Aberrations in incentive learning and responding to heroin in male rats after adolescent or adult chronic binge-like alcohol exposure

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Abstract

BACKGROUND AND PURPOSE: Binge drinking is a serious problem among adolescents and young adults despite its adverse consequences on the brain and behavior. One area that remains poorly understood concerns the impact of chronic intermittent ethanol (CIE) exposure on incentive learning.

METHODS: Here we examined the effects of CIE exposure during different developmental stages on conditioned approach and conditioned reward learning in rats experiencing acute or protracted withdrawal from alcohol. Two or 21 days after adolescent or adult CIE exposure, male rats were exposed to pairings of a light stimulus (CS) and food pellets for 3 consecutive daily sessions (30 CS-food pellet pairings per session). This was followed by conditioned approach testing measuring responses (food trough head entries) to the CS-only presentations and by conditioned reward testing measuring responses on a lever producing the CS and on another producing a tone. We then measured behavioral sensitization to repeated injections of heroin (2 mg/kg/day for 9 days).

RESULTS: Adolescent and adult alcohol-treated rats showed significantly impaired conditioned reward learning regardless of withdrawal period (acute or prolonged). We found no evidence of changes to conditioned approach learning after adolescent or adult exposure to CIE. Finally, in addition to producing long-term impairments in incentive learning, CIE exposure enhanced locomotor activity in response to heroin and had no effect on behavioral sensitization to heroin regardless of age and withdrawal period.

CONCLUSIONS: Our work sets a framework for identifying CIE-induced alterations in incentive learning and inducing susceptibility to subsequent opioid effects.

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conditioned reward; conditioned approach; conditioned reinforcement; chronic intermittent ethanol exposure; alcohol; incentive learning; heroin sensitization

1. Introduction

Binge drinking is a recurring cause of alcohol-related deaths in the United States (Stahre et al., 2014) with approximately a quarter of the population aged 12 years or older engaging in this risky behavior (Johnston et al., 2019; Substance Abuse and Mental Health Services Administration, 2014). Most adults with alcohol use disorder (AUD) began drinking during adolescence, a developmental period characterized by elevated risk of substance use initiation, peer pressure, risk-taking and greater susceptibility to the adverse consequences of use (Casey and Jones, 2010). Individuals diagnosed with AUD often show deficits in retrieval of verbal and non-verbal information, performance of visuospatial tasks and executive cognitive functioning (Brown et al., 2000; Garcia-Moreno et al., 2008; Sanhueza et al., 2011; Winward et al., 2014). Similarly, preclinical studies report deficits in attention set shift learning (Fernandez and Savage, 2017; Gass et al., 2014), reversal learning (Badanich et al., 2016; Kuzmin et al., 2012) and operant extinction (Broadwater and Spear, 2013; Gass et al., 2014) in animals subjected to chronic intermittent ethanol (CIE) exposure, an animal model of binge drinking, during adolescence. These tasks employ cortico-striatal circuits (Dalton et al., 2014; Floresco et al., 2008; Ghods-Sharifi et al., 2008; McAlonan and Brown, 2003; Ragozzino et al., 1999) whose functions and structures are altered by alcohol, especially when CIE exposure occurs during early adolescence (Broadwater et al., 2018; Coleman et al., 2014; Galaj et al., 2019; McMurray et al., 2016; Renteria et al., 2018; Shan et al., 2019).

