Received: 20 February 2024 | Revised: 19 September 2024 | Accepted: 25 October 2024

DOI: 10.1111/adb.70009

Addiction Biology SSA WILEY

ORIGINAL ARTICLE

Sex-associated differences in incentive salience and drinking behaviour in a rodent model of alcohol relapse

Aileen Hakus[1,2] | Jerome Clifford Foo[3] | Marta Casquero-Veiga[4,5] | Asude Zülal Gül[1] | Franziska Hintz[1] | Marion Rivalan[6] | York Winter[2] | Josef Priller[1,7,8,9] | Ravit Hadar[1] | Christine Winter[1]

[1]Department of Psychiatry and Psychotherapy, Charité - Universitätsmedizin Berlin, Berlin, Germany

[2]Department of Biology, Humboldt University, Berlin, Germany

[3]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

[4]Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Madrid, Spain

[5]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

[6]Charité - Universitätsmedizin Berlin, Berlin, Germany

[7]Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité - Universitätsmedizin Berlin, and DZNE, Berlin, Germany

[8]Department of Psychiatry and Psychotherapy, School of Medicine and Health, Technical University of Munich and DZPG, Munich, Germany

[9]University of Edinburgh and UK DRI, Edinburgh, UK

Correspondence
Christine Winter, Department of Psychiatry and Psychotherapy, Charité - Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany.
Email: christine.winter@charite.de

Funding information
Open Access funding enabled and organized by the DFG SFB TRR 265 grant (B04 to CW and JP) and e:Med Target-OXY to JCF (031L0190A). JP was also supported by BMBF 01EE2303B. MCV was supported by Fundación Tatiana Pérez de Guzmán el Bueno as scholarship holder of this institution, and EU Joint Programme - Neurodegenerative Disease Research (JPND).

Abstract

The ability of environmental cues to trigger alcohol-seeking behaviours is thought to facilitate problematic alcohol use. Individuals' tendency to attribute incentive salience to cues may increase the risk of addiction. We sought to study the relationship between incentive salience and alcohol addiction using non-preferring rats to model the heterogeneity of human alcohol consumption, investigating both males and females. Adult rats were subjected to the alcohol deprivation effect (ADE) paradigm, where they were given voluntary access to different alcohol solutions with repeated interruptions by deprivation and reintroduction phases over a protracted period (five Alcohol Deprivation Cycles). Before each Alcohol Deprivation Cycle, rats were tested in the Pavlovian Conditioned Approach (PCA) paradigm, which quantifies the individual salience toward a conditional cue and the reward, thus allowing us to trace the process of attributing incentive salience to reward cues. During the final Alcohol Deprivation Cycle (ADE5), animals were tested for compulsive-like behaviour using quinine taste adulteration. We investigated sex differences in drinking behaviour and PCA performance. We observed thatb females drank significantly more alcohol than males and displayed more sign-tracking (ST) behaviour in the PCA, whereas males showed goal-tracking (GT) behaviour. Furthermore, we found that high drinkers exhibited more ST behaviour. The initial PCA phenotype was correlated with later alcohol consumption. Our findings indicate a complex relationship between incentive salience and alcohol addiction and emphasize the importance of considering both sexes in preclinical research.

KEYWORDS
alcohol addiction, alcohol deprivation effect model, goal tracker, incentive salience, Pavlovian conditioned approach, sign-tracker

1 | INTRODUCTION

Alcohol addiction is the result of a complex interplay between social, psychological and biological factors[1,2] and has devastating health and economic consequences. Harmful alcohol consumption accounts for 8.6% and 1.7% of the global disease burden in men and women, respectively. While numerous environmental and genetic factors associated with problematic alcohol-drinking and addiction have been identified,[3] a clarification of how these factors differ by sex is needed.[4]

It has been proposed that the development of alcohol dependence is mediated by the degradation of executive control over behaviour and a transition from goal-directed to more habitual and compulsive drug-seeking.[5] Furthermore, drug-related stimuli can acquire significance when presented and induce excessive emotional and motivational responses.[6] The tendency to attach excessive motivational importance to stimuli may predispose individuals to develop addictive behaviour and is observed to vary between different types of drinkers, for example, heavier drinkers show a more substantial attentional bias toward alcohol-related cues than low drinkers.[7] Over time and after repeated alcohol exposure, neutral cues become associated with reward drive and reinforce reward-seeking behaviour by attributing incentive-motivating properties.[8] Previously neutral cues can trigger craving and drug-seeking behaviour, eventually leading to relapse.[9]

Understanding how incentive salience and relapse processes are related may provide essential insights into addiction mechanisms. A paradigm to assess the process of attributing salience to reward stimuli is the Pavlovian conditioned approach (PCA).[10–13] The PCA paradigm allows to trace the process of attributing incentive salience to reward cues and to identify different phenotypes. While some animals interact exclusively with unconditioned stimuli (so-called 'goal-trackers' (GT)), others tend to engage with conditioned stimuli (so-called 'sign-trackers' (ST)). ST animals appear to assign higher incentive salience to stimuli associated with reward and are thought to be more prone to developing drug addiction.[8,14,15]

To evaluate the mechanisms underlying alcohol addiction, we focused on the alcohol deprivation effect (ADE) paradigm, a validated preclinical model of relapse to alcohol.[16,17] In this model, animals are given long-term access to alcohol, with repeated periods of deprivation. When alcohol is reintroduced after a deprivation period, it leads to a transient increase in consumption (the ADE), accompanied by an eventual shift in preference toward more concentrated alcohol solutions.[17,18] Further, investigations in alcohol-preferring rats have found that after several deprivation periods, rats show compulsive-like drinking behaviour and continued alcohol consumption despite the presence of taste-aversive stimuli like quinine, suggesting the loss of control and behavioural inflexibility.[17,19–21]

Both PCA and ADE studies have often been conducted in male, alcohol-preferring animals, which may provide an incomplete picture and limit the generalizability and translational value of findings. Drinking patterns in human males and females differ, with females drinking less than males.[22] In contrast, in both rats[23] and mice,[24] alcohol intake is higher in females than in males. Thus, it is important to conduct research on females to examine the attribute incentive salience, the consumption of alcohol and the development of compulsive drinking behaviours. A longitudinal approach will give a better understanding of the sex-dependent developmental trajectories of drinking behaviour and associated biomarkers.

In the present study, we aimed to assess whether early behavioural markers were associated with addiction-like behaviour, and whether they inform later developmental trajectories of drinking behaviour in alcohol non-preferring rats. In both male and female nonalcohol-preferring rats, we used the PCA and ADE experimental paradigms to investigate the development of incentive salience attribution and the loss of control over alcohol consumption. In addition, the relationship between performance on the PCA task and drinking behaviour over time was examined.

