n = 33). (i) Sampling and choice schedule. (j) Discrete choice. Heroin-versus-social interaction with incremental time of access to the social partner. Mean ± SEM preference score across all the access time conditions. (k) Discrete choice. Individual data of mean of preference score comparing before and after abstinence (n = 16). *$P < 0.05$, significantly different from 1 versus 1.

administration training, (2) number of bursts and (3) drug seeking. We define a burst as the self-administration of more than three infusions in less than 5 min (D'Ottavio et al., 2025). Next, we set a minimum of two and a maximum of five clusters when applying *k*-means clustering, using Classify module in SPSS. The elbow method indicated that the optimal number of clusters was two. Therefore, we only used two clusters in the analysis. After clustering, we compared the resulting two groups, which we labelled as 'addiction vulnerable' and 'addiction resilient' groups for (1) total intake, (2) number of bursts, (3) drug seeking and (4) heroin preference. We also calculated the 'Addiction Severity' score as the *z* score sum of (1) total intake, (2) number of bursts and (3) drug seeking and determined if the addiction severity predicts the magnitude of heroin preference. For this purpose, we correlated the addiction severity score with *z* score heroin preference (*z* score of preference over the first three consecutive days of choice). (*Note:* We used the mean preference score of the first three consecutive choice sessions after heroin self-administration training.) In both experiments, the choice behaviour was stable during these three choice sessions.

2.7 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24 (Alexander et al., 2023).

3 | RESULTS

3.1 | Experiment 1. Effect of heroin access time on choice for heroin versus social interaction

The goal of Experiment 1 was to test the hypothesis that the unit-dosing strategy for heroin is suboptimal in a heroin-versus-social choice procedure. We compared two choice procedures: (1) a standard procedure where the rats chose between 1 unit-dose of heroin and 1 min of social interaction and (2) a revised procedure where the rats chose between 5 min of heroin self-administration and 5 min of social interaction.

We first trained the rats for heroin and social self-administration. During training, the rats increased the number of social and heroin rewards earned over time (Figure 1d). Next, we randomly assigned the rats to two groups for each of the discrete choice conditions (1 vs. 1 unit-dose or 5 vs. 5-min access); there were no differences in heroin intake and heroin seeking between the two groups (Figure 1d,e and Table S1).

In the '5 vs 5' choice, the rats had 5 min of access to heroin and typically self-administered multiple infusions, rarely opting for just a single unit-dose. The number of infusions was significantly higher in the '5 vs 5' choice trials than in the '1 vs 1' choice trials (Figure 1f).

The preference score was also significantly different between the '1 vs 1' and '5 vs 5' choices (Table S1). In the '1 vs 1' choice, the rats increased their preference for social rewards over sessions, showing a strong preference for social over heroin (preference score near 1) (Figure 1g,h). In contrast, during the '5 vs 5' choice, the rats decreased their preference for social rewards over sessions and showed overall indifference towards the two rewards (preference score near 0.5) (Figure 1g,h). However, in the '5 vs 5' choice the indifference was influenced by significant inter-individual variability in preferences (data not normally distributed), with one subpopulation of rats preferring heroin over social interaction, while the other subpopulation showed a strong preference for social interaction over heroin (Figure 1h). In the '5 vs 5' choice, preference for heroin remained stable when we increased access to the social partner (Figure 1j), and after 4 weeks of abstinence (Figure 1k).

3.2 | Experiment 2. Generality of the effect of heroin access time during choice to female rats and screen-based social interaction

The goal of Experiment 2 was to test if the results of Experiment 1 would generalize to females and to the limited-contact, screen-based social interaction procedure (Venniro et al., 2019). We first trained the rats for heroin and social self-administration. During training, the rats increased the number of social and heroin rewards earned over time (Figure 2d). We found no differences in heroin intake or seeking between the two sexes (Figure 2d,e and Table S1). The number of infusions during choice trials was significantly higher in females than in males (Figure 2f). However, there was no difference in heroin preference between the two sexes (Figure 2g,h).

Overall, as in the 5 versus 5 min access condition in Experiment 1, the data indicate indifference between the two rewards (preference score near 0.5) due to considerable individual variability in preferences. In both males and females, we observed one subpopulation of rats preferring heroin over social interaction, while another preferring social interaction over heroin (Figure 2h).

When we increased the number of responses required on the heroin lever, there was no change in heroin preference, with the rats showing inflexibility in their preference even at a higher cost to earn heroin infusions (i.e., FR32) (Figure 2i,j).

