



The neurokinin-1 receptor mediates escalated alcohol intake induced by multiple drinking models

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ABSTRACT

We have previously demonstrated that the neurokinin-1 receptor (NK1R) is upregulated in the central nucleus of the amygdala of alcohol preferring (P) rats and that this receptor mediates escalated alcohol consumption in this strain. However, it is unclear if non-genetic models of escalated consumption are also mediated by NK1R signaling, and if so, what brain regions govern this effect. In the experiments presented here, we use two methods of inducing escalated alcohol intake in outbred Wistar rats: yohimbine pretreatment and intermittent alcohol access (Monday, Wednesday, and Friday availability; 20% alcohol). We found that escalated alcohol consumption induced by both yohimbine injection and intermittent access is attenuated by systemic administration of the NK1R antagonist L822429. Also, when compared to continuous alcohol access or access to water alone, NK1R expression was increased in the nucleus accumbens (NAC) and dorsal striatum, but not the amygdala. Escalated consumption induced by intermittent access was attenuated when the NK1R antagonist L822429 was infused directly into the dorsal striatum, but not when infused into the NAC. Taken together, these results suggest that NK1R upregulation contributes to escalated alcohol consumption that is induced by genetic selection, yohimbine injection, and intermittent access. However there is a dissociation between the regions involved in these behaviors with amygdalar upregulation contributing to genetic predisposition to escalated consumption and striatal upregulation driving escalation that is induced by environmental exposures.

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1. Introduction

In 2014, 16.3 million adults aged 18 and older had an alcohol use disorder, representing 6.8% of the United States population (SAMHSA, 2015b). Additionally, alcohol misuse cost the United States economy \$249 billion dollars in 2010 (Sacks et al., 2015). In

order to alleviate these burdens, the FDA has approved three medications to treat alcohol use disorders: acamprosate, disulfiram, and naltrexone (SAMHSA, 2015a). These medications have been shown to reduce alcohol consumption and increase abstinence, but these effects are moderate and inconsistent (Winslow et al., 2016). Because of these inconsistencies, it is important to investigate other potential treatment options.

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2011, 2014, 2015). Additionally, we and others have observed an effect of this system on escalated consumption, but not baseline alcohol intake (Augier et al., 2014; Ayanwuyi et al., 2015; Barson et al., 2015; Schank et al., 2011, 2013; Thorsell et al., 2010).

NK1Rs are located in a wide range of brain regions such as the striatum, amygdala, and hippocampus, all of which are involved in the regulation of affective disorders (Commons, 2010; Rigby et al., 2005). In examining the role of the NK1R in these regions in stress-elicited alcohol seeking and escalated consumption, we have found that upregulation of the NK1R in the central nucleus of the amygdala (CeA) of alcohol preferring (P) rats drives their escalated alcohol consumption (Schank et al., 2013). In contrast, stress-induced reinstatement of alcohol seeking in outbred Wistar rats appears to be driven by the actions of the NK1R in the NAC shell (Schank et al., 2015).

In addition to selective breeding for high alcohol preference, as utilized in generating the P rat and other preferring strains, escalated consumption of alcohol can be induced in outbred animals by manipulating the access schedule or injecting a stress-inducing pharmacological agent. Specifically, intermittent access in the two-bottle choice paradigm, which consists of cycles of drinking and abstinence, leads to a rapid increase in ethanol consumption whereas continuous access to ethanol does not. This method, first reported by Wise and colleagues (Wise, 1973), and rediscovered by Bartlett and colleagues more recently (Simms et al., 2008), has been adopted as a reliable method to induce escalated consumption in preclinical alcohol research. This method has significant translational value, as two of the FDA-approved treatments for alcohol use disorder, acamprosate and naltrexone, decrease alcohol consumption in rats with intermittent access to alcohol, but not in rats given continuous access to alcohol (Simms et al., 2008). In regards to pharmacological induction of escalated alcohol consumption, injection of the anxiogenic compound yohimbine, an α_2 adrenergic antagonist that increases noradrenergic output, induces increased rates of alcohol self-administration (Le et al., 2005, 2013; Williams et al., 2016). While our previous work demonstrates a role of the NK1R in the CeA in escalated consumption of P rats, it is unclear if this receptor contributes to escalated consumption induced by the non-genetic models described above, and if so, what brain regions govern this effect.

The current study investigated the role of NK1R in escalated drinking induced by yohimbine injection in the operant self-administration paradigm and in escalated intake induced by intermittent access in the two-bottle choice paradigm. Furthermore, we assessed changes in NK1R expression in stress-sensitive brain regions following exposure to intermittent access and targeted the NK1R in specific regions using intracranial infusion of the NK1R antagonist L822429.

2. Materials & methods

2.1. Animals and housing

Adult male, Wistar rats (200–225 g at time of arrival) were individually housed on a reversed light cycle (lights on at 8:00 a.m.). The animals were acclimatized to housing conditions for one week and handled for an additional week before the start of experimentation. Food and water were provided *ad libitum* except where otherwise stated. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Georgia.