One topic that has remained poorly investigated concerns the impact of adolescent or adult CIE exposure on incentive learning or the learning about stimuli that gain reward value through association with naturally rewarding stimuli. Given that chronic alcohol intake affects neural circuits that overlap with those involved in reward-related learning this becomes an important question from both basic brain/behavior mechanisms and pathological alcohol use perspectives. A standard procedure to investigate incentive learning in animals is the conditioned reward paradigm. Here, stimuli (conditioned stimuli, CSs) that are associated with primary rewards (unconditioned stimuli, USs) acquire the ability to produce effects similar to the US including the capacity to elicit conditioned approach responses and to function as conditioned rewards in their own right (Beninger and Ranaldi, 1992; Skinner, 1938). We have proposed and demonstrated through a series of studies that the acquisition by a CS of the capacity to function as such depends on its acquired capacity to activate the same neural system – ventral tegmental area (VTA) dopamine (DA) neurons – that are unconditionally activated by the primary reward (Galaj et al., 2017; Galaj and Ranaldi, 2018; Kest et al., 2012; Ranaldi, 2014; Ranaldi et al., 2011; Zellner et al., 2009; Zellner and Ranaldi, 2010). Pedunculopontine tegmental, basolateral amygdala and prefrontal cortical afferents to the VTA DA system, a system that plays a critical role in conditioned reward learning (Burns et al., 1993; Everitt and Robbins, 1992; Pears et al., 2003, Ranaldi 2014),
are severely impacted by CIE exposure (Arendt et al., 1988; Broadwater et al., 2018; Coleman et al., 2014; Jury et al., 2017; McGinnis et al., 2019; Morales et al., 2018; Pereira et al., 2019; Schindler et al., 2016; Vetreno et al., 2014). Also, there is evidence that repeated alcohol administration can cause long-term sensitization associated with adaptive changes in midbrain DA neuronal output to and cholinergic signaling in the striatum (Miller and Kamens, 2019; Nestby et al., 1997; Tschumi et al., 2019; Xu and Kang, 2019). Therefore, if CIE exposure sensitizes the VTA DA neuronal system and affects signaling to this system – a system critical for mediating CS effects – then it would be expected that animals exposed to CIE might show differential responding to CSs compared to animals not exposed to CIE.

A few studies have shown that adolescent alcohol exposure amplified the incentive value of CSs, as demonstrated by increases in sign tracking (engagement with the CS) and decreases in goal tracking (US-directed behaviors) (Kruse et al., 2017; Madayag et al., 2017; McClory and Spear, 2014), increasing vulnerability to impulsive responding and further drug and alcohol abuse (Tomie, 1996; Tomie and Morrow, 2018; Tomie and Sharma, 2013). However, these alcohol-induced behavioral alterations were tested only in adult animals. There is also evidence that alcohol consumption during adolescence in rats enhances conditioned reinforcement (conditioned reward) in adulthood, but this deficit was tested only in a sub-population of high sign-trackers (Kruse et al., 2017). Adolescent alcohol bingeing effects on conditioned reward in a broader population remain unknown. Thus, to date, the effects of adolescent or adult CIE exposure on incentive learning during acute withdrawal and in adolescence as well as later in life have not been assessed.

In addition to overlapping with neural substrates underlying reward-related learning, chronic alcohol intake affects the same neural circuits that undergo plasticity during sensitization to drugs of abuse, including heroin. Sensitization refers to progressively greater responses (behavioral or physiological) to repeated intermittent exposure to the same dose of a drug. Alcohol and other drugs of abuse share some common mechanisms of action (Di Chiara and Imperato, 1988) and with repeated intermittent exposure induce sensitization (Galaj et al., 2013; Masur et al., 1986; Morrison et al., 2011; Post et al., 1988; Steketee et al., 1991), a phenomenon thought to contribute to the pathogenesis of addiction (Kalivas and Stewart, 1991; Nestby et al., 1997; Robinson and Berridge, 1993). In fact, in previous studies we have demonstrated that chronic intermittent heroin treatment produces locomotor sensitization and enhances responding for conditioned reward during acute (WD 2 = 2 days) and protracted (WD 30 = 30 days) withdrawal periods in rats (Galaj et al., 2013; Morrison et al., 2011; Ranaldi et al., 2009). Given that chronic alcohol or heroin affects the same underlying neural substrate – the mesolimbic DA system – and that both of these drugs are often co-abused (Backmund et al., 2003), it is imperative to determine the consequences of earlier alcohol exposure on behavioral responses to heroin.

In the present study we determined whether CIE exposure to alcohol during different developmental periods (adolescence vs. adulthood) and during acute or protracted withdrawal (1) alters reward-related learning as assessed in the conditioned reward and conditioned approach paradigms and (2) affects locomotor responses to heroin and or the development of behavioral sensitization to the locomotor responses to heroin.
2. Methods

The housing conditions and care of the animals were consistent with those specified by the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 2011). All experiments were approved by the Queens College Institutional Animal Care and Use Committee.

2.1. Subjects

Male Sprague Dawley rats (n=64) purchased from Envigo (NJ, US) arrived at our animal facility on postnatal day P21. All animals were housed in pairs in a temperature- and humidity-controlled colony under a 12-h light/dark cycle (lights on at 7:00 pm). Rats had free access to water and standard rat chow (Purina Lab Diet 5012) except during Experiment 1 where they received daily rations of food in order to maintain weights at 85% of their free feeding values. Alcohol treatment and behavioral experimentation occurred during the animals' dark cycle (2-6 pm).