2 | MATERIALS AND METHODS

2.1 | Animals

We followed EU guidelines (European Communities Council Directive 2010/63/EU), and all procedures were approved by the local state authority (Landesamt für Gesundheit und Soziales [LaGeSo], Berlin) guidelines. Experiments were conducted using PND60 female (alcohol-drinking $n = 13$, controls $n = 8$) and male (alcohol-drinking $n = 15$, controls $n = 8$) RccHan Wistar rats (Envigo, Netherlands). Sample size was predetermined by power analyses. All animals were single housed in a 24 h light/dark cycle (lights on at 6 am, lights off at 6 pm). Single housing of animals allows the accurate recording of high-resolution alcohol consumption at the level of the individual. Room temperature and humidity were kept constant (temperature: 20–24°C, relative humidity: 45%–65%). Standard laboratory rodent chow (Ssniff, Soest, Germany) and water were provided ad libitum throughout the experimental period. All efforts were made to reduce animal suffering and the number of animals used.

2.2 | ADE procedure

On postnatal day 60 (PND60), all rats were provided ad libitum access to tap water and 5%, 10% and 20% ethanol solutions (v/v, prepared from 96% ethanol [TechniSolv, VWR Chemicals, France] diluted in tap water) in a 4-bottle free-choice paradigm. Special bottle caps (Zoonlab GmbH, Germany; bent by infra e motion: Fritz Kutschera) were used to minimize spillage and evaporation. The positions of the bottles were changed regularly to avoid location preferences.

Rats underwent five Alcohol Deprivation Cycles. After an initial 8 weeks of continuous voluntary alcohol access, the first 2-week deprivation period was introduced, followed by the reintroduction of

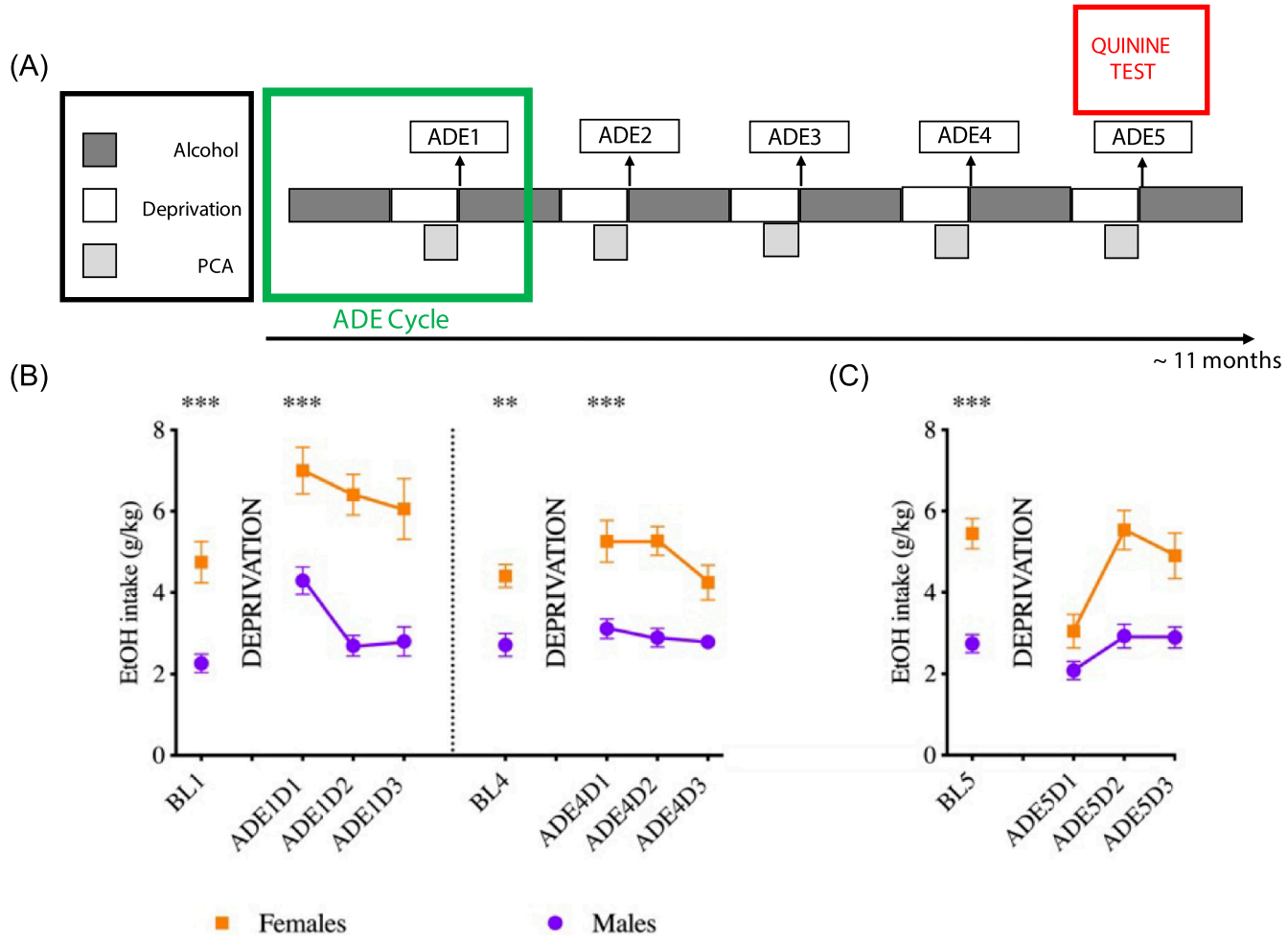

FIGURE 1 Long-term drinking over the experimental period measured by daily total ethanol (EtOH) intake (g/kg/d) before and after an EtOH withdrawal period of 2 to 4 weeks. (A) Experimental design showing the time of alcohol and deprivation periods as well as Pavlovian conditioned approach (PCA), defined as the alcohol deprivation cycle. At the end the quinine test was performed (B) alcohol deprivation Cycle 1 and 4. (C) Quinine test (alcohol deprivation Cycle 5). BL intake is the mean of the last 5 days of baseline drinking. Note that quinine is only in the bottles on D1, with increased consumption from D2 likely due to its removal. Error bars indicate SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ indicate statistically significant differences between males and females. Abbreviations: ADE5D1, quinine; ADE, alcohol deprivation effect; BL, baseline; D, day; EtOH, ethanol; females, orange; males, purple.

alcohol. Over the next 11 months, rats underwent subsequent deprivation (3–4.5 weeks) and reintroduction phases (6–8 weeks) (Figure 1A). Ethanol consumption and animal weights were measured daily for 5 days before and after each deprivation phase. Baseline ethanol intake (BL) was calculated as the daily average across the 5 days (grams of total pure ethanol consumed per day and kg of body weight (g/kg/day) preceding a deprivation period). Upon reintroduction of alcohol, consumption was measured daily to compare with baseline consumption. For the first 24 h of the 5th alcohol-reintroduction phase, quinine was added to the three different alcohol solutions (5%, 10%, 20%) at a concentration of 0.1 g/L. Note that quinine is in the bottles only on Day 1 (D1), with increased consumption from Day 2 as a result of the removal of quinine. No method was used for randomization to allocate animals to experimental groups.