We also compared the seven choice sessions—comprising of the four sessions during heroin self-administration and the first three sessions after self-administration—between Experiment 1 (full-contact social interaction) and Experiment 2 (screen-based social interaction). The analysis showed a significant choice session × social contact condition interaction (Table S1), indicating that heroin preference emerged more rapidly when social interaction was limited to screen-based contact (Figure 1g versus Figure 2g). This finding agrees with recent evidence on the importance of physical interaction in the rewarding effects of social interaction (Liu et al., 2025).

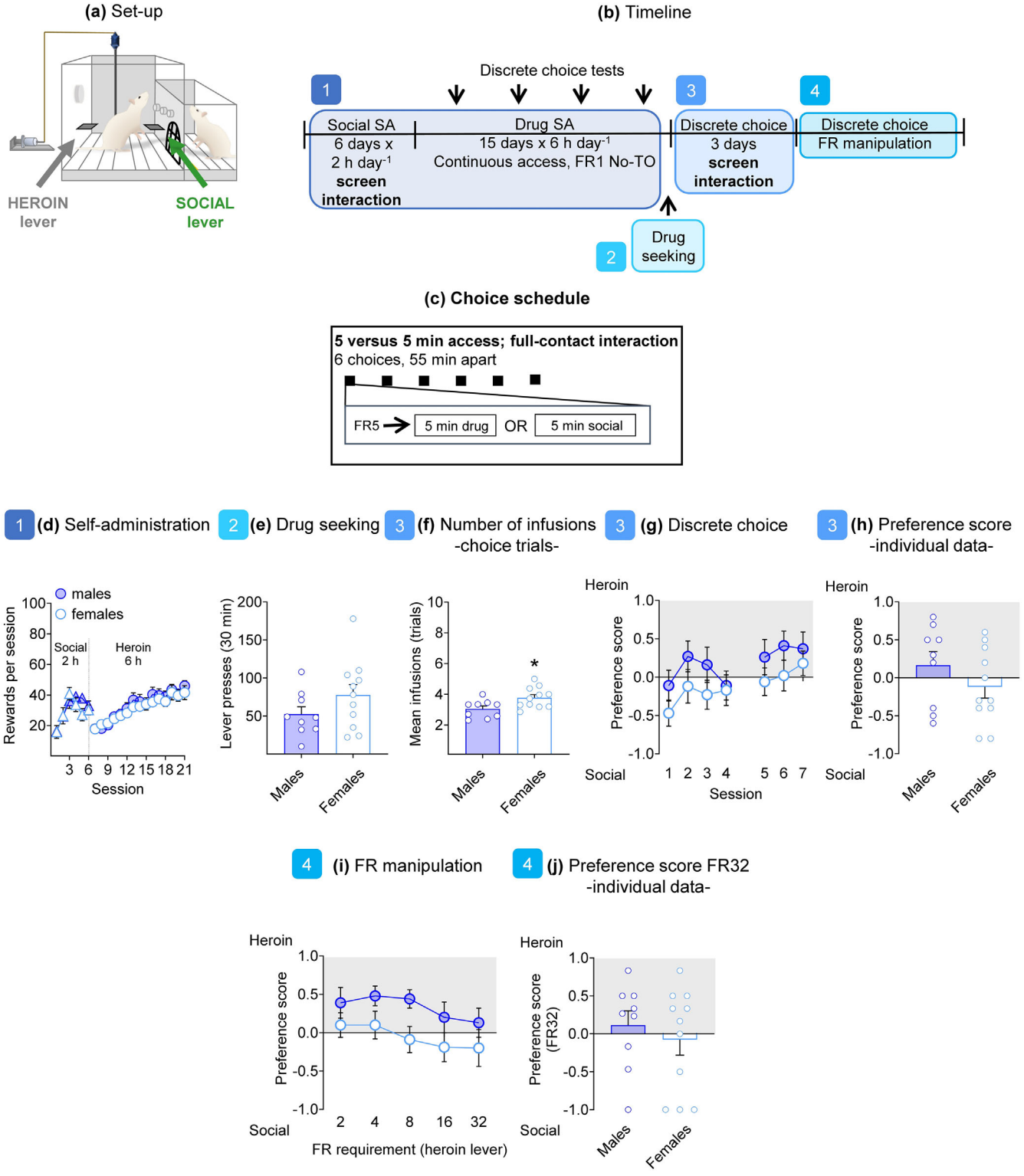

FIGURE 2 Experiment 2. Generalization of the effect of heroin access time during choice to screen-based social interaction and female rats. (a) Experimental setup. (b) Experimental timeline. (c) Choice schedule. (d) Social and drug self-administration. Mean ± SEM number of social and drug rewards per session. (e) Drug-seeking test. Individual data of number of lever presses on the active lever during the 30-min extinction tests. (f) Number of infusions during choice trials. Individual data of mean number of infusions during choice trials. (g) Discrete choice. Mean ± SEM number of preference score. Score of −1 indicates a preference for social interaction, and score of 1 indicates a preference for heroin. (h) Discrete choice. Individual data of mean of preference score during the last three sessions of social choice (males: n = 9; females: n = 11). (i) Fixed ratio (FR) manipulation for heroin access in the discrete choice. Mean ± SEM number of preference score. (j) Discrete choice. Individual data of mean of preference score during the last three sessions of social choice: FR2 for social and FR32 for heroin. Individual data of mean of preference score for males (left) and females (right) (males: n = 9; females: n = 10). *$P < 0.05$, significantly different from males.

3.3 | Cluster analysis of addiction vulnerability and resilience

Heroin preference was positively correlated with burst-patterned heroin taking, heroin intake and heroin seeking, with heroin intake being the strongest predictor of heroin preference (Figure 3c). We then used cluster analysis to identify 'addiction-vulnerable' and 'addiction-resilient' groups, based on total heroin intake during training, total number of burst events, and non-reinforced heroin seeking during early abstinence across the two experiments. Vulnerable and resilient rats did not show differences in the acquisition and maintenance of social self-administration (Figure 3d). Vulnerable rats showed higher heroin intake, higher heroin seeking and more burst events than resilient rats (Figure 3d–g). We also summed the *z* scores of total heroin intake during training, number of bursts during training and heroin seeking during early abstinence to create a putative 'heroin addiction severity index'. This severity index was significantly higher in vulnerable rats (Figure 3h). Additionally, across all experimental subjects, the addiction severity index was positively correlated with heroin preference (Figure 3i). Finally, vulnerable rats had a greater preference for heroin than resilient rats (Figure 3j).

