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ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

ORIGINAL ARTICLE

Adverse social experiences in adolescent rats result in persistent sex-dependent effects on alcohol-seeking behavior

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Abstract

Background: Accumulating clinical evidence suggests that women with prior exposure to adverse childhood experiences are more susceptible to heavy drinking and other health-related behaviors. Yet, preclinical studies investigating sex-dependent effects of adolescent adverse social experiences (ASEs) on later alcohol-seeking behavior are lacking. This is mainly due to the unavailability of valid animal models and a shortage of studies that compare effects in males and females. Therefore, we sought to investigate the sex-dependent effects of ASE on adult alcohol-seeking behavior, locomotion, and reward sensitivity in male and female rats.

Methods: We recently developed a rat model for childhood/adolescent peer rejection that allows us to study the long-term consequences of ASEs. Adolescent Wistar rats were reared from postnatal day (pd) 21 to pd 50 either within a group of Fischer 344 rats (ASE) or within a group of Wistar rats (control). Wistar rats housed with Fischer 344 rats do not reciprocate social play in adolescence. This reduced play across adolescence mimics peer rejection and results in chronic dysregulation of social and pain-related behaviors. We tested adult male and female rats in the reinstatement paradigm for cue-induced alcohol-seeking behavior, circadian locomotor activity, and sucrose consumption long after the termination of the peer rejection condition.

Results: Peer rejection induced persistent sex-dependent changes in alcohol cue-induced reinstatement. Females showed an increased reinstatement effect while peer-rejected males demonstrated a decrease. Sex differences were observed in locomotor activity or reward sensitivity to sucrose.

Conclusions: Peer rejection has long-lasting sex-dependent consequences on alcohol-seeking behavior without affecting locomotion or sweet reward sensitivity. Our results suggest that peer-rejected female rats represent a vulnerable population in which to study relapse-like behaviors that are similar to clinical findings, while males seem to buffer the peer rejection effect and demonstrate resilience to later life alcohol-seeking behaviors, as measured by the reinstatement effect. Finally, we provide a novel approach to investigate the molecular and neurobiological underpinnings of ASEs on alcohol and other drug-seeking behaviors.

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KEYWORDS
adverse social experiences, alcohol seeking, peer rejection, relapse behavior, sex dependent

INTRODUCTION

Increasing evidence from human studies shows that adverse childhood experiences (ACEs) can result in altered brain structure and function (reviewed in Herzog & Schmahl, 2018). These changes, in turn, may result in several mental health issues later in life. The impact of multiple ACEs is best illustrated by the fact that the risk for suicide attempts is massively increased with an odds ratio of almost 18 (Hughes et al., 2019). ACEs can also alter reward processing (Hanson et al., 2016) and cause sleep disturbances (Hasler et al., 2012) that in turn can lead to alcohol-related problems such as early alcohol drinking initiation, alcohol binging, and problematic alcohol use (lifetime) (Bellis et al., 2019; Benjet et al., 2016; Erdozain et al., 2015; Hughes et al., ,2017, 2019). Epidemiological data initially suggest that men are more prone for alcohol dependence compared with women (Oberleitner et al., 2015). However, when a history of ACEs was considered, the lifetime diagnosis for alcohol dependence was reversed in men (6.6%) and women (10.8%). Women with a history of ACEs also respond more negatively to other alcohol-related problems compared to men with a history of ACEs (Craig et al., 2019; Cunradi et al., 2020; Edwards et al., 2003; Fang & McNeil, 2017; Peltier et al., 2019). Additionally, women with a history of ACEs have been found to demonstrate greater cue reactivity to alcohol in experimental settings (Hartwell & Ray, 2013), suggestive of an increased propensity for relapse when compared to men with a history of ACEs. Further research has also eluded that women with a history of ACEs show increased vulnerability for alcohol relapse compared to men with ACEs (Heffner et al., 2011; Hyman et al., 2008; Kennedy et al., 2013). These findings highlight the importance of considering ACEs and sex in predicting alcohol-related problems and suggest that women with ACEs may have gender-specific mechanisms contributing to higher rates of alcohol relapse and alcohol dependence. It is thus of high relevance to develop translational approaches that allow us to study the underlying molecular and neurobiological mechanisms that mediate the effects of ACEs on alcohol-related problems later in life.

Theoretical models have proposed that individuals with ACEs are at an increased risk of later alcohol-related problems due to latent vulnerability (McCrory & Mayes, 2015). The core idea posits that the response to adversity although initially adaptive become maladaptive in the long-term. For example, being rejected by your peers during adolescence is distressful and may initially lead to devaluing social contact to protect one's self-worth. In the long term, peer rejection may lead to less social contact and therefore less adaptive social skills later in life that can in turn increase the risk of later alcohol-related problems. Indeed, research has indicated that peer rejection during adolescence is associated with future somatic and mental health issues including substance use problems (Finkelhor et al., 2015; Pinchevsky et al., 2014). Two potential mediating factors that increase risk of later alcohol-related problems are ACE-induced circadian dysregulation and reward system alterations (Hanson et al., 2016; Hasler et al., 2012). Although emerging evidence supports a relationship, there is still a significant gap in our understanding of the mechanisms to explain the association between peer rejection and later alcohol-related problems.