2.2. Operant self-administration

Rats self-administered alcohol (10% v/v) in standard operant chambers (MedAssociates) on an FR1 schedule with a 5 s timeout/cue light presentation that followed alcohol delivery. Prior to initiation of alcohol self-administration, a saccharin fading procedure in the absence of the cue light was used to initiate lever pressing, as previously described (Schank et al., 2011, 2012a, 2013, 2014, 2015). Sessions were 30 min in duration and run once daily, five days per week. The rats reached stable lever pressing after 15–20 days at which point the rats were injected with either vehicle (2-hydroxypropyl β -cyclodextrin, 45% w/v) or the NK1R antagonist L822429 (30 mg/kg, i.p. at 2 ml/kg). We have used this dose of L822429 in previous studies and have observed no non-specific or sedative effects in Wistar rats at this dose (Schank et al., 2011, 2013). Thirty minutes after these injections, rats were injected with vehicle (sterile water) or yohimbine (2.5 mg/kg, i.p. at 1 ml/kg). Self-administration sessions began 30 min after the yohimbine injections. Each rat received each treatment in a random, counterbalanced order with 2–3 days of self-administration without pretreatment between each treatment session.

2.3. Two-bottle choice

Continuous Access: Rats were given continuous access to one bottle of 20% alcohol and one bottle of water in their home cage for 4–5 weeks. Bottles were equipped with stainless steel drinking spouts and were placed on the wire lid of the home cage.

Intermittent Access: The intermittent access paradigm was carried out as described by de Guglielmo et al. (2016). Briefly, rats were given access to two bottles with stainless-steel drinking spouts, one of which contained water at all times. Rats were given access to 20% (v/v) alcohol in one of the two bottles on Monday, Wednesday, and Friday. All other days, both bottles contained water. This access schedule induces escalation and rats exposed to this procedure achieve blood alcohol levels as high as 100–150 mg/dl (Carnicella et al., 2009; Simms et al., 2008).

At the beginning of the dark cycle, bottles for all rats were weighed to calculate consumption. In order to avoid side preference, the position of the alcohol bottle was switched each time alcohol was available. Each rat was weighed weekly in order to calculate the grams of alcohol intake per kilogram of body weight. Two weeks before the treatment phase was to begin, bottles were weighed 2 h after bottle presentation in addition to the 24 h time point to establish a baseline measure for the first 2 h of the drinking session. This is a useful measure in the event that treatment effects are transient due to limited drug half-life.

An additional group of rats was presented with water in both bottles for the duration of the experiment and was used to establish control levels for NK1R gene expression.

2.4. Systemic L822429 - two bottle choice

After baseline-drinking rates reached stability, the treatment phase began. Rats were injected with L822429 (15 or 30 mg/kg) or vehicle (2-hydroxypropyl β -cyclodextrin, 45% w/v; i.p. at 2 ml/kg) 60 min before being given access to alcohol. Two hours after being presented with alcohol, the bottles were weighed. Each rat received each treatment in a random, counterbalanced order with 2–3 days of self-administration without pretreatment between each treatment session. Because a subset of these rats were the same as those used for gene expression measures, the group of rats receiving water only was also treated with the NK1R antagonist in the same manner, even though they did not have access to alcohol. After 2–4 days of alcohol access without pretreatment, all rats were sacrificed

at the start of the dark phase on the next scheduled day of alcohol availability. Brains were extracted, specific regions were dissected, snap frozen on isopentane, and stored at -80°C until processing.

2.5. qPCR

Quantitative polymerase chain reaction was carried out as described in Schank et al. (2014). Rats were decapitated and brain tissue was prepared in 2 mm sections using a Kopf tissue slicer. The following regions were dissected: nucleus accumbens (NAC), dorsal striatum, amygdala (AMG), lateral septum (LS) and prefrontal cortex (PFC). Samples were obtained using biopsy punches (AMG: 2 mm) or free hand dissection (dorsal striatum, NAC, LS, PFC). Samples were snap frozen in chilled isopentane, and stored at -80°C until analyzed.

Total RNA was extracted using RiboPure kit (Ambion, Grand Island, NY) according to the manufacturer's instructions. RNA concentrations were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Purified RNA (0.5 μg for AMG, 1 μg for all other regions) was reverse-transcribed using a first-strand cDNA synthesis kit (Invitrogen, Grand Island, NY) according to the manufacturer's instructions. Reactions were in triplicate, using a 1/10 dilution of each cDNA with specific TaqMan probe/primers and TaqMan universal master mix (Applied Biosystems, Foster City, CA), and run on an Applied Biosystems QuantStudio6 PCR machine.

FAM-labeled probes and forward and reverse primers for *Tacr1* (Rn00562004_m1*; transcript for NK1R) and *Gapdh* (Rn99999916_s1; housekeeping gene) were selected from Assay on Demand (Applied Biosystems). Cycling parameters were 2 min at 50°C for reverse transcription, 10 min at 95°C for heat inactivation of the reverse transcriptase and activation of the DNA polymerase, and 40 cycles of 15 s at 95°C and 1 min at 60°C . Data were analyzed using Statistica 13. Reactions were normalized to the *Gapdh* endogenous control and compared between groups using the $2^{-\Delta\Delta\text{Ct}}$ method.