4 | DISCUSSION

The ideal animal model for testing addiction treatments should use vulnerable laboratory animals whose drug-taking behaviour is minimally influenced by non-drug rewards, closely resembling patients in need of treatment (Ahmed, 2018; Heilig et al., 2019; Venniro et al., 2021). However, in drug-versus-social choice models, the rats consistently prefer social interaction over drugs (Venniro et al., 2019; Venniro & Shaham, 2020). Here, we refined this model by training the rats to self-administer heroin without timeout and allowing them to choose between social interaction and a longer duration of heroin availability enabling burst-patterned heroin taking. This approach identify individual differences in heroin preference, independent of social interaction conditions (full-contact versus screen-based) or effort manipulations. Additionally, heroin preference correlated with putative markers of 'addiction severity': high heroin intake, burst-patterned heroin taking and heroin seeking during abstinence. In contrast, 'addiction severity' was not associated with social self-administration.

4.1 | Burst-patterned heroin taking versus single heroin unit-dose during choice

As in our previous study (Venniro et al., 2019), the rats consistently preferred social interaction over a single heroin infusion (0.075 mg kg^{-1}). However, when heroin was available for 5 min, enabling burst-patterned heroin taking, a subset of the rats shifted their preference towards heroin. Below, we propose an explanation for these differences and suggest refinements to drug versus social choice procedures to better capture individual variability in drug preference.

In a recent study (D'Ottavio et al., 2025), we found that removing the timeout period following heroin infusions in the classical extended-access drug self-administration procedure (Ahmed et al., 2000) unexpectedly led to burst-patterned heroin taking. This pattern was characterized by three or more infusions within ~5 min, followed by pause periods, resembling behaviour observed in rats given intermittent heroin access (D'Ottavio et al., 2023; O'Neal et al., 2020). In contrast, the standard limited-access and extended-access procedures, which includes a timeout period after heroin infusions, causes a different intake pattern—initial 'loading dose' followed by stable, regular inter-infusion intervals (D'Ottavio et al., 2025; Wise et al., 1995). We also found that, compared to the rats in the timeout condition, those in the no-timeout condition showed greater motivation to obtain heroin (measured by progressive ratio schedule), increased heroin seeking during abstinence, and preference for the no-timeout condition over the timeout condition (measured in a choice procedure) (D'Ottavio et al., 2025).