To explore the relationship between peer rejection and later alcohol-related problems, we used our clinically relevant rat model of adolescent peer rejection which results in adverse social experiences (ASEs) (Schneider et al., 2014, 2016a). Our peer rejection-like model moderates adolescent rat's ability to properly engage in adequate and reciprocal social play. Specifically, our model recapitulates aspects of peer rejection as Wistar rats housed with Fischer 344 rats are rejected through nonreciprocity of social play. Specifically, Wistar rats housed with Fischer 344 rats receive dramatically less playful responses although initiating more attacks and producing more prosocial 50kHz ultrasonic vocalizations (indicating their willingness to play) than their control counterparts (Schneider et al., 2016b). In short, play-deprived Wistar rats attempt to play with their Fischer 344 cage mates; however, their efforts are not reciprocated. This imitates aspects of human peer rejection, where individuals attempt to interact with their peers but are in some form excluded much like our rats. Although peer rejection is a complex phenomenon that can be induced in a multitude of ways in humans (Kawamoto et al., 2015), we have found several similar features in our peer-rejected female rats and humans. These changes include deficits in social interactions, social memory, pain-sensitivity, emotional reactivity, processing of socially transmitted information, and neurochemical (endocannabinoid system) dysregulation (Bungert et al., 2015; Schaefer et al., 2014; Schneider et al., ,2014, 2016a). Furthermore, considering that peer rejection has been linked to alcohol problems (Pinchevsky et al., 2014), it is plausible that peer-rejected rats may also demonstrate vulnerability to alcohol-seeking behaviors.

The goal of the present study was to determine whether peer rejection during childhood and adolescence induces changes to alcohol self-administration and reinstatement of alcohol-seeking behavior in a sex-dependent manner. We subjected male and female rats to peer rejection across adolescence (pd 21–50) and then exposed them to voluntary alcohol self-administration in adulthood (pd 90). We tested both male and female rats in the cue-induced reinstatement test to assess for relapse-like behavior. As mentioned earlier, potential mediating factors that increase risk of later alcohol-related problems are ACE-induced circadian dysregulation and reward system alterations (Hanson et al., 2016; Hasler et al., 2012). Therefore, we sought to evaluate whether peer-rejected rats would show circadian dysregulation

assessed by the home-cage circadian locomotor activity and also alterations in reward sensitivity in a sucrose preference test.

MATERIALS AND METHODS

Animals

Forty female Wistar rats, 24 male Wistar rats, 120 female Fischer 344 rats, and 72 male Fischer 344 rats were used. All rats used in the current study were delivered to our institute on pd 21, Wistar RccHan rats from Envigo (Horst, Netherlands) and Fischer 344 rats from Charles River (Sulzfeld, Germany). Animals were housed in groups of 4 throughout their adolescence into adulthood (unless stated otherwise) in standard type IV rat cages (Ehret, Emmendingen, Germany) under a 12-h artificial light–dark cycle. Standard laboratory rat food (Ssniff, Soest, Germany) and tap water were provided ad libitum throughout the experimental period (unless stated otherwise). All experimental procedures were approved by the Committee on Animal Care and Use (Regierungspräsidium Karlsruhe, Germany) and were carried out in accordance with the local Animal Welfare Act and the European Communities Council Directive of 22 September 2010 (2010/63/EU).

Peer rejection model

The study design was based on our previous model (Schneider et al., 2016a) of peer rejection. In the present study, Wistar rats were subjected to either control or peer rejection condition throughout childhood and adolescence immediately after weaning (pd 21–50). In the control condition, male and female Wistar rats were housed in same-sex groups of 4 under standard housing conditions in separate rooms. In the peer rejection condition, 1 Wistar rat was housed with 3 age- and sex-matched Fischer rats. The Wister rats and the less playful Fischer 344 have a mismatch in their social play behaviors, Fischer 344 rats inadequately reciprocate social play initiated by the Wistar rat mimicking aspects of peer rejection. On pd 50, control Wistar rats were regrouped with unfamiliar Wistar rats. Peer-rejected Wistar rats were grouped with unfamiliar Wistar rats from the same rearing condition, thereby terminating the peer rejection procedure. After the peer rejection period, animals were allowed to recuperate with other "rejected" Wistar rats into adulthood. All animals began alcohol self-administration training in adulthood (Figure 1).

Cue-induced reinstatement of alcohol seeking

Operant alcohol self-administration apparatus

Cue-induced reinstatement of alcohol seeking was carried out in operant chambers (MED Associates Inc.) enclosed in ventilated sound-attenuating cubicles in control/peer rejection female (n = 20/20) and male (n = 12/12) rats. The chambers were equipped with a response lever on each side panel of the chamber. Responses at the active lever activated a syringe pump that delivered a ~30 µl drop of fluid into a liquid receptacle next to it. Responses at the inactive lever were recorded but had no programmed consequences. A light stimulus (house