2.2.1 | PCA

PCA tests were performed at the end of each deprivation phase to determine incentive salience phenotypes (Figure 1A). Rats' individual performances in PCA test indicate three different behavioural phenotypes: sign-trackers (ST), which interact with conditioned stimuli (CS), attributing incentive salience to cues; goal-trackers (GT), which interact with the reward directly; and intermediate trackers (IT), which show both behaviours.[25,26] Control groups not exposed to the ADE paradigm ($n = 16$, 8F/8M) also completed the PCA procedure in parallel.

Operant conditioning chambers

Four operant conditioning chambers (20.5 × 24.1 cm floor area, 29.2 cm high; IMETRONIC, Pessac, France) at the Animal Behavioural

Phenotyping Facility (ABPF, Charité Berlin Mitte, Germany) were used for PCA training. Each chamber had a food pellet magazine containing an infrared sensor. A single retractable lever was located at 2.5 cm either right or left from the pellet magazine. The position of the lever on either side of the food receptacle was counterbalanced across boxes to eliminate an effect of side bias. Operation of a pellet dispenser delivered 45-mg banana-flavoured food pellets (BioServe, #F0059, Frenchtown, NJ, USA) into the food magazine. Two days before starting PCA procedures, 25 pellets were placed in each home cage to familiarize the animals with the food. Each operant conditioning chamber was located in a sound-attenuating cabinet.

PCA—Procedure

PCA sessions were conducted between 06:00 AM and 06:00 PM. The week before starting the PCA paradigm, animals were acclimatized to the behavioural facility. Each PCA block lasted six consecutive days, with 1 day of PCA pretraining followed by 5 days of PCA training (one session per day).[25]

PCA—Pretraining

During the first 5 min of pretraining, rats were habituated to the conditioning boxes. Afterward, 25 food pellets were delivered on a variable interval (VI) 60-s schedule (average interval: 30 s, range: 0–60 s). Pretraining sessions lasted approximately 17.5 min. All rats consumed all food pellets.

PCA—Training

Each training session consisted of 25 trials. Each trial consisted of a 1-min waiting period, followed by the presentation of the lever (conditioned stimulus; CS) into the chamber for 8 s and simultaneous illumination with an LED light. Retraction of the lever was immediately followed by the response-independent delivery of one food pellet (unconditioned stimulus; USA). The CS was presented on a random interval 90-s schedule (average interval: 90 s, range: 30–150 s). Each session lasted approximately 40 min.

PCA—Behavioural characterization

PCA index scores were calculated for each Alcohol deprivation cycle to assign animals the different phenotypes (ST, IT, GT).[25] Briefly, for each session, a PCA score was calculated based on the number of times animals interacted with the lever or waited at the pellet dispenser for the reward. Output parameters (number and latency of lever presses and magazine entries) were recorded using POLY software (Imetronic, Pessac, France) and used to pre-calculate Response Bias, Latency Score and Probability Difference for each animal and eventually to determine the PCA scores. PCA scores from the last 2 days were averaged to obtain the final PCA Index Score.

Animals whose scores ranged from 0.5 to 1.0 were classified as STs; those whose scores ranged from −0.5 to −1.0 were categorized as GTs. Intermediate responders ranged between −0.5 and 0.5 (for more details, see previous works[25]). The term 'performance' refers to how often the animal engages with either the lever or the pellet dispenser to be classified as ST or GT.

2.3 | Statistical analyses

Statistical tests were performed using GraphPad Prism software (Version 9.1, La Jolla CA, USA, 2020). A probability p-value of <0.05 was considered significant.

Mixed-effects models were used to analyse longitudinal data, specifying a compound symmetry covariance matrix assuming equal variance and covariance and using Restricted Maximum Likelihood (REML) estimation, with the Geisser–Greenhouse procedure to correct for violations of the assumption applied when required. Sidak tests were performed to correct for multiple comparisons.

Pearson correlation analyses were conducted for the whole sample and stratified by sex. No animal was excluded from our dataset. The investigator who ran the analysis was blind to the group allocation during the experiments and when analysing the data.

2.3.1 | Drinking behaviour

To test for the presence of ADEs (a significantly higher alcohol intake after reintroduction compared to baseline), alcohol consumption (g/kg) was specified as the dependent variable, with sex (M/F) and day (BL and ADE D1) as fixed factors and individual rat and residuals as random factors. The same model was specified for all Alcohol Deprivation Cycles (including the final ADE + quinine). Pearson correlations were also calculated to examine the relationship between BL and ADE drinking. Alcohol solution preference (5%, 10% and 20%) during cycles was calculated using a preference index (PI) following:

$$\mathrm{PI} = (5\% \times \mathrm{vol5\%}) + (10\% \times \mathrm{vo10\%}) + (20\% \times \mathrm{vol20\%})/\mathrm{vol5\%} + \mathrm{vol10\%} + \mathrm{vol20\%}$$

A mixed model was used to test for effects of time points and sex on preferences.

2.3.2 | PCA

Proportions of different PCA phenotypes over time were examined. To investigate the development of PCA index scores over time, PCA score was specified as the dependent variable, with sex (M/F), time point (Alcohol Deprivation Cycle during which the PCA test was conducted) and the interaction between sex and time point as fixed factors and individual rat and residuals as random factors.

2.3.3 | Drinking and PCA performance

To examine the relationship between PCA performance and drinking, Pearson correlations between drinking (during BL and ADE phases) and corresponding PCA performance within the same cycle (e.g., the correlation between PCA1 and BL1/ADE1) were calculated.

PCA performance results were defined as the average of the last 2 days of PCA training during the respective PCA block. To examine the ability of initial PCA performance to predict later drinking outcomes, we also tested the correlation of PCA score at the first cycle (PCA1) and later drinking (BL4/ADE4, BL5/ADE5). We define 'initial' as the first PCA phenotype that emerged during the first PCA test (phenotype is calculated from five consecutive training days and described as PCA1).

3 | RESULTS

3.1 | Drinking behaviour

Descriptively, the ADE was most pronounced at ADE1 in both males and females and decreased over subsequent Alcohol Deprivation Cycles (Figure 1B). During the second and third cycles, no changes were observed in PCA performance compared to the first PCA block, and ADEs did not reach statistical significance (data not shown).

ADEs were observed during the first day of ADE1 (main effect of day, $F[1.26] = 36.5$, $p < 0.001$). During ADE4, the magnitude of the ADE approached but did not reach significance ($F[1.25] = 3.80$, $p = 0.06$).

Female rats drank more ethanol than male rats (main effect of sex, ADE1: $F(1.28) = 37.2$, $p < 0.001$; ADE4: $F(1.26) = 24.2$, $p < 0.001$).

No significant interaction effects between sex and day were observed during ADE1 ($F(1.26) = 0.206$, $p = 0.65$) or ADE 4 ($F[1.25] = 0.688$, $p = 0.41$).

Preference scores of ethanol concentrations did not significantly differ over time points ($F[1.94; 27.2] = 2.09$, $p = 0.14$), or according to sex ($F[1.00; 14.0] = 0.091$, $p = 0.77$).