2.2. Chronic intermittent ethanol treatment

Rats were subjected to a chronic intermittent ethanol (CIE) exposure or water exposure procedure adapted from our previous study and other reports (Shan et al., 2019; Vore et al., 2017). Rats were randomly assigned to either adolescent or adult CIE or water treatment groups. During postnatal days P28 – P47 rats in the adolescent groups received daily intragastric gavages of either ethanol, administered at a dose of 4 g/kg (at 25% v/v concentration), or distilled water, on four 3-day on/2-day off cycles. Rats assigned to adult CIE groups received the same treatments on P70 – P89. To determine whether acute or protracted withdrawal periods affect behavior of these CIE exposure animals we further divided groups based on the number of withdrawal days (WD 2 and WD 21). As used in this report, the terms withdrawal days or periods refer to the time between the final alcohol administration and the initiation of behavioral testing rather than to physical dependence.

2.3. Apparatus

Conditioned approach and conditioned reward experiments took place in 8 conditioning chambers, each measuring 30 x 21 x 18 cm (l x w x h) and placed in a ventilated, sound attenuating cubicle with an operating fan that circulated air and masked outside noise. The conditioning chambers were equipped with 2 removable levers, a tone generator, a light mounted above the left lever and a food trough (5 x 5 cm) into which food pellets (45 mg each; Bio-Serv, Inc., Frenchtown, NJ, US) could be dropped from a food dispenser. A photosensor in the food trough detected head entries.

Locomotor activity in the heroin sensitization experiments was measured in open field chambers measuring 43 x 43 x 30 cm. Each chamber was equipped with 32 photo-emitters positioned 6 cm above the floor and along two adjacent walls (16 in each wall), each paired directly opposite a photocell. Locomotor activity counts were registered when adjacent beams were broken consecutively.
2.4. Reward-related learning paradigm with conditioned approach and conditioned reward

Behavioral experimentation began 2 or 21 days after the last gavage treatment (starting on P49 or P89 for WD 2 groups and P68 or P110 for WD 21 groups). The entire procedure consisted of four phases: pre-exposure, conditioning, conditioned reward test and conditioned approach test. The two latter tests were counterbalanced such that half of the animals in each group were tested for conditioned approach, followed by a conditioned reward test the next day while for the other half the order of testing was reversed.

Pre-exposure—During the pre-exposure phase rats were placed in the operant conditioning chambers for 40-min sessions held once a day for 3 consecutive days. Pressing one lever produced a 3-s tone presentation while pressing the other lever resulted in a 3-s light presentation above that lever. The number of presses on each lever was recorded, providing operant levels of responding on each lever. No criteria or limitations to lever pressing were set.

Conditioning—During 1-h-long conditioning sessions, held once a day for 3 consecutive days, the levers were absent and 30 light stimulus [eventual conditioned stimulus (CS)] presentations were presented under a random time schedule and on average 150 s apart. Each 3-s CS was followed by the delivery of a food pellet into the food trough. A day following the last conditioning session rats received one 30-min session during which no food or CS presentations occurred.

Conditioned approach test—During the CS-only test (1-h long), measuring conditioned approach, levers were absent and rats received 30 CS presentations only (no food pellets) under the same schedule as during conditioning sessions.

Conditioned reward test—During the conditioned reward test (40 min long) both levers were present and pressing one lever produced the tone and pressing the other the light, as in the pre-exposure phase. No food pellets were delivered during this test.

2.5. Heroin sensitization

In this experiment we wanted to determine whether adolescent or adult CIE exposure affects locomotor activity responses to heroin in rats. Two days after the conditioned reward test the same groups (n=8 in each) were subjected to a chronic intermittent heroin treatment regimen during which the rats were injected intraperitoneally with heroin (2 mg/kg) followed 5 min later by placement into locomotor activity chambers for 30 min. This was repeated on 9 consecutive days.

2.6. Data Analysis

In Experiment 1a, to analyze conditioned reward, the number of responses made on each lever during the three pre-exposure sessions was averaged and the number of responses on each lever during the conditioned reward test was measured for each rat. Responding on the light and tone levers was analyzed using a mixed design, 5-way analysis of variance (ANOVA) with CIE treatment, age and withdrawal period as between-groups factors and
phase and lever as repeated-measures factors. Significant 3 or more factor interactions were followed by interaction comparisons and post-hoc Bonferroni tests.