These findings suggest that, like humans (Chessick, 1960; McAuliffe & Gordon, 1974), the rats prefer to self-administer heroin in a burst-pattern. The stability of this burst-patterned heroin taking across training sessions indicates that rats voluntarily regulate heroin intake to achieve their preferred drug concentrations in brain (D'Ottavio et al., 2025; Roberts & Zimmer, 2020; Tsibulsky & Norman, 1999). Progressive ratio procedures further suggest a voluntary effort to maximize drug-induced effects, most likely tied to optimal brain-drug concentrations (D'Ottavio et al., 2025; Roberts & Zimmer, 2020). These results suggest that in a choice procedure, the availability of a suboptimal heroin dose [a single unit-dose—a fraction of the rats' 'preferred' drug dose (Desai et al., 2023; Morgan et al., 2009; Roberts et al., 2013)] may have biased the choice towards the social reward (Venniro & Shaham, 2020). We further predicted that individual differences in heroin versus social choice would emerge if the rats had access to their preferred burst-patterned heroin taking.

Our findings confirm these hypotheses, showing strong preference for heroin over social interaction in some rats. Furthermore, we found that individual differences in heroin preference were independent of social interaction conditions (full-contact or screen-based), remained stable across repeated tests, persisted even when the effort required to access social interaction increased (increasing FR requirements) and were maintained during abstinence. We did not detect sex differences in heroin preference, a finding that should be interpreted with caution because the smaller number of females, only included in Experiment 2, limits the statistical power to detect such differences.

The observation that low social preference persists during protracted abstinence (4 weeks), extends previous studies showing that chronic exposure to high opioid doses decreases unconditioned social interaction in mice during prolonged abstinence (Goeldner et al., 2011; Pellissier et al., 2018; Pomrenze et al., 2022; Valentinova