3.2 | PCA performance

PCA performance was established before ADE measurements (the first cycle) and did not change significantly over time ($F[2.11, 55.0] = 1.42$, $p = 0.25$). Figure 2B illustrates phenotype proportions throughout the experiment. All other rats that started as either sign-trackers (predominantly female rats) or goal-trackers (only males) maintained the phenotypes across the 5 cycles (5 PCA). None of the female rats were classified as GT at any time point. Only a single male rat began as an IT (PCA1 Index Score: 0.36) and showed ST behaviour during the remainder of the experiment (Figure 2B). No interaction effect between sex and timepoint was observed ($F[3, 78] = 0.148$, $p = 0.93$) (Figure 2A).

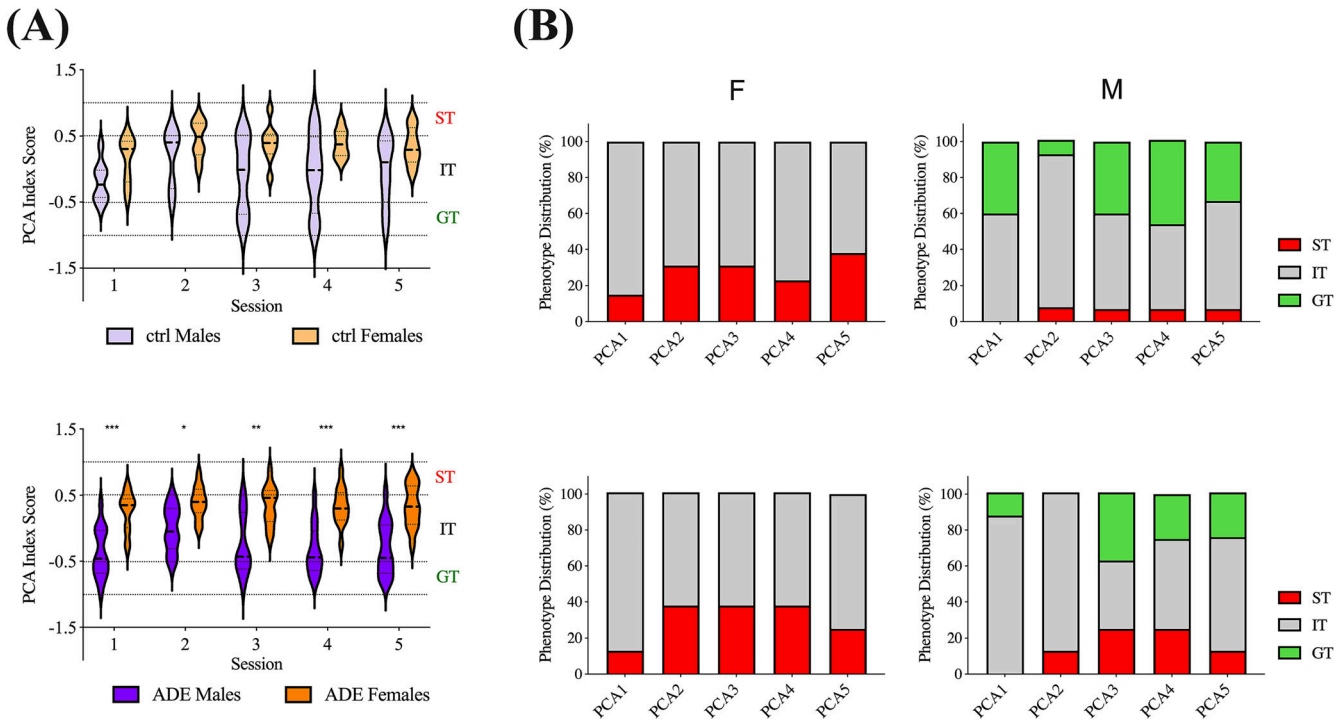

FIGURE 2 Pavlovian conditioned approach (PCA) paradigm. (A) Index Scores and phenotypes for ctrl (up) and ADE (below) animals during the experiment. (B) The percentage distribution of phenotypes averaged over the final 2 days of PCA training for ctrl (up) and ADE (below) animals stratified by sex. Error bars represent ±S.E.M. *$p = 0.05$; **$p < 0.05$; ***$p < 0.001$; *ST Phenotype Score ranged from score ≥0.5 (red) and GT Phenotype Score ≤0.5 (green).* Abbreviations: ADE, alcohol drinker; ctrl, control; F, females; GT, goal tracker; IT, intermediate tracker; M, males; PCA, Pavlovian conditioned approach; ST, sign tracker.

In controls not exposed to alcohol, a similar effect of sex was observed ($F[1.14] = 4.37$, $p = 0.047$), and PCA performance also did not change over time.

3.3 | PCA behaviour and drinking behaviour

Table S1 shows correlations between PCA index scores and drinking behaviour within the same Alcohol Deprivation Cycle in the whole group and stratified by sex. A significant positive correlation was observed between PCA4 and ADE4 drinking behaviour for all animals ($r[27] = 0.511$; $p = 0.005$) (see Figure 3A and Table S1). Interestingly, when stratified by sex, no significant associations between PCA performance and drinking behaviour were observed (during any Alcohol Deprivation Cycle), suggesting that differences between males and females drive the associations.

3.4 | Initial PCA and later drinking

When looking at the whole group of rats, we found a significant positive correlation between PCA1 and later relapse drinking ADE4D1 ($r[27] = 0.489$; $p = 0.008$) (Figure 3B; Table S2). When looking at each sex independently, we found no association between PCA1 and drinking during reintroduction.

3.5 | Quinine

Considering the whole group of animals, no ADEs were observed during quinine adulteration; instead, a reduction of ADE5D1 drinking compared to baseline (BL5) was observed (main effect of day, $F(1.26) = 28.7$, $p < 0.001$). Female rats drank more than male rats (the main effect of sex, $F(1.26) = 35.2$, $p < 0.001$), and quinine had a larger effect on female drinking (interaction of sex and timepoint, $F(1.26) = 10.3$, $p = 0.003$) (Figure 4A), reducing consumption to a greater degree. Post hoc Sidak tests showed that the reduction of alcohol intake in females was significant ($p < 0.001$). Drinking was also reduced in males, but the difference was not significant ($p = 0.660$) (Figure 4A).

At the group level, compulsive-like drinking was not observed. At the individual level, we observed increased drinking during reintroduction in one female and four male rats (Figure 4B).

Additionally, we observed no association between PCA1 score and ADE5D1 drinking ($r[28] = 0.030$, $p = 0.883$) in the whole sample. When stratifying by sex, we found a significant negative correlation between PCA1 and ADE5D1 drinking ($r[13] = -0.569$; $p = 0.042$) in females (Figure 5, Table S2), but not in males ($r[15] = 0.106$, $p = 0.707$).