To analyze conditioned approach (Experiment 1b), each 60-min conditioning session and the CS-only test session were divided into 3 periods: non-CS, Pre-CS (6 s prior to onset of CS) and CS (6 s beginning with the onset of the CS) periods. The total numbers of food trough head entries during the Pre-CS and CS periods were counted for each session and used to calculate difference scores (CS minus Pre-CS head entries) for each session. This difference score indicates the magnitude of conditioned approach learning. These data were analyzed using a 4-way ANOVA with CIE treatment, age and withdrawal period as between-groups factors and session as a repeated-measures factor. A significant main effect of session was followed by post hoc Bonferroni tests. The difference scores (CS minus Pre-CS head entries) for the CS-only test session were analyzed using a 3-way ANOVA with CIE treatment, age and withdrawal period as between-groups factors.

For the heroin sensitization experiment the data consisted of the total number of locomotor activity counts made during each of nine sessions. Statistical analyses were conducted on the data from all sessions using a 4-way ANOVA with CIE treatment, age and withdrawal period as between-groups factors and session as a repeated measures factor.

3. Results

3.1. Conditioned reward.

During the pre-exposure phase, in all 8 groups (n=8 in each group), the numbers of lever presses on the light and tone levers were low with slightly more responding on the light lever than on the tone (see Fig. 1A-D). In the test phase, all groups showed increases in light lever responding with no or very little change in tone lever responding, as compared to the pre-exposure phase. However, in the test phase, all alcohol-treated groups showed smaller pre-exposure to test increases in responding on the light lever, with no change in responding on the tone lever, than the corresponding water-treated control groups (Fig. 1A-D).

A 5-way ANOVA revealed a significant phase x lever x treatment interaction \([F_{1, 56} = 14.41, p < .001]\). Interaction comparisons of lever by treatment at each phase revealed a significant interaction in the test phase \([F_{1,56} = 30.36, p < .05]\) but not in the pre-exposure phase. Tests of simple effect of treatment at each level of lever in the test phase revealed a significant treatment effect on the light lever \([F_{1,56} = 72.43, p < .05]\) but not on the tone lever. Thus, in general, alcohol treated rats showed significantly less conditioned reward responding than water-treated rats, regardless of age or withdrawal period.

3.2. Conditioned approach

During the three conditioning sessions groups that were previously treated with alcohol during adolescence or in adulthood and assigned to WD 2 or WD 21 had similar difference scores to their respective water-treated control groups and for all groups the difference scores appeared to increase with subsequent sessions (Fig. 2A-D). A 4-way ANOVA revealed no significant effects. During the CS-only test all four groups previously treated with alcohol showed similar CS-PreCS difference scores to their respective water-treated
counterpart groups (Fig. 2A-D). For the CS-only test a 3-way ANOVA with CIE treatment, age and withdrawal period as between-groups factors revealed no significant effects.

### 3.3. Heroin locomotor activity

We then wanted to determine whether a history of CIE exposure during adolescence or in adulthood would affect locomotor responses to or the development of sensitization to those locomotor responses to heroin. To test these possibilities we subjected all animals to chronic intermittent heroin treatment (2 mg/kg) for 9 consecutive days. All groups showed similar progressively increasing levels of locomotor activity in response to heroin across the 9 treatment sessions (Fig. 3A-D). A 4-way revealed a significant main effect of treatment \( F_{1,56} = 4.31, p < .05 \), a significant main effect of withdrawal \( F_{1,56} = 11.68, p < .01 \) (Fig. 3A-D) and a significant main effect of session \( F_{8,448} = 10.66, p < .001 \). Thus, in general, rats previously treated with alcohol showed significantly greater locomotor responses to heroin.

### 4. Discussion

The major findings of this study are the following: (1) Rats exposed to alcohol during adolescence or adulthood showed a significant reduction in conditioned reward learning; (2) We found no evidence that adolescent or adult CIE exposure affects conditioned approach during acute or protracted withdrawal periods; (3) Regardless of the onset, prior alcohol exposure increased behavioral responses to heroin and had no effect on the development of behavioral sensitization to heroin.