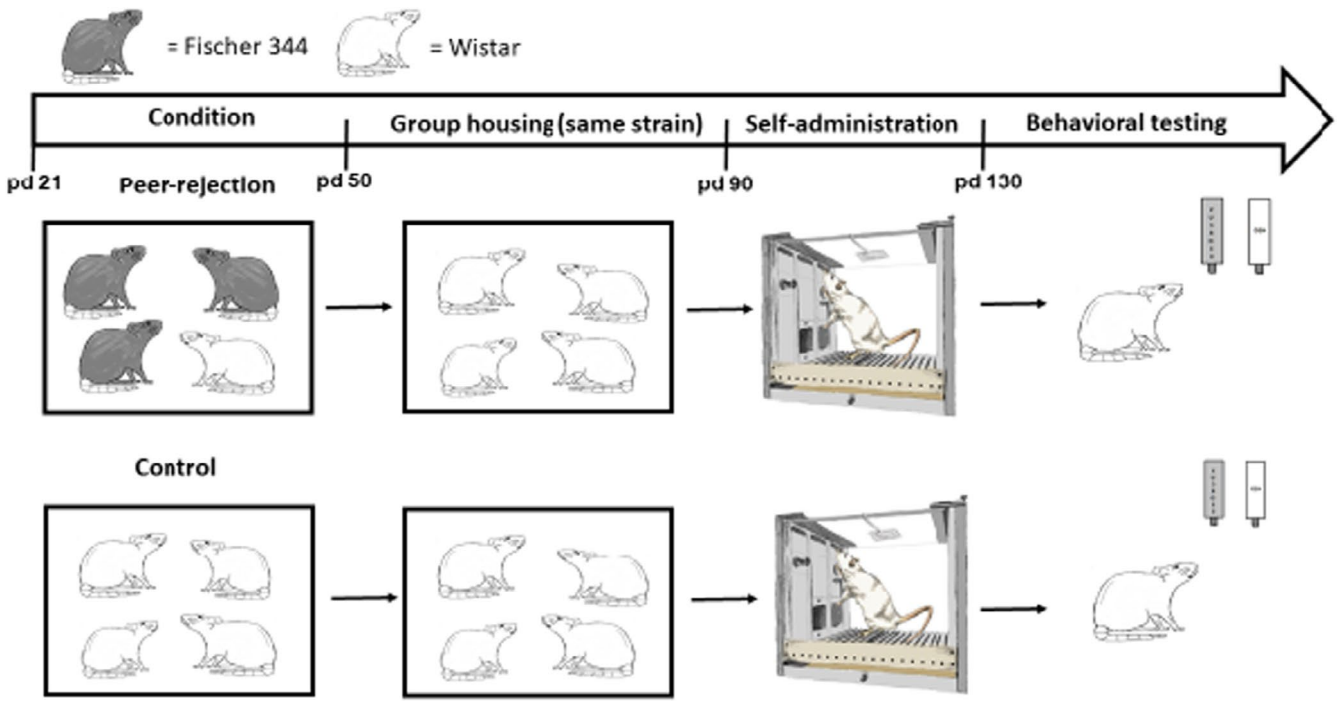

FIGURE 1 Experiment timeline for peer rejection, self-administration, and behavioral testing

light) was mounted above the left and right response levers of the self-administration chamber and served as a discriminative stimulus. The house light indicated the availability of the reward (alcohol/water) as well as an indicator of "time-out" (blinking-light) during which lever presses did not result in reward delivery although lever responses were recorded. Fluid delivery, presentation of stimuli, and data recording were controlled with Med-PC-V software (MED Associates Inc.).

Alcohol self-administration acquisition and extinction phase

All animal training and testing sessions were performed during the dark phase of their light/dark cycle. Animals were trained to self-administer either 10% (v/v) alcohol or water in daily 30-min sessions using a fixed ratio 1 (FR 1) schedule. The purpose of the acquisition phase was to train the animals to discriminate between the availability of alcohol (reinforcement) and water (nonreinforcement). Discriminative stimuli predicting alcohol or water availability were presented during each alcohol or water self-administration session (one 30-min session/day). An orange flavor extract served as the contextual stimulus (S+) for alcohol, whereas water availability was signaled by a lemon grass extract (S−). These olfactory stimuli were generated by dripping few drops of the respective extract into the bedding of the operant chamber before each session. In addition, each lever press resulting in alcohol delivery was accompanied by a 5-s blinking-light conditioned stimulus (CS+), whereas a 5-s constant-light stimulus (CS−) was presented with water delivery. The 5-s period served as a "time-out," during which responses were recorded but not reinforced. At the end of each session, the bedding of the chamber was changed and trays were thoroughly cleaned. During the first 2 days of acquisition, animals were kept fluid deprived for 22 hr per day. Subsequently, alcohol and water sessions were conducted without fluid deprivation in a random manner until the animals received a total of 10 alcohol and 10 water sessions.

After completing the acquisition phase, rats were subjected to daily 30-min extinction sessions for five consecutive days, which in total was sufficient to reach reduced response rates approximating the extinction criterion of 20% of the last acquisition sessions. Extinction sessions began by extending the levers without presenting olfactory discriminative stimuli. Responses at the previously active lever activated the syringe pump, without resulting in the delivery of either alcohol or water or the presentation of response-contingent cues (stimulus blinking-light or constant-light).

Alcohol cue-induced reinstatement

Reinstatement testing began 2 days after the final extinction session. During the reinstatement tests, rats were presented with the same conditions as during acquisition, without reward delivery (alcohol or water). Contextual cues predicting alcohol (S+) or water (S−) availability were also presented during the reinstatement session. Reinstatement sessions were initiated by the extension of both levers and the presentation of either the alcohol- (S+) or water- (S−) associated discriminative stimulus. Responses at the active lever were followed by the activation of the syringe pump and the presentation of the CS+ (blinking-light) in the S+ condition or the CS− (constant-light) in the S− condition. Half of animals were tested under the S+/CS+condition on day 1 and under the S−/CS− condition on day 2. Conditions were reversed for the other half of animals. The number of responses on both the active (i.e., alcohol-associated lever for S+/CS+condition and water-associated lever for S−/CS− condition) and inactive levers (i.e., water-associated lever for S+/CS+condition and alcohol-associated lever for S−/CS− condition) was recorded throughout the experiment. Further behavioral testing took place for all animals after reinstatement testing.