4 | DISCUSSION

The present study examined the relationship between incentive salience and alcohol consumption in a longitudinal preclinical model of alcohol relapse in both male and female non-preferring RccHan Wistar rats. We observed clear sex differences in alcohol-drinking and found that females drank more alcohol compared to males. Regarding incentive salience behaviour, female rats showed sign-tracking behaviour, while male rats exhibited more goal-tracking behaviour. In general, drinking patterns and PCA phenotypes were established from the beginning of the experiment and remained constant throughout the

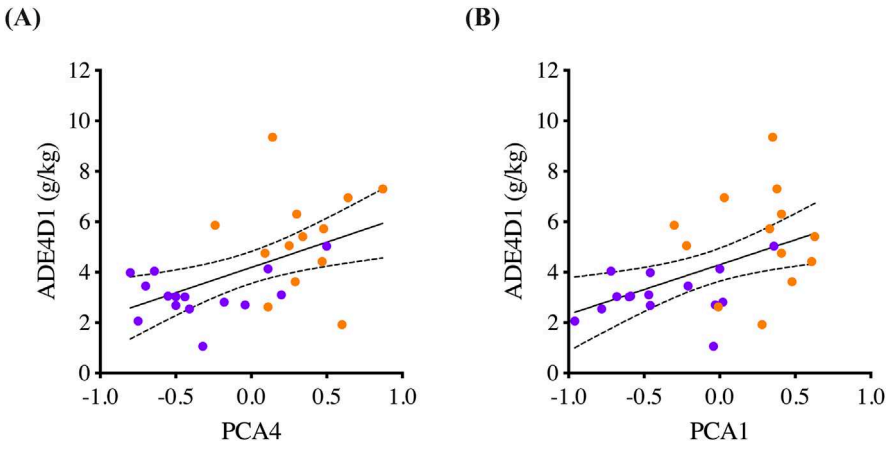

FIGURE 3 Correlation between Pavlovian Conditioned Approach (PCA) and ADE. (A) Alcohol deprivation Cycle 4 showed a significant correlation between PCA and its respective ADE, for example, PCA4 and ADE4D1 for the whole group (males = purple; females = orange). There was no correlation when restricted to within sexes. (B) Between the initial PCA index score and later ADE, there was a significant positive correlation between initial PCA and ADE4, for example, for PCA1 and ADE4D1 for the whole group. Data represent alcohol intake (g/kg) *and comprise PCA index scores: STs (score ≥0.5), IRs (−0.5 < score <0.5), as well as GTs (score ≤−0.5).* Pearson's correlation coefficient (r) between drinking conditions at the same and different cycles for the whole group of animals. The stripped lines indicate two confidence bands (95% confidence). The solid line shows the best fit line. Abbreviations: ADE, alcohol deprivation effect; D, day; PCA, Pavlovian conditioned approach.

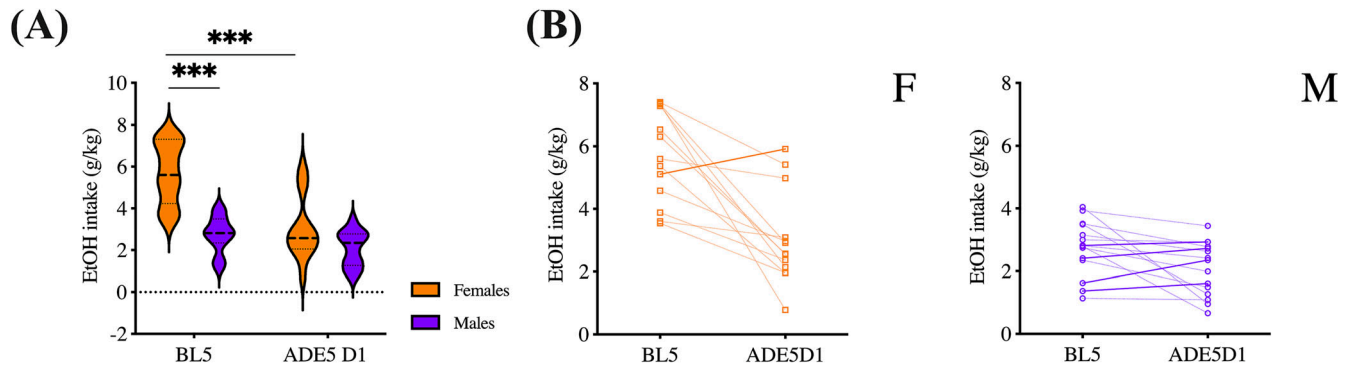

FIGURE 4 Characterization of compulsive drinking by quinine adulteration test. Compulsive drinking, BL5 with ADE5D1$_{Quinine}$ (A) stratified by sex and (B) individual analyses for each animal per group, females (left panel) and males (right panel). Straighter, darker lines in each graph represent animals with increased drinking during ADE5D1$_{Quinine}$ vs. BL5. Abbreviations: ADE5D1, Quinine; ADE, alcohol deprivation effect; BL, baseline; D, day; EtOH, ethanol; F, females (orange); M, males (purple).

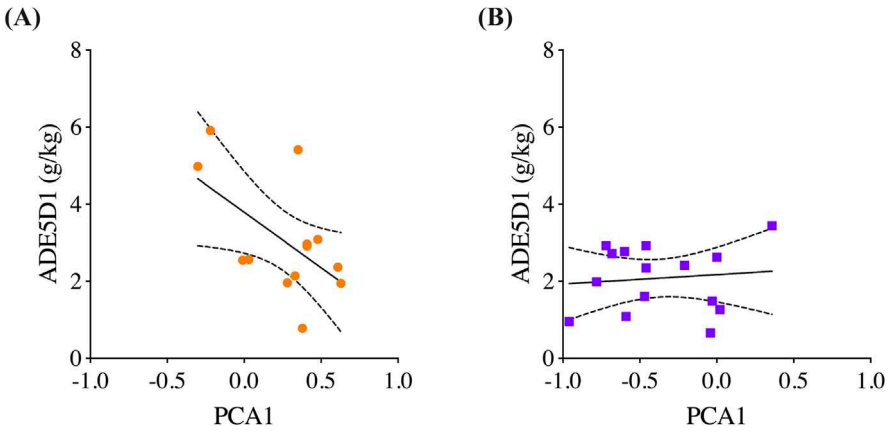

FIGURE 5 Correlation between initial Pavlovian conditioned approach (PCA) index score and drinking. (A) For females, there was a significant negative correlation in alcohol deprivation Cycle 5 between PCA1 and ADE5D1. (B) No significant correlation was found between the initial PCA index score and drinking for males. Data represent alcohol intake (g/kg) *and PCA index scores: STs (score ≥0.5), IRs (−0.5 < score <0.5), as well as GTs (score ≤−0.5)*—Pearson's correlation coefficient (*r*) between initial PCA (PCA1) and drinking condition. The stripped lines indicate two confidence bands (95% confidence). The solid line shows the best fit line. **p* < 0.05; ***p* < 0.01; ****p* < 0.001. Abbreviations: ADE5D1, quinine; ADE, alcohol deprivation effect; D, day; PCA, Pavlovian conditioned approach.

experiment. We did not find evidence for compulsive-like drinking in response to the quinine challenge.