Our results indicate that CIE exposed animals showed impaired conditioned reward learning rather than suppressed motoric activity. We base our conclusions on several findings. First, water- and alcohol-treated animals showed similar levels of lever pressing during the pre-exposure phase and similar numbers of total head entries during conditioning sessions (data not shown). Second, CIE animals showed no weight loss during the alcohol treatment or withdrawal periods and ate all food pellets during conditioning sessions. Thus, the most parsimonious interpretation of these results is that alcohol exposure induced a deficit in incentive learning. This deficit appears to be long lasting as alcohol-treated adolescent rats, in comparison to water-treated rats, showed significantly less responding for conditioned reward during protracted withdrawal (WD 21). Interestingly, we observed similar deficits in rats exposed to alcohol in adulthood, suggesting that alcohol-induced alterations in incentive learning are not age-specific. Indeed, CIE exposure in adulthood has been associated with deficits in other types of learning (Badanich et al., 2016; Broadwater and Spear, 2013; Fernandez et al., 2017; Kuzmin et al., 2012; Slawecki, 2006). Our data suggest that alcohol binge drinking, regardless of the age of initiation, can negatively impact reward-related learning.

Interestingly, alcohol-treated rats responded less than water-treated counterparts for a food-paired stimulus (the light) when that stimulus was the conditioned reward outcome of lever pressing but responded similarly to water-treated rats when the same food-paired stimulus was a CS eliciting a conditioned approach response. A major difference between both protocols is that in the conditioned approach experiments the dependent measure – CS-
elicited head entries during the CS-only test – did not involve new learning as this was the same response learned during conditioning whereas for the conditioned reward experiments the dependent measure – CS-reinforced lever pressing – involved the learning of a new response-outcome contingency. It seems that this type of incentive learning may be affected by chronic binge-like alcohol intake. However, the lever press response in the conditioned reward test requires more effort than the head entry response in the conditioned approach experiment so it remains possible that the alcohol treatment affected the motivation to perform the more difficult response rather than the capacity to learn the new response. Although reasonable, this explanation seems less likely than the learning explanation given that, before conditioning, all animals showed similar levels of lever pressing indicating that motivation to explore (i.e., press) the levers was similar between alcohol and water groups.

Alcohol remains the most commonly abused substance by adolescents, despite adverse consequences on the developing brain and behavior. Two major long-term consequences of adolescent alcohol use are impairment in decision making (Brown et al., 2000) and increased vulnerability to addiction (Brown and Tapert, 2004; Grant et al., 2001). These findings match those of preclinical studies reporting cognitive deficits (Badanich et al., 2016; Coleman et al., 2014; Fernandez et al., 2017; Galaj et al., 2019; Gass et al., 2014; Kuzmin et al., 2012) and increased alcohol intake (Matthews et al., 2008; Pascual et al., 2009; Strong et al., 2010) in adult rats or mice after adolescent alcohol exposure [but see (Vetter et al., 2007)]. Undeniably, adolescent CIE exposure has long-term repercussions that are evident in adulthood. However, our findings demonstrate that adolescent and adult CIE exposure leads to severe deficits in incentive learning, as manifested by significantly smaller increases in pre-exposure to test responding on the light (conditioned reinforced) lever during acute (WD2) and prolonged (WD21) withdrawal.

In contrast to our findings, a previous study reported that animals with a history of adolescent alcohol consumption showed enhancement of conditioned reinforcement, as indicated by greater responding for the CS even in the absence of primary reward, as compared to their controls (Kruse et al., 2017). The differences in findings between the studies likely derive from differences in methodologies. While animals in the present study were exposed to intermittent alcohol treatment via intragastric administration, which closely models binge drinking, animals in the other study were given gel matrix consisting of water, gelatin, polycose and 190-proof ethanol (10%) to be consumed voluntarily. In addition, in the previous study alcohol-induced alterations in incentive learning processes were evaluated only in sign trackers (Kruse et al., 2017), who typically show strong preference for cues (CS) (Flagel et al., 2009). Here we provide a comprehensive assessment of the effects of CIE exposure during different developmental periods on incentive learning in a broader population of rats (i.e., one not restricted to a specific behavioral phenotype). We found that alcohol-treated animals show a persistent deficit in learning within the complex conditioned reward paradigm, which requires the intact ability of rats to shift behavioral strategies from nose poking (conditioning phase) to lever pressing (test phase), learn response-outcome contingencies and engage in goal-directed behavior.