Circadian locomotor activity measurements by the E-motion system

In order to test home-cage locomotor activity, control/peer-rejected female (n = 12/12) and male (n = 12/12) rats were separated into single cages for 3 days and their number of movements was monitored by the use of an infrared sensor connected to a recording and data storing system (Infra-e-motion, Henstedt-Ulzburg, Germany). Home-cage locomotion was measured after alcohol reinstatement. An Infra-e-motion device was placed above each cage (30 cm from the bottom) so that the rat could be detected at any position inside the cage. The device was sampling every second whether the rat was moving or not. The sensor could detect body movement of the rat of at least 1.5 cm from 1 sample point to the next. The data measured by each Mouse-E-Motion device were downloaded onto a personal computer and processed with Microsoft Excel.

Sucrose consumption test

To study potential alterations in reward sensitivity, sucrose consumption was measured in control/peer-rejected female (n = 12/12) and male (n = 12/12) rats that were kept single-housed. All rats were placed into separate single house cages. Two preweighed bottles, 1 containing tap water and the other containing the assigned sucrose solution, were placed on each cage. After 24 h, bottles were removed and reweighed and liquid consumption was measured as the difference in the weight (g) of each bottle before and after the test. Sucrose preference was calculated using the formula: sucrose preference = [consumed sucrose solution/total liquid consumed (sucrose solution + water)] × 100. All rats were given ad libitum access to 1 bottle with tap water and another bottle with sucrose solution for 24 hr. Male rats received 0.35% (w/v) sucrose solution, while female rats were given 0.15% (w/v) sucrose. We have found that

females consume more sucrose solution than males at equal concentrations to sweet tasting solutions and therefore used a lower the concentration for testing females (unpublished data). The positions of bottles were counterbalanced to avoid location preferences, and subsequent 24-hour intake of water and sucrose solution was measured.

Statistical analysis

Data obtained from the self-administration and cue-induced alcohol-seeking experiments were analyzed using a mixed multifactorial ANOVA with repeated measures [between-subjects factors were as follows: sex (female vs. male) and condition (control vs. peer-rejected), and within-subject factors were as follows: lever (active vs. inactive) and session (extinction vs. reinstatement)]. Locomotor activity data were analyzed using 1-hr recordings using 3-way ANOVA with repeated measures [between-subjects factors were as follows: sex (female vs. male) and group (control vs. peer-rejected) and within-subjects factor was as follows: time (day)]. Furthermore, 24-hr locomotor activity, dark-phase and light-phase data were analyzed using a 2-way ANOVA. One-way ANOVA was used for analysis of sucrose intake and preference [between-subjects factor: group (control vs. peer-rejected)]. Post hoc testing (Tukey's HSD) was performed when appropriate. We assigned level of statistical significance was set to $p < 0.05$. All analyses were conducted on using SPSS Statistic version 26.0, and graphs were illustrated on GraphPad Prism version 6.0.

RESULTS

Cue-induced reinstatement of alcohol seeking

Female control rats reached 97 ± 13 alcohol-associated lever responses (S+/CS+condition) and 21 ± 2 water-associated lever responses (S−/CS− condition), while male control rats had 100±21 alcohol-associated lever responses (S+/CS+ condition) and 28±6 water-associated lever responses (S−/CS− condition) by the end of the acquisition phase. During the acquisition phase, peer-rejected females consumed the most alcohol 0.9g/kg (control females consumed 0.8 g/kg), while their male counterparts consumed the least amount of alcohol per body weight 0.43 g/kg (control males consumed 0.54 g/kg). On average, female peer-rejected rats reached 124 ± 12 alcohol-associated lever responses (S+/CS+ condition) and 29 ± 4 water-associated lever responses (S−/CS− condition) and male peer-rejected rats had 80±16 alcohol-associated lever responses (S+/CS+ condition) and 16 ± 2 water-associated lever responses (S−/CS− condition) by the end of the acquisition phase.

Neither alcohol- nor water self-administration acquisition differed significantly between condition (peer-rejected vs. control) animals for water, $F(1, 60) = 0.053$, $p = 0.81$, or ethanol, $F(1, 58) = 0.15$, $p = 0.69$. No differences were observed between sexes in acquisition of alcohol, $F(1, 60) = 0.55$ $p = 0.45$ (Figure 2) or water, $F(1, 60) = 3.44$, $p = 0.06$, self-administration. Analysis of alcohol intake (g/kg) across acquisition demonstrated a significant main effects of sex, $F(1, 60) = 9.983$ $p = 0.0.02$, were female animals consumed more alcohol than their male counterparts.