We observed sex differences in alcohol consumption throughout the experiment, both at baseline and reintroduction, with female rats drinking significantly more alcohol than males. This is in line with previous research in both alcohol-preferring and non-preferring rats.[24,27–31] In fact, female rats were shown to be more susceptible to the reinforcing effects of alcohol than male rats.[32,33] In analogy, human studies have observed that women have a higher risk of relapse-like behaviour than men.[34] In most human epidemiological studies, men drink more than women, but what is generally not taken into account is that women have lower body mass than men. In rodent studies, including ours, this is taken into account as alcohol consumption is quantified in g/kg. Interpretation of results from alcohol research in humans is also further complicated by sociocultural factors.[35,36] It should also be noted that the levels of alcohol consumption in women are increasing faster than in men, underscoring the need for research including female animals.[37]

In contrast to alcohol-preferring rats,[17,18] the RccHan rats used in our experiments did not transition to increased preference for higher concentration solutions with higher alcohol concentrations, suggesting that they may overall be less susceptible to alcohol addiction. We did not find the development of stronger ADEs over time, but instead a weakening, which may mirror behaviour in people who consume alcohol but do not become addicted or who do not lose control over alcohol consumption.

PCA performance was largely unchanged throughout the experiment, with different behavioural phenotypes already emerging at the first testing. There was a marked sex difference in PCA phenotypes, with females showing ST behaviour and males showing more GT behaviour. These findings are in line with results reported for

Sprague-Dawley rats[25,38,39] and for Wistar rats.[40,41] Previous studies found that STs attribute more salience to drug-paired cues and are more susceptible to cue-induced reinstatement of drug-seeking behaviour than GTs. Those studies were mainly conducted with male Sprague-Dawley rats,[11,14,42–44] but PCA in females indicated that they exhibit more and faster ST behaviour.[27,45]

Research previously showed that ethanol-associated cues (e.g., auditory stimuli) can lead rats treated with ethanol to seek rewards that are not associated with ethanol.[46] Drug consumption can reinforce nondrug-related reward behaviour.[47–49] Thus, environmental stimuli may elicit maladaptive responses under some circumstances.

We observed more ST behaviour at higher levels of alcohol intake after reintroduction. Together with the fact that PCA phenotypes were established early and did not change over time, these phenotypic characteristics might serve as early markers of eventual alcohol-drinking/relapse. However, when stratified by sex, the correlation between PCA index score and drinking behaviours was not significant, suggesting that sex differences drive the associations when considering the whole sample (i.e., females drank more and had more sign-tracking behaviour than males). It was expected that STs would develop addictive behaviour differently than GTs and that PCA phenotypes could be used as a type of marker. However, we found that PCA performance was already established at the outset and did not change over the experiment and that sex was a strong determinant of both drinking behaviour and PCA performance.

There are other studies investigating how the sign-tracker phenotype is related to human behavioural traits such as compulsivity, impulsivity and addiction. The ST phenotype is also associated with high impulsivity, compulsive tendencies and addiction tendencies.[50] This suggests a co-segregation or simultaneous occurrence of these behaviours. Hence, the sign-tracker phenotype may serve as an indicator or marker of impulsivity, compulsivity and addiction-related behaviours.

Previous ADE studies have broadly used alcohol-preferring strains, such as P rats[51] or Wistar rats,[21,52] while previous PCA studies mostly used Sprague-Dawley and Wistar rats.[53] The rat strain employed in the present study, namely, RccHan Wistar rats, is less prone to alcohol consumption but shows more variability-related individual differences in voluntary alcohol-drinking and is therefore believed to better reflect the human population.[54] Indeed, in our study, we did not find the development of stronger ADEs over time, underscoring the value of using RccHan Wistar rats as a translational model. This may even have the potential to examine protective factors against loss of control in future studies.

Overall, compulsive-like drinking was not observed in the present study. In females, we found that more ST behaviour was correlated with less drinking during quinine adulteration. Previous studies in alcohol-preferring (P) rat strains have demonstrated that high alcohol intake does not necessarily lead to or predict the development of compulsive alcohol-seeking[19] or compulsive drinking.[55] It was also shown that habitual alcohol exposure did not predict the transition to compulsivity in alcohol-preferring rats.[19] Our results are in line with this, as female rats who drank more throughout the experiment but did not develop stronger compulsivity as male rats.

The present study has several limitations. The sample sizes of the subgroups are small, and larger independent samples will be needed to validate the results. We found correlations between alcohol consumption and specific PCA behaviour in alcohol-drinking subtypes.

While other studies have observed compulsive-like behaviour with quinine, we found that drinking in the RccHan rat strain is not as strongly affected by quinine. Different behavioural tests, for example, foot-shock resistance,[56–58] might be better suited to examine compulsive drinking. However, due to ethical considerations and the German/EU law (2010/63/EU), alternatives need to be found. Another limitation is that we did not determine the exact aversive threshold in this rat strain. However, quinine concentrations of 0.1 g/L were shown to be effective in many studies. Still, we cannot exclude that variability between strains and facilities could have affected our results.

4.1 | Conclusion

In this study, we observed sex differences in both incentive salience attribution and alcohol-drinking behaviour in rats. Humans also differ in their susceptibility to cues that contribute to problematic alcohol use. Thus, incentive salience behaviour may serve as a predictor of later drinking outcomes. Further studies are needed to better understand the sex-related differences in the processes contributing to drinking behaviour. In addition, the molecular and cellular mechanisms underlying the behavioural changes and sex differences needed to be explored.

AUTHOR CONTRIBUTIONS

Aileen Hakus conducted experiments, analysed data and wrote the manuscript. Jerome Clifford Foo analysed data and wrote the manuscript. Marta Casquero-Veiga, Franziska Hintz and Asude Zülal Gül contributed to conducting experiments. Marion Rivalan and York Winter provided conditioned boxes. Ravit Hadar conceptualized the project and wrote the manuscript. Josef Priller conceptualized the project. Christine Winter conceptualized the project and supervised Aileen Hakus and wrote the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We thank Jennifer Altschüler and Jessica Albrecht for their excellent technical assistance. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusion are present in the paper and/or the Supplementary Materials.