A number of studies have shown that CIE exposure or voluntary alcohol consumption can disrupt cue-reward associative learning and promote a sign-tracking phenotype, as measured

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in a conditioned approach paradigm (Kruse et al., 2017; McClory and Spear, 2014; Spoelder et al., 2017). The present study used a design that measured only one type of conditioned approach response (e.g., food trough head entries), whereas other studies employed designs that allowed the differentiation of sign tracking from goal tracking responses (Kruse et al., 2017; Madayag et al., 2017; McClory and Spear, 2014; Spoelder et al., 2017). Thus, it is impossible to make a direct comparison between the present and previous findings. Other important differences between the studies include that, in the present study, conditioned approach was measured in the absence of primary reward delivery whereas in other studies reward delivery continued. Another reason for discrepant outcomes between previous and present reports on the effects of alcohol on incentive learning might be related to the number of conditioning sessions. Previous studies used 6-8 days of conditioning (Kruse et al., 2017; Madayag et al., 2017; McClory and Spear, 2014; Spoelder et al., 2017) whereas we used only 3. It remains possible that the CIE rats may have been latently limited in the amount of conditioned approach learning, a state that could have been revealed with a greater number of learning sessions. Lastly, rats in the present study were food restricted, while in other studies they were not (Kruse et al., 2017; Madayag et al., 2017; McClory and Spear, 2014; Spoelder et al., 2017).

We were interested in the link between exposure to alcohol and incentive learning because we hypothesize that chronic binge-like intake of alcohol affects the same neural circuits that are involved in reward-related learning. We have proposed and demonstrated through a series of studies that the neural circuitry involved in this type of reward-related learning involves coincident NMDA and mACh receptor stimulation in the VTA (Ranaldi, 2014; Sharf and Ranaldi, 2006; Zellner et al., 2009; Zellner and Ranaldi, 2010). The pedunculopontine tegmental and laterodorsal tegmental nuclei that give rise to the main cholinergic projections to VTA DA cells are severely affected by chronic alcohol consumption (Pereira et al., 2019). Similarly, alcohol exposure leads to persistent alterations in the prefrontal cortex (Alasmari et al., 2018; Jury et al., 2017; McGinnis et al., 2019; Morales et al., 2018; Pascual et al., 2009; Varodayan et al., 2018) whose glutamatergic neurons are known to innervate the VTA DA system and control reward-directed behaviors. Thus, it is conceivable that CIE exposure, by disrupting glutamatergic and cholinergic inputs to the VTA DA system, impairs conditioned reward learning. If the strength of CS-reward learning depends on the degree of activation of VTA DA neurons, as we have previously demonstrated (Galaj and Ranaldi, 2018), then alcohol-induced disruption in glutamatergic and cholinergic inputs most likely interferes with the strengthening of the CS-related synaptic control of reward neuronal signals and conditioned reward learning.

It is also noteworthy that the conditioned reward paradigm requires higher-order reward-associative processing and, to some extent, cognitive flexibility in that animals need to adapt their behavior to changes in stimulus/response/reward relations from stimulus-reward (conditioning phase) to response-reward (test phase) contingencies. CIE exposure impacts the developing prefrontal cortex by inducing persistent alterations in neuronal morphology and function (Coleman et al., 2014; Jury et al., 2017; Liu and Crews, 2015; McGinnis et al., 2019; Morales et al., 2018; Vargas et al., 2014; Vetreno et al., 2014) that have been associated with behavioral inflexibility and deficits in decision making (Fernandez et al., 2017; Fernandez and Savage, 2017; Galaj et al., 2019). Thus, it is possible that alcohol-
induced cortical functional deficits might partially be responsible for behavioral deficits observed in the conditioned reward paradigm.