For the reinstatement test, a mixed multifactorial ANOVA with repeated measures revealed a main effect of session, $F(1, 60) = 106.43$, $p < 0.001$, lever, $F(1, 60) = 149.08$, $p < 0.001$, sex, $F(1, 60) = 4.80$, $p = 0.03$, condition × sex, $F(1, 60) = 23.83$, $p < 0.001$, condition × sex × lever, $F(1, 60) = 10.79$, $p = 0.002$, and session × lever × condition × sex, $F(1, 60) = 11.32$, $p < 0.01$, interactions, indicating that all animals increased their pressing for the alcohol lever during the S+/CS+test compared with the last extinction session (Figure 3). Post hoc analysis of the interaction effects revealed that female peer-rejected (vs. control) rats responded significantly more on the active lever during the S+/CS+test, condition, $F(1, 38) = 7.35$, $p = <0.001$ (Figure 3A), while the opposing effect was observed in peer-rejected (vs. control) males, condition, $F(1, 22) = 4.87$, $p = 0.038$ (Figure 3C). No significant differences were observed in responding on the inactive lever during S+/CS+tests between

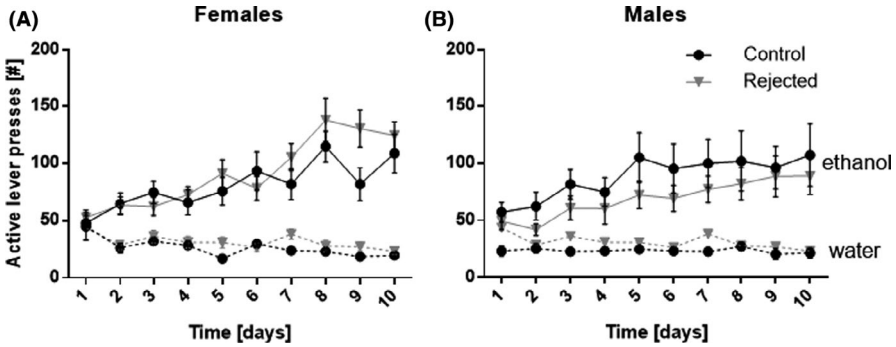

FIGURE 2 Average number of alcohol-paired (S+/CS+) and water-paired lever responses for each session (day) of self-administration under a FR1 schedule of reinforcement in females ($n = 20/20$) (A) and males ($n = 12/12$) (B). The alcohol-paired (ethanol) lever responses were greater than the (water) lever responses, but no group differences were observed in alcohol or water consumption between controls and peer-rejected animals. Data are presented as means ± S.E.M

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groups (Figure 3B,D). A main effect of condition, $F(1, 60) = 5.28$, $p = 0.003$, was observed in the S−/CS− test (water responding). This effect was mainly driven by a lower responding on the active lever in peer-rejected males (Table 1).

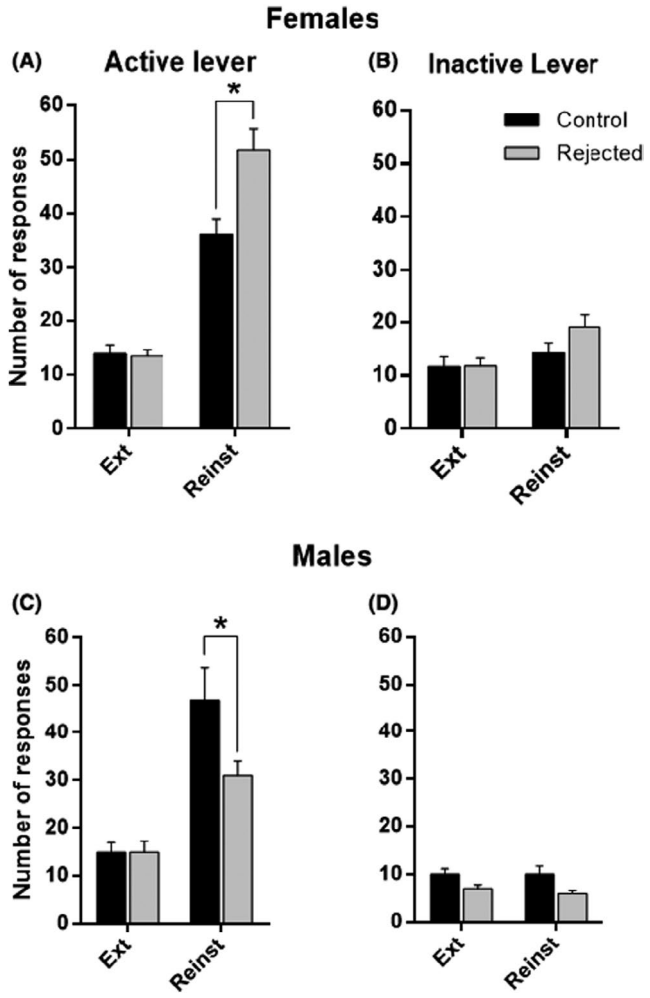

FIGURE 3 Alcohol cue-induced reinstatement in control and peer-rejected female (A-B, $n = 20/20$) and male (C-D, $n = 12/12$) rats. Data are shown as the average number of lever presses on the active (A, C) and inactive (B, D) levers during the last 3 extinction sessions (Ext) and as the number of responses after the presentation of stimuli previously paired with either alcohol (Reinst). Data are presented as means ± SEM. * indicates significant group differences, $p < 0.01$

Home-cage circadian locomotor activity measurements by the E-motion system

A 3-way ANOVA with repeated measures revealed a significant main effect of day, $F(1, 42) = 5.92$, $p = 0.004$, sex, $F(1, 42) = 11.705$, $p = 0.001$, and day × sex × group, $F(1, 42) = 6.814$, $p = 0.001$, interaction. These data indicate that activity increased on day 3 which was most pronounced in female, $F(1, 42) = 11.705$, $p = 0.001$, locomotor activity. Average 24-hour activity differed between sexes, $F(1, 42) = 0.009$, $p = 0.92$ (Figure 3), where the effect was also strongest in female rats. All rats showed a rodent typical increase in locomotion during the dark phase and reduction in activity during the light phase. We did not observe differences in dark- (04:00–16:00) or light-phase (16:00–04:00) locomotor activity (Figure 4).