ORCID

Aileen Hakus https://orcid.org/0000-0002-5355-8660
Marta Casquero-Veiga https://orcid.org/0000-0003-0859-9495

REFERENCES

1. Gautier M, Pabst A, Maurage P. Social decision making in severe alcohol use disorder: scoping review and experimental perspectives. *Alcohol Clin Exp Res*. 2021;45(8):1548-1559. doi:10.1111/ACER.14664
2. Kendler KS, Schmitt E, Aggen SH, Prescott CA. Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. *Arch Gen Psychiatry*. 2008;65(6):674-682. doi:10.1001/ARCHPSYC.65.6.674
3. Reilly MT, Noronha A, Goldman D, Koob GF. Genetic studies of alcohol dependence in the context of the addiction cycle. *Neuropharmacology*. 2017;122:3-21. doi:10.1016/J.NEUROPHARM.2017.01.017
4. Becker JB, McClellan ML, Reed BG. SEX DIFFERENCES, GENDER AND ADDICTION. *J Neurosci Res*. 2017;95(1–2):136-147. doi:10.1002/JNR.23963
5. Everitt BJ, Dickinson A, Robbins TW. The neuropsychological basis of addictive behaviour. *Brain Res Brain Res Rev*. 2001;36(2–3):129-138. doi:10.1016/S0165-0173(01)00088-1
6. Berridge KC, Robinson TE. Liking, wanting and the incentive-sensitization theory of addiction. *Am Psychol*. 2016;71(8):670-679. doi:10.1037/amp0000059
7. Field M, Cox WM. Attentional bias in addictive behaviors: a review of its development, causes, and consequences. *Drug Alcohol Depend*. 2008;97(1–2):1-20. doi:10.1016/J.DRUGALCDEP.2008.03.030
8. Yager LM, Robinson TE. A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue. *Psychopharmacology (Berl)*. 2013;226(2):217-228. doi:10.1007/S00213-012-2890-Y
9. Milton AL. Drink, drugs and disruption: memory manipulation for the treatment of addiction. *Curr Opin Neurobiol*. 2013;23(4):706-712. doi:10.1016/J.CONB.2012.11.008
10. Fitzpatrick CJ, Morrow JD. Individual variation in the attribution of incentive salience to social cues. *Sci Rep*. 2020;10(1):2583. doi:10.1038/S41598-020-59378-5
11. Flagel SB, Akil H, Robinson TE. Individual differences in the attribution of incentive salience to reward-related cues: implications for addiction. *Neuropharmacology*. 2009;56(Suppl 1):139-148. doi:10.1016/J.NEUROPHARM.2008.06.027
12. Overby PF, Daniels CW, Del Franco A, et al. Effects of nicotine self-administration on incentive salience in male Sprague Dawley rats. *Psychopharmacology (Berl)*. 2018;235(4):1121-1130. doi:10.1007/S00213-018-4829-4
13. Robinson TE, Flagel SB. Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. *Biol Psychiatry*. 2009;65(10):869-873. doi:10.1016/J.BIOPSYCH.2008.09.006
14. Saunders BT, Robinson TE. A cocaine cue acts as an incentive stimulus in some, but not others: implications for addiction. *Biol Psychiatry*. 2010;67(8):730-736. doi:10.1016/J.BIOPSYCH.2009.11.015
15. Yager LM, Pitchers KK, Flagel SB, Robinson TE. Individual variation in the motivational and neurobiological effects of an opioid Cue. *Neuropsychopharmacology*. 2014;40(5):1269-1277. doi:10.1038/npp.2014.314
16. Spanagel R, Hölter SM. Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? *Alcohol Alcoholism (Oxford, Oxfordshire)*. 1999;34(2):231-243. doi:10.1093/ALCALC/34.2.231
17. Vengeliene V, Bilbao A, Spanagel R. The alcohol deprivation effect model for studying relapse behavior: a comparison between rats and mice. *Alcohol (Fayetteville, NY)*. 2014;48(3):313-320. doi:10.1016/J.ALCOHOL.2014.03.002
18. Wolffgramm J. An ethopharmacological approach to the development of drug addiction. *Neurosci Biobehav Rev*. 1991;15(4):515-519. doi:10.1016/S0149-7634(05)80142-3
19. Giuliano C, Puaud M, Cardinal RN, Belin D, Everitt BJ. Individual differences in the engagement of habitual control over alcohol seeking predict the development of compulsive alcohol seeking and drinking. *Addict Biol*. 2021;26(6):e13041. doi:10.1111/ADB.13041
20. Spanagel R, Hölter SM, Allingham K, Landgraf R, Zieglgänsberger W. Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat. *Eur J Pharmacol*. 1996;305(1–3):39-44. doi:10.1016/0014-2999(96)00174-4
21. Villarín Pildaín L, Vengeliene V, Matthäus F. New measurement criteria for studying alcohol drinking and relapse in rodents. *In Silico Pharmacol*. 2013;1(1):1-10. doi:10.1186/2193-9616-1-13
22. Wilsnack RW, Wilsnack SC, Kristjanson AF, Vogeltanz-Holm ND, Gmel G. Gender and alcohol consumption: patterns from the multinational GENACIS project. *Addiction*. 2009;104(9):1487-1500. doi:10.1111/j.1360-0443.2009.02696.x
23. Mittal N, Fleming SM, Martinez A, et al. Sex differences in cognitive performance and alcohol consumption in high alcohol-drinking (HAD-1) rats. *Behav Brain Res*. 2020;381:112456. doi:10.1016/J.BBR.2019.112456
24. Tambour S, Brown LL, Crabbe JC. Gender and age at drinking onset affect voluntary alcohol consumption but neither the alcohol deprivation effect nor the response to stress in mice. *Alcohol Clin Exp Res*. 2008;32(12):2100-2106. doi:10.1111/J.1530-0277.2008.00798.X
25. Fitzpatrick CJ, Morrow JD. Pavlovian conditioned approach training in rats. *J vis Exp*. 2016;2016(108):e53580. doi:10.3791/53580
26. Morrison SE, Bamkole MA, Nicola SM. Sign tracking, but not goal tracking, is resistant to outcome devaluation. *Front Neurosci*. 2015;9(DEC):468. doi:10.3389/FNINS.2015.00468/BIBTEX
27. Anderson RI, Spear LP. Autoshaping in adolescence enhances sign-tracking behavior in adulthood: impact on ethanol consumption. *Pharmacol Biochem Behav*. 2011;98(2):250-260. doi:10.1016/J.PBB.2011.01.004
28. Bertholomey ML, Nagarajan V, Torregrossa MM. Sex differences in reinstatement of alcohol seeking in response to cues and yohimbine in rats with and without a history of adolescent corticosterone exposure. *Psychopharmacology (Berl)*. 2016;233(12):2277-2287. doi:10.1007/S00213-016-4278-X
29. Peña-Oliver Y, Giuliano C, Economidou D, et al. Alcohol-preferring rats show goal oriented behaviour to food incentives but are neither sign-trackers nor impulsive. *PLoS ONE*. 2015;10(6):e0131016. doi:10.1371/JOURNAL.PONE.0131016
30. Priddy BM, Carmack SA, Thomas LC, Vendruscolo JCM, Koob GF, Vendruscolo LF. Sex, strain, and estrous cycle influences on alcohol drinking in rats. *Pharmacol Biochem Behav*. 2017;152:61-67. doi:10.1016/J.PBB.2016.08.001
31. Randall PA, Stewart RT, Besheer J. Sex differences in alcohol self-administration and relapse-like behavior in long-Evans rats. *Pharmacol Biochem Behav*. 2017;156:1-9. doi:10.1016/J.PBB.2017.03.005
32. Becker JB, Hu M. Sex Differences in Drug Abuse. *Front Neuroendocrinol*. 2008;29(1):36-47. doi:10.1016/J.YFRNE.2007.07.003
33. Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. *Trends Pharmacol Sci*. 2004;25(5):273-279. doi:10.1016/j.tips.2004.03.011
34. Robbins SJ, Ehrman RN, Childress AR, O'Brien CP. Comparing levels of cocaine cue reactivity in male and female outpatients. *Drug Alcohol Depend*. 1999;53(3):223-230. doi:10.1016/S0376-8716(98)00135-5
35. Lui CK, Kerr WC, Mulia N, Ye Y. Educational differences in alcohol consumption and heavy drinking: an age-period-cohort perspective. *Drug Alcohol Depend*. 2018;186:36-43. doi:10.1016/J.DRUGALCDEP.2017.12.046