Over the last several decades, it has become clear that repeated administration of alcohol, opiates or psychostimulants induces enduring sensitization (Galaj et al., 2013; Masur et al., 1986; Morrison et al., 2011; Post et al., 1988; Steketee et al., 1991), itself thought to contribute to the development and maintenance of addiction (Nestby et al., 1997; Robinson and Berridge, 1993; Wise and Bozarth, 1987). In the present study we have shown that chronic intermittent heroin treatment increases behavioral response in rats regardless of previous alcohol exposure and the developmental stage (age) or withdrawal period in which the heroin treatment occurs. That is, all groups of rats showed similar increases in their response to heroin. However, alcohol-exposure was not without effect on subsequent heroin exposure in that animals previously exposed to alcohol were sensitized to the locomotor effects of heroin. That is, we found that rats with CIE treatment resulted in significantly greater locomotor responses to heroin, regardless of age or withdrawal period, compared to their water-treated counterparts. These findings are in line with previous reports that prior repeated exposure to alcohol in adult rats can enhance the locomotor effects of morphine 3 weeks later (Nestby et al., 1997). Adolescent rodents are generally known to be less sensitive to the acute effects of alcohol (Hefner and Holmes, 2007; Silveri and Spear, 1998; Vetter-O’Hagen et al., 2009; White et al., 2002; Willey et al., 2012) and require higher doses of it to show behavioral sensitization than do adults (Stevenson et al., 2008). This seems counterintuitive, as greater alcohol-induced damage has been associated with adolescent-versus adult-onset CIE exposure. Nevertheless, the process of behavioral sensitization has been postulated to produce enduring neuroplasticity in the mesolimbic system that contributes to the pathogenesis of drug abuse and addiction (Kalivas et al., 1998; Kalivas and Duffy, 1987; Robinson and Berridge, 2000, 1993; Wise and Bozarth, 1987). Some changes that might be responsible for the alcohol-induced enhancement of heroin’s locomotor effects observed here include enhanced responsiveness of DA neurons to the drug and to depolarizing currents, postsynaptic changes in DA-sensitive striatal GABAergic neurons and enhanced reactivity of striatal cholinergic neurons modulating DA signals (Kalivas and Stewart, 1991; Nestby et al., 1997; Robinson et al., 1988; Tjon et al., 1995). Thus, the convergence of effects of both repeated alcohol and repeated heroin on mesolimbic DA circuitry might explain why in the present study we observed an enhancement of heroin’s locomotor effects after alcohol treatment.

The limitation of this study is that our subjects were male rats only. It is well established that females are more sensitive to the acute effects of alcohol and more vulnerable to alcohol-induced deficits than males (Ceylan-Isik et al., 2010; Hommer, 2003). Thus, it is possible that consequences of CIE exposure on incentive motivational learning and response to heroin are more detrimental in females than males. We are currently conducting a follow up study to determine sex differences in CIE exposure-induced alterations.

Together with previous work we have begun to outline an emerging profile of adolescent and adult CIE exposure-induced alterations in incentive learning and opioid stimulant effects. We propose that these aberrations are likely associated with alcohol-induced neuroadaptations in afferent signaling to the VTA DA system as well as with alterations in...
DA neuronal structure and function. It is likely that CIE exposure-induced alterations in reward-related learning and opioid stimulation are not restricted to a single alcohol-induced neuroadaptation, but rather arise from multiple changes within the mesocorticollimbic circuits, some of which may partially contribute to deficits in conditioned reward while others to enhancement in heroin locomotor stimulation. As our findings suggest, long-term consequences of CIE exposure may include alterations in incentive learning and increased propensity for the emergence of drug abuse and addiction.

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Figure 1. 
A–D shows impaired conditioned reward learning in rats exposed to CIE exposure during adolescence or adulthood with the deficit being more pronounced during acute (WD 2) than protracted withdrawal (WD 21). Mean (± SEM) number of presses on the lever producing the light stimulus and on the lever producing the tone stimulus during pre-exposure and test sessions in A: adolescent CIE exposure WD 2, B: adolescent CIE exposure WD 21, C: adult CIE exposure WD 2 and D: adult CIE exposure WD 21 groups. * represents a significantly smaller phase by lever interaction in the alcohol groups compared to water groups (p < .001).
Figure 2.
A-D shows the effects of adolescent and adult CIE exposure on food conditioned approach learning. Mean (± SEM) difference scores (CS minus Pre-CS head entries) during the 3 conditioning sessions and CS-only test in A: adolescent CIE exposure WD 2, B: adolescent CIE exposure WD 21, C: adult CIE exposure WD 2 and D: adult CIE exposure WD 21 groups.
Figure 3.
A-D shows the effects of adolescent or adult CIE exposure on heroin sensitization. Mean (± SEM) locomotor activity counts during 9 sessions in response to heroin injections (2 mg/kg) in A: adolescent CIE exposure WD 2, B: adolescent CIE exposure WD 21, C: adult CIE exposure WD 2 and D: adult CIE exposure WD 21 groups. The analysis indicated a significant main effect of treatment, session and withdrawal.