Sucrose consumption test

Analysis of sucrose preference did not reveal significant differences between control and peer-rejected animals in either females, $F(1, 22) = 2.847$, $p = 0.106$, or males, $F(1, 22) = 0.687$, $p = 0.416$. Further analysis of sucrose solution (ml/kg) intake during the 24-hr free-choice sucrose did not differ between rejected or control animals in females, $F(1, 22) = 3.235$, $p = 0.086$, or males, $F(1, 22) = 0.143$, $p = 0.709$ (Figure 5).

DISCUSSION

In this study, we investigated how peer rejection across adolescence affected alcohol-seeking behavior, circadian locomotor activity, and sucrose consumption in adult female and male Wistar rats. We observed no differences in either acquisition of alcohol, water self-administration, and extinction learning between control and peer-rejected animal groups across sexes. However, peer rejection had sex-dependent effects on alcohol cue-induced reinstatement. Specifically, our data suggest that peer rejection enhanced later life alcohol cue-induced reinstatement only in peer-rejected female Wistar rats (vs. control) but had an opposing effect on rejected males. These data suggest that ASEs specifically alter mechanisms involved in alcohol relapse-like behaviors in a sex-dependent manner. There are several candidate mechanisms that could drive these

TABLE 1 Water extinction and reinstatement data from female ($N = 20/20$) and male ($N = 12/12$) rats. Data are indicated as mean ±SEM

Water cue-induced extinction and reinstatement					
Factors		Extinction		Reinstatement	
Sex	Condition	Active	Inactive	Active	Inactive
Female	Control	12 ± 1.80	14 ± 1.78	14 ± 1.78	16 ± 1.39
	Peer-rejected	12 ± 1.48	13 ± 1.39	19 ± 1.78	15 ± 1.35
Male	Control	10 ± 1.20	15 ± 1.90	10 ± 2.29	13 ± 1.67
	Peer-rejected	7 ± 0.74	15 ± 1.90	11 ± 1.80	16 ± 1. 79

sex-dependent effects on alcohol cue-induced reinstatement differences. First, peer rejection results in pronounced alterations in the endocannabinoid system, especially in cannabinoid receptor 1 (CB1) expression as well as within the oxytocin system in reward-related brain regions (Schneider et al., 2014, 2016a). Both systems are critically involved in relapse-like behavior (Spanagel, 2020), and sex-dependent modulation of this behavior has been described as well (Hansson et al., 2018; Hansson & Spanagel, 2021).

Another potential explanation could be that female rats are more physiologically reactive to peer rejection during adolescence compared with male rats and therefore present a more vulnerable population to relapse-like behaviors in adulthood. Indeed, sex differences in vulnerability and resilience to adolescent stress are beginning to emerge. Males in general (humans included) are at a greater risk of experiencing negative effects of stress across adolescence. While female offspring have a compensatory mechanism that protects them against the effects of stress during adolescence, however, these effects are unmasked later in life (i.e., after menopause) (Hodes & Epperson, 2019). Nonetheless, a history of ACEs in both sexes increases the increased risk of alcohol abuse and dependence in different directions (Craig et al., 2019; Peltier et al., 2019). For example, only in women the intensity of ACEs is correlated with an increase in relapse to drugs (Hyman et al., 2008). In conclusion, our findings and rat model of peer rejection provide good face validity to the human condition as only in women the intensity of ACEs is correlated with an increase in relapse to alcohol and other drugs (Heffner et al., 2011; Hyman et al., 2008; Kennedy et al., 2013).

With respect to the considerable overlap between peer rejection and relapse-like behaviors in humans and rats, we want to highlight that male rats seem to be more resilient to the peer rejection procedure (e.g., no changes in social interaction and pain threshold; unpublished observations) while peer-rejected female rats, much like prepubertal girls(Benenson et al., 2013; Stroud et al., 2017), seem to be more sensitive to peer rejection (Stroud et al., 2017) and its long-term effects. Importantly, chronic effects of peer rejection in humans are associated with heightened neural responses to social exclusion in the dorsal anterior cingulate cortex (dACC) (Will et al., 2016), a region identified in alcohol cue reactivity and alcohol relapse (Noori et al., 2016; Zhao et al., 2020). This presents a potential anatomical correlate for the sex-dependent changes observed to alcohol seeking in peer-rejected animals that may be driven by changes in the dACC.