36. Zemore SE, Karriker-Jaffe KJ, Mulia N. Temporal trends and changing racial/ethnic disparities in alcohol problems: results from the 2000 to 2010 National Alcohol Surveys. *J Addict Res Ther*. 2013;4(04). doi:10.4172/2155-6105.1000160
37. Keyes KM, Jager J, Mal-Sarkar T, Patrick ME, Rutherford C, Hasin D. Is there a recent epidemic of women's drinking? A critical review of national studies. *Alcohol Clin Exp Res*. 2019;43(7):1344-1359. doi:10.1111/ACER.14082
38. Madayag AC, Stringfield SJ, Reissner KJ, Boettiger CA, Robinson DL. Sex and adolescent ethanol exposure influence Pavlovian conditioned approach. *Alcohol Clin Exp Res*. 2017;41(4):846-856. doi:10.1111/ACER.13354
39. Pohořalá V, Enkel T, Bartsch D, Spanagel R, Bernardi RE. Sign- and goal-tracking score does not correlate with addiction-like behavior following prolonged cocaine self-administration. *Psychopharmacology (Berl)*. 2021;238(8):2335-2346. doi:10.1007/S00213-021-05858-Z
40. López P, Alba R, Orduña V. Individual differences in incentive salience attribution are not related to suboptimal choice in rats. *Behav Brain Res*. 2018;341:71-78. doi:10.1016/J.BBR.2017.12.028
41. Serrano-Barroso A, Vargas JP, Diaz E, O'Donnell P, López JC. Sign and goal tracker rats process differently the incentive salience of a conditioned stimulus. *PLoS ONE*. 2019;14(9):e0223109. doi:10.1371/JOURNAL.PONE.0223109
42. Flagel SB, Watson SJ, Robinson TE, Akil H. Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology (Berl)*. 2007;191(3):599-607. doi:10.1007/S00213-006-0535-8
43. Saunders BT, Yager LM, Robinson TE. Cue-evoked cocaine 'craving': role of dopamine in the accumbens core. *J Neurosci*. 2013;33(35):13989-14000. doi:10.1523/JNEUROSCI.0450-13.2013
44. Yager LM, Robinson TE. Individual variation in the motivational properties of a nicotine cue: sign-trackers vs. goal-trackers. *Psychopharmacology (Berl)*. 2015;232(17):3149-3160. doi:10.1007/S00213-015-3962-6
45. Hammerslag LR, Gulley JM. Age and sex differences in reward behavior in adolescent and adult rats. *Dev Psychobiol*. 2014;56(4):611-621. doi:10.1002/DEV.21127
46. Corbit LH, Janak PH. Ethanol-associated cues produce general Pavlovian-instrumental transfer. *Alcohol Clin Exp Res*. 2007;31:766-774.
47. Shields CN, Gremel CM. Prior chronic alcohol exposure enhances Pavlovian-to-instrumental transfer. *Alcohol*. 2021;96:83-92.
48. LeBlanc KH, Maidment NT, Ostlund SB. Impact of repeated intravenous cocaine administration on incentive motivation depends on mode of drug delivery. *Addict Biol*. 2014;19:965-971.
49. Ostlund SB, LeBlanc KH, Kosheleff AR, Wassum KM, Maidment NT. Phasic mesolimbic dopamine signaling encodes the facilitation of incentive motivation produced by repeated cocaine exposure. *Neuropsychopharmacology*. 2014;39:2441-2449.
50. Schettino M, Ceccarelli I, Tarvainen M, Martelli M, Orsini C, Ottaviani C. From skinner box to daily life: sign-tracker phenotype co-segregates with impulsivity, compulsivity, and addiction tendencies in humans. *Cogn Affect Behav Neurosci*. 2022;22(6):1358-1369. doi:10.3758/s13415-022-01014-y
51. McKinzie DL, Nowak KL, Yorger L, et al. The alcohol deprivation effect in the alcohol-preferring P rat under free-drinking and operant access conditions. *Alcohol Clin Exp Res*. 1998;22(5):1170-1176. doi:10.1111/J.1530-0277.1998.TB03718.X
52. Vengeliene V, Bachteler D, Danysz W, Spanagel R. The role of the NMDA receptor in alcohol relapse: a pharmacological mapping study using the alcohol deprivation effect. *Neuropharmacology*. 2005;48(6):822-829. doi:10.1016/J.NEUROPHARM.2005.01.002
53. Fitzpatrick CJ, Gopalakrishnan S, Cogan ES, et al. Variation in the form of Pavlovian conditioned approach behavior among outbred male Sprague-Dawley rats from different vendors and colonies: sign-tracking vs. goal-tracking. *PLoS ONE*. 2013;8(10):e75042. doi:10.1371/JOURNAL.PONE.0075042
54. Momeni S, Segerström L, Roman E. Supplier-dependent differences in intermittent voluntary alcohol intake and response to naltrexone in Wistar rats. *Front Neurosci*. 2015;9(NOV):424. doi:10.3389/FNINS.2015.00424
55. Vengeliene V, Bilbao A, Molander A, Spanagel R. Neuropharmacology of alcohol addiction. *Br J Pharmacol*. 2008;154(2):299-315. doi:10.1038/BJP.2008.30
56. Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. *Science*. 2004;305(5686):1014-1017. doi:10.1126/SCIENCE.1099020/SUPPL_FILE/DEROCHEGAMONET.SOM.PDF
57. Everitt BJ, Robbins TW. Drug addiction: updating actions to habits to compulsions ten years on. *Annu Rev Psychol*. 2016;67(1):23-50. doi:10.1146/ANNUREV-PSYCH-122414-033457
58. Hopf FW, Lesscher HMB. Rodent models for compulsive alcohol intake. *Alcohol (Fayetteville, NY)*. 2014;48(3):253-264. doi:10.1016/J.ALCOHOL.2014.03.001

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hakus A, Foo JC, Casquero-Veiga M, et al. Sex-associated differences in incentive salience and drinking behaviour in a rodent model of alcohol relapse. *Addiction Biology*. 2025;30(1):e70009. doi:10.1111/adb.70009