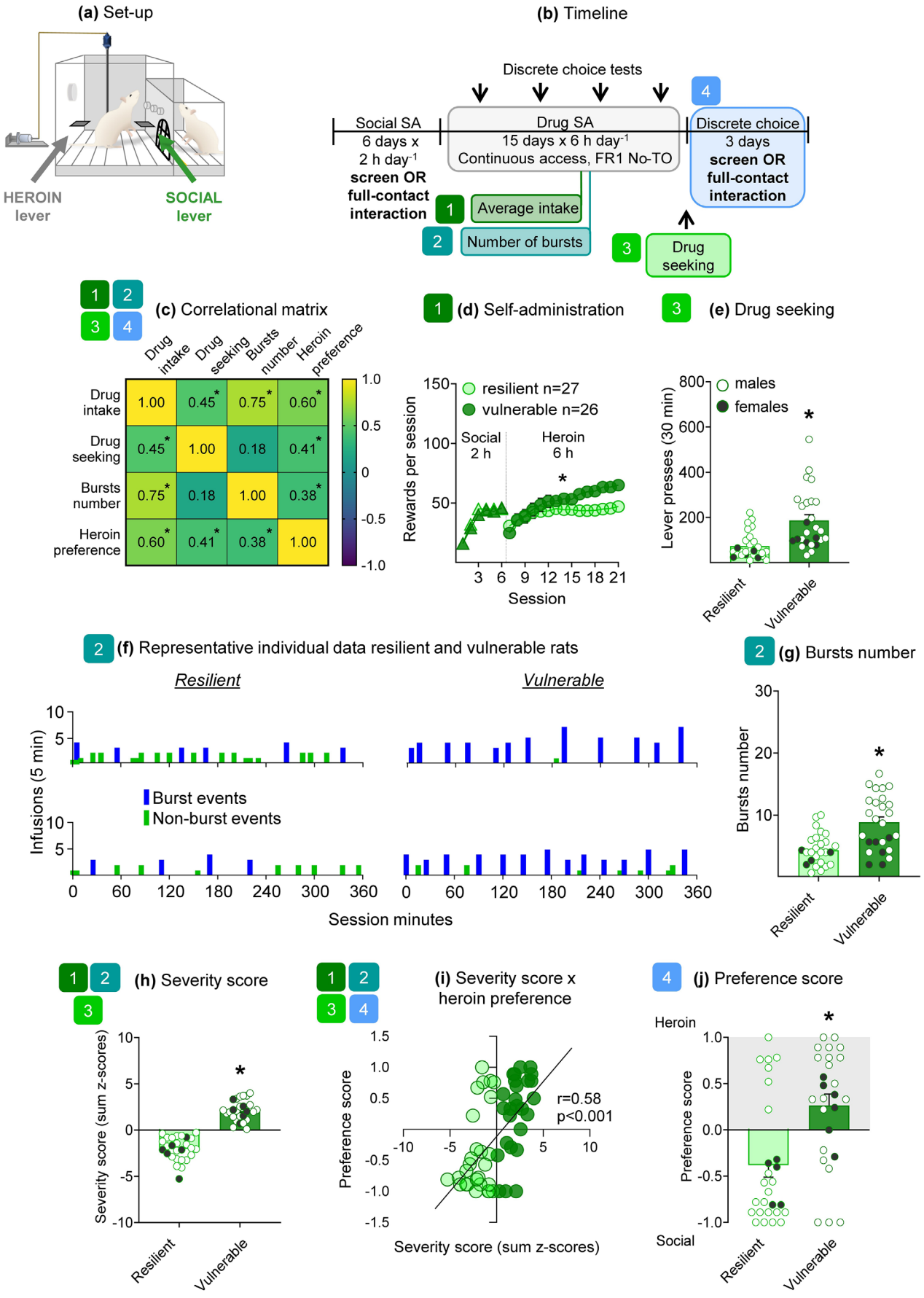

FIGURE 3 Legend on next page.

et al., 2019). Together, these results suggest that under our experimental conditions, heroin versus social preference reflects a stable behavioural trait rather than a transient behavioural state.

Overall, our findings demonstrate that when the rats voluntarily engage in burst-patterned heroin taking, some exhibit behaviour resembling the human condition—prioritizing drug intake over social interactions (American Psychiatric Association, 2013; Heilig et al., 2016; Hogarth, 2020).

4.2 | 'Addiction severity' predicts heroin preference

As discussed above, combining the burst-inducing no-timeout heroin self-administration procedure (D'Ottavio et al., 2025) with a modified choice procedure allowing burst-patterned heroin taking resulted in significant individual differences in heroin preference. Based on these findings, we retrospectively tested whether markers of addiction severity in rat models—high drug intake, burst-patterned heroin taking and high relapse to drug seeking during abstinence (Ahmed & Koob, 1998; Belin et al., 2009; Deroche-Gamonet et al., 2004; Venniro et al., 2020)—would predict heroin preference.

To examine this question, we conducted a cluster analysis using a composite 'addiction severity score' based on heroin intake, burst number and heroin seeking measures. This analysis identified two distinct populations we termed 'addiction vulnerable' and 'addiction resilient' rats. The vulnerable group showed significantly higher total heroin intake, more bursts per session and greater lever pressing during the heroin-seeking test. More importantly, the vulnerable rats showed significantly greater heroin preference than the resilient rats, and across both groups, the 'addiction severity score' was significantly correlated with heroin preference. However, while heroin preference was largely predicted by drug taking and seeking, some rats with high severity score preferred social interaction. Future studies should incorporate heroin preference as an additional measure of addiction severity, capturing individual vulnerability more accurately. Of note, the differences in heroin versus social choice are unlikely to be due to preexisting social reward differences, because social self-administration prior to heroin exposure was similar in both groups.

A key question for future research is whether heroin preference under our experimental conditions correlates with other classical measures of addiction severity, such as resistance to punishment and progressive ratio responding (Augier et al., 2018; Deroche-Gamonet et al., 2004). This is particularly relevant because, in our previous study using the DSM-IV based model, where addiction severity was assessed with a composite score of drug seeking during no-drug periods, resistance to punishment and progressive ratio responding, we found that the severity score did not predict preference for a single unit dose of **methamphetamine** over social interaction (Venniro et al., 2018). We speculate that the discrepancy between our studies stems from the use of a 'suboptimal' methamphetamine dose (a single unit dose) in the earlier study. However, it is also possible that the discrepancy reflects underlying differences between opioid and psychostimulant reward mechanisms (Badiani et al., 2011; Celentano et al., 2009).

Taken together, our findings support incorporating drug versus social preference as a measure of addiction severity in addiction models, alongside established measures of opioid intake, relapse and sensitivity to adverse consequences.