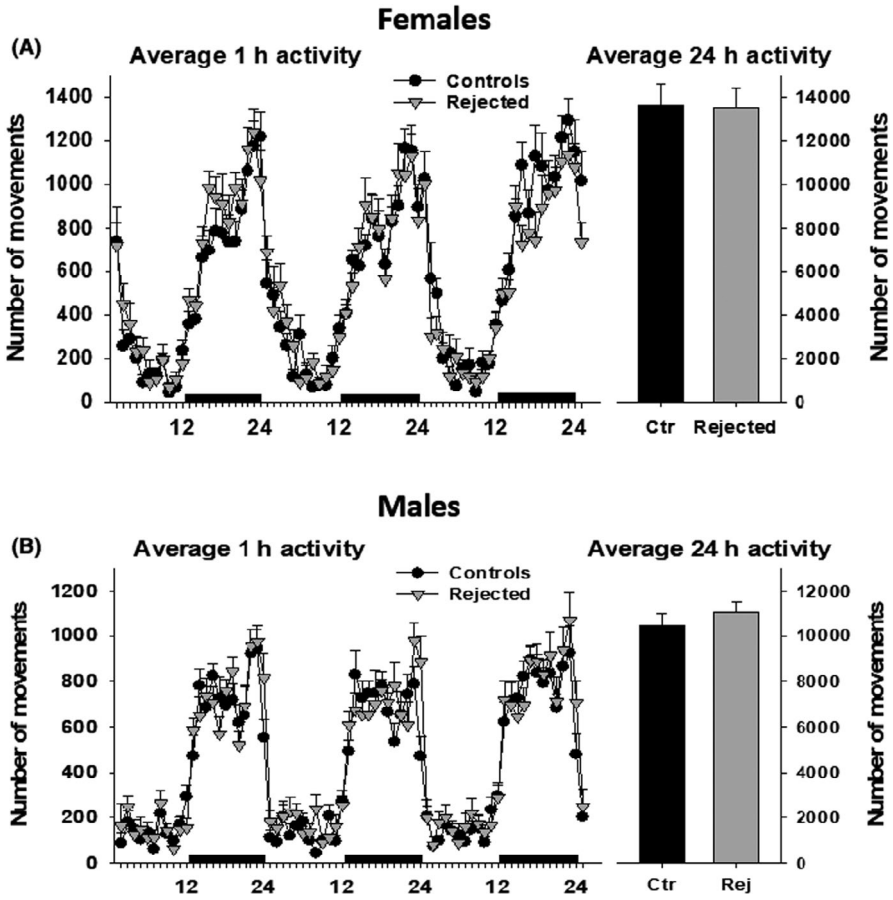

FIGURE 4 Circadian activity recordings in control and peer-rejected female (A) (n = 12/12) and (B) (n = 12/12) male rats measured as the number of movements during 3 consecutive days in the home-cage (black horizontal bars mark the dark (active) phases of the circadian cycle). Left figures demonstrate total number of movements during each consecutive hour, and right figures represent total number of movements during 24 hr. All data are expressed as means ± SEM

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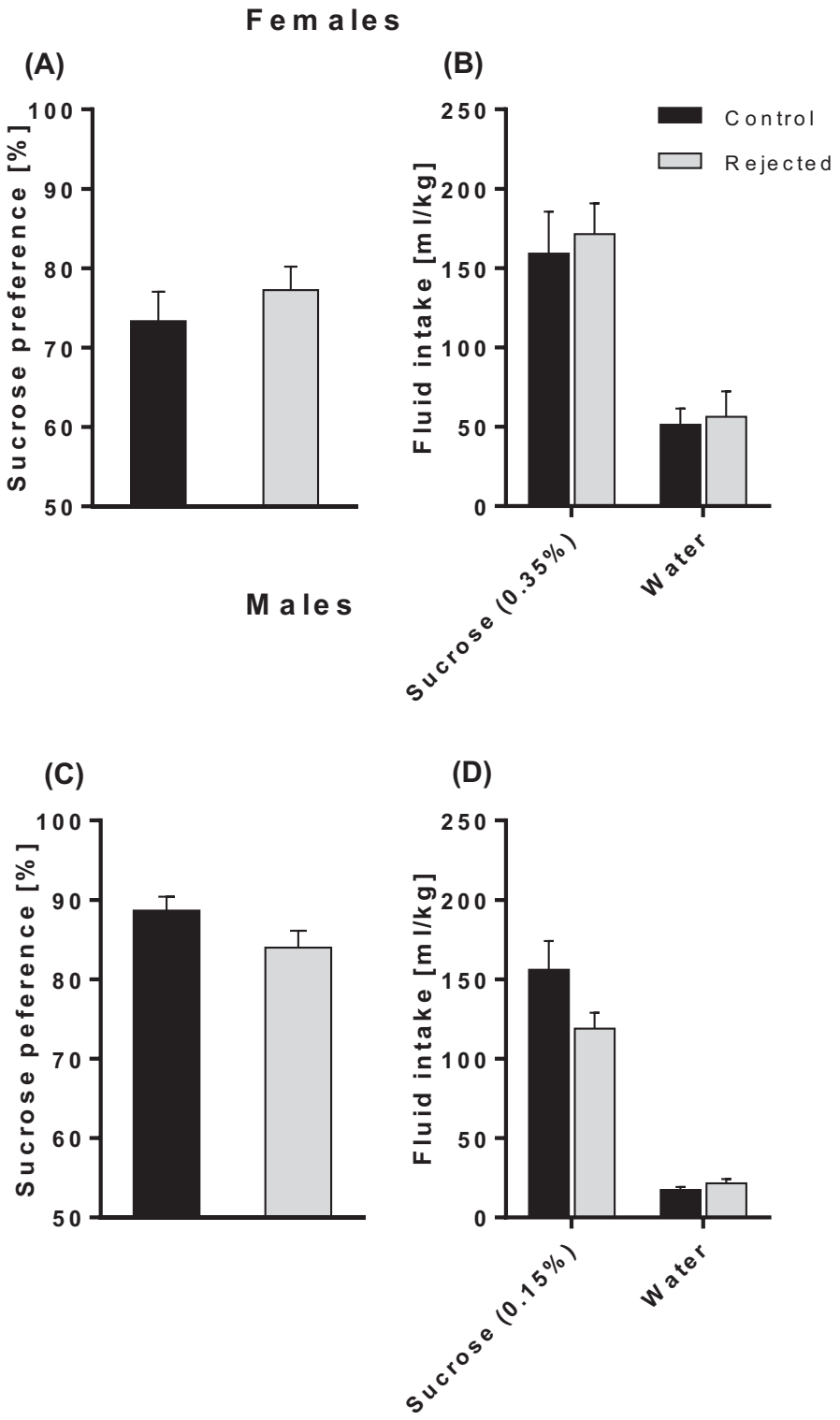

FIGURE 5 Sucrose preference (%) and intake of water and sucrose (ml/kg) in control and peer-rejected (A, B) female (*n* = 12/12) and (C, D) male (*n* = 12/12) rats. All animals had free 24-hr access to a water bottle and a bottle containing either 0.15% sucrose solution (female rats) or 0.35% sucrose solution (male rats). For direct sex comparisons, these concentrations were chosen to have similar levels of sucrose intake and preference in female and male control rats. All data are expressed as means ± SEM

Peer rejection has been linked to poor sleep during adolescence (Tu et al., 2019). This dysregulation of sleep in turn is associated with a myriad of health risks including increased alcohol consumption in both rodents and humans (reviewed by Jagannath et al., 2013). Moreover, alcohol can also influence circadian-related gene expression and circadian rhythmicity (Perreau-Lenz & Spanagel, 2015). As our peer rejection model combines voluntary alcohol self-administration, we hypothesized that peer rejection may impact

circadian rhythms and induce circadian dysregulation. Our analysis revealed a main effect of sex on locomotion. The main effect of sex was driven by a higher locomotion in females, who are in general more active than male rats making this finding unsurprising and well-established in the field (reviewed in; Rosenfeld, 2017). Our data indicate that peer rejection and alcohol self-administration do not induce long-term circadian dysregulation. Other studies that have reported circadian dysregulation after ASEs (Wells et al., 2017) have used more severe stressors such as chronic social defeat. This along with the fact that our circadian measurements were assessed 3 weeks after the end of alcohol self-administration may explain the null findings.

Alongside circadian dysregulation, ACEs have been reported to induce alterations within the reward system. The cumulative stress of ACEs is often associated with a blunted reward response in humans (Hanson et al., 2016) and rodents (Willner et al., 1987) alike. Similar to individuals with ACEs, humans with alcohol problems show comparable neurobiological alterations in their reward processing (Novick et al., 2018), suggesting that some of the underlying neurobiological mechanisms of ACEs and AUD may overlap. For instance, both populations show a blunted response to reward in the ventral striatum, a region known to be involved in reward learning and decision-making (Cox & Witten, 2019; Wrase et al., 2007). We had previously demonstrated that peer-rejected rats show a decreased affinity for social reward (Schneider et al., 2016a). To build on this, we assessed whether these deficits may be generalizable (i.e., palatable foods). To assess alterations in reward sensitivity, we tested animals in the sucrose consumption test. However, we found no differences in sucrose consumption or sucrose preference in peer-rejected rats. These results suggest that there is not a general alteration in reward sensitivity in peer rejection. One limitation with our approach to assess reward sensitivity is the ability of the test to observe subtle changes in sucrose consumption/preference. Using an operant paradigm with the matching law principle (Sanchis-Segura et al., 2005) or intracranial self-stimulation could provide better sensitivity and be more discriminative in detecting subtle changes between peer-rejected and control animals.

Although our peer rejection produces similar effects to those seen in humans, there are some limitations that need to be considered. First, our model is not a pure peer rejection model where the animal is directly shun from the group (i.e., bullying). Our peer rejection model induces more subtle modes of social exclusion, where Wistar rats are indirectly rejected by their Fischer 344 cage mates by depriving social play during an important time in development. Second, there is a need to develop methods to evaluate in detail the long-term consequences of the peer rejection procedure to better determine the severity of the rejection in male animals.

In conclusion, changes in alcohol-seeking behavior in peer-rejected rats reflect a quantitative sex difference that represents a vulnerability in females and a resilience in male rats to relapse-like behaviors. Similar observations have been observed in humans. Although our model cannot fully replicate the human condition of ACEs, it recapitulates behavioral deficits that impact alcohol-seeking behavior later in life. Finally, our model provides an approach with good face validity to studying the molecular and neurobiological mechanisms of ASEs on alcohol and potentially on other drugs of abuse across all phases of addiction-like behaviors in male and female rats.

We conclude that the presented ecologically valid model of peer rejection can be used in future studies to study the molecular and neurobiological underpinnings of persistent sex-dependent effects of adolescent ASEs on later alcohol-seeking and relapse-like behavior.

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CONFLICT OF INTEREST

The authors whose names are listed above certify that they have NO affiliations with or involvement in any organization or entity with any financial or non financial interest in the subject matter or materials discussed in this manuscript.

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