In conclusion, we have introduced, a behavioural procedure that can be used to identify a subgroup of rats showing a strong, stable and inflexible preference for heroin over rewarding social interaction. This putative heroin preference trait is associated with high heroin intake, burst-patterned heroin taking and increased relapse vulnerability, mirroring severe heroin addiction in humans.

As previously discussed, individuals who transition from recreational opioid use to addiction often show harmful drug-taking behaviours, including intense drug use, heightened craving and high drug valuation (Griffiths et al., 1994; Harding, 1988; Hogarth, 2020; McAuliffe & Gordon, 1974). Furthermore, evidence indicates that individuals with opioid addiction show broader impairments in social functioning, including diminished empathy, impaired social cognition and smaller social networks (Carlyle et al., 2020; Preller et al., 2014; Tobler et al., 2016). These studies suggest that the severity of opioid use contributes to decreased engagement in adaptive social behaviours (Christie, 2021; Heilig et al., 2016).

However, the origins of these social impairments remain unclear—specifically, whether they are preexisting vulnerabilities that predispose individuals to addiction or the consequences of prolonged drug use (Verdejo-Garcia, 2014). Distinguishing between these factors is challenging, making laboratory animal studies essential for understanding the interplay between social deficits, opioid use and addiction development.

Finally, the neurobiological mechanisms underlying individual differences in heroin preference are not known. Previous studies have reported that chronic **morphine** exposure or heroin self-administration decreases social interaction in mice (Goeldner

FIGURE 3 Addiction severity predicts heroin preference: cluster analysis across Experiments 1 and 2. (a) Experimental setup. (b) Experimental timeline. (c) Correlational matrix. (d) Social and drug self-administration in resilient and vulnerable rats. Mean ± SEM number of social and drug rewards per session. (e) Drug-seeking test. Individual data of number of lever presses on the active lever during the 30-min extinction tests in resilient and vulnerable rats. (f) Representative individual data resilient and vulnerable rats. Blue: burst events; green: non-burst events. (g) Bursts number. Individual data of mean number of bursts during choice trials in resilient and vulnerable rats. (h) Addiction severity score. Individual data of severity *z* score in rats from resilient and vulnerable clusters. (i) Correlation between severity *z* score index and choice. (j) Preference score. Individual data of mean social preference scores during the last three sessions of social choice in rats from resilient and vulnerable clusters. (vulnerable: n = 26; resilient: n = 27). In (g-j), females (n = 11); males (n = 44). *$P < 0.05$, significantly different from the resilient group. [Correction added on 2 August 2025, after first online publication: Figure 3 has been updated in this version.]

et al., 2011; Piccin et al., 2022; Pomrenze et al., 2022; Wang et al., 2025). These opioid-induced social deficits have been linked to alterations in 5-HT and **dynorphin/κ opioid receptor** transmission in the nucleus accumbens (Goeldner et al., 2011; Pomrenze et al., 2022) and decreased medial prefrontal cortex response to social interaction (Wang et al., 2025). In addition, there is suggestive evidence from a knockout mouse study for the involvement of **corticotropin-releasing factor receptor 1** (Piccin et al., 2022). However, these studies have not directly examined individual differences in opioid-induced social deficits or their relationship to opioid addiction vulnerability. Thus, it is not known if the neurobiological mechanisms identified in these studies also contribute to individual differences in heroin preference in a translationally relevant operant choice model using rats.

AUTHOR CONTRIBUTIONS

Ginevra D'Ottavio: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); visualization (equal); writing—original draft (equal). **Alana Sullivan:** Formal analysis (equal); investigation (equal). **Sara Pezza:** Investigation (supporting). **Maria Chiara Ruano:** Investigation (supporting). **Jacopo Modoni:** Investigation (supporting). **Ingrid Reverte:** Investigation (supporting). **Claudia Marchetti:** Investigation (supporting). **Soami F. Zenoni:** Investigation (supporting). **Marco Venniro:** Writing – review and editing (supporting). **Michele S. Milella:** Writing – review and editing (supporting). **Fernando Boix:** Conceptualization (supporting); writing—review and editing (supporting). **Yavin Shaham:** Funding acquisition (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); writing—original draft (equal). **Daniele Caprioli:** Conceptualization (equal); funding acquisition (equal); methodology (equal); project administration (lead); resources (equal); supervision (equal); writing—original draft (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any conflicts of interest (financial or otherwise) related to the content of the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

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