2.6. Intracranial infusion

Intracranial infusions were carried out as described by Schank et al. (2015); (Schank et al., 2013). Briefly, after 2 weeks of intermittent access to alcohol, rats were implanted with bilateral cannulas directed at the NAC or dorsal striatum. Treatment groups were matched for baseline drinking. Coordinates for the NAC were: AP: +1.7 mm, ML: \pm 2.8 mm, DV: -5.8 mm, 10° angle; injectors extended 1 mm beyond the cannula tip for a final DV coordinate of -6.8 mm, and were intended to target the border between the core and shell subregions. Coordinates for the dorsal striatum were: AP: -0.7 mm, ML: ± 3.6 mm, DV: -4.0 mm; injectors extended 1 mm beyond the cannula tip for a final DV coordinate of -5.0 mm, and aimed for the more lateral portions of this region. Following 5 days of recovery, intermittent access to alcohol restarted and continued for 3 weeks.

Rats received intracranial infusions of vehicle (2-hydroxypropyl β -cyclodextrin, 45% w/v) or L822429 (7.5 μg) in a volume of 0.5 μl /side, delivered over 1 min, with the injector left in place 1 min after the infusion. Infusions were delivered 10 min before rats were presented with alcohol on a normal drinking day. Rats had an additional drinking day following the infusion day and then underwent a second test day with the opposite treatment. Infusions were given in a randomized, counterbalanced order. After one more day of drinking, cresyl violet was injected, rats decapitated, brains removed and snap frozen on dry ice and stored at -20°C . Brains were sectioned and imaged to ensure proper cannula placement. Placements were determined by an

investigator that was blind to treatment conditions. Rats were removed from analysis if cannulae in both hemispheres of the brain were misplaced.

2.7. Statistics

Operant self-administration experiments were analyzed using two way ANOVA with the within subjects factors of L822429 treatment and yohimbine treatment. Analysis of escalation in two bottle choice experiments was performed using a two way ANOVA with the between subjects factor of alcohol access condition and the within subjects factor of day. Analysis of NK1R antagonism in two bottle choice experiments was performed using a two way ANOVA with the between subjects factor of alcohol access condition and the within subjects factor of L822429 dose. qPCR data was analyzed using a one way ANOVA with the between subjects factor of alcohol access condition. Intracranial infusion experiment was analyzed using a one way ANOVA with the within subjects factor of L822429 treatment. Except where stated, Newman-Keuls test was used for post-hoc group comparisons.

3. Results

3.1. Systemic injection of L822429 attenuates yohimbine-induced escalation of alcohol self-administration

After training for alcohol self-administration and establishment of a stable baseline, escalated self-administration was induced by yohimbine injection. The NK1R antagonist L822429 was administered prior to this treatment. We found that L822429 decreased alcohol self-administration in rats ($n = 14$) treated with yohimbine (Fig. 1). Specifically, two-way ANOVA analysis of the alcohol deliveries obtained during self-administration sessions showed a main effect of L822429 ($F(1,13) = 17.9$, $p = 0.001$), a main effect of yohimbine ($F(1,13) = 10.1$, $p = 0.007$), and a L822429 \times yohimbine interaction ($F(1,13) = 10.4$, $p = 0.007$). Posthoc Newman-Keuls tests identified a statistically significant increase in alcohol deliveries in the vehicle-yohimbine treatment compared to all other treatments ($p < 0.001$, Fig. 1A) indicating that yohimbine induced an escalation in alcohol intake and that L822429 reversed this effect. Two-way ANOVA analysis of the active lever responses during the self-administration test sessions supported these findings, revealing a main effect of L822429 ($F(1,13) = 20.3$, $p = 0.0006$), a main effect of yohimbine ($F(1,13) = 10.1$, $p = 0.007$), and a L822429 \times yohimbine interaction ($F(1,13) = 9.3$, $p = 0.009$). Posthoc tests indicated that there was a statistically significant increase in active lever presses in the vehicle-yohimbine treatment compared to all other treatments ($p < 0.001$, Fig. 1B). Inactive lever pressing was not affected by treatment. Specifically, two-way ANOVA analysis of inactive lever responses showed no effect of L822429 ($F(1,13) = 1.7$, $p = 0.21$), no effect of yohimbine ($F(1,13) = 0.3$, $p = 0.60$), and no L822429 \times yohimbine interaction ($F(1,13) = 0.3$, $p = 0.62$, Fig. 1C).

3.2. Systemic injection of L822429 blocks the escalated alcohol consumption observed in rats with intermittent access to alcohol

Next, we induced escalated alcohol intake by exposing rats to an intermittent access schedule in the two bottle choice paradigm of voluntary alcohol consumption. When compared to rats with continuous access ($n = 13$), rats with intermittent access to alcohol ($n = 15$) exhibited an escalation in alcohol intake. Specifically, two way ANOVA revealed a main effect of day ($F(13, 338) = 5.4$, $p < 0.0001$), a main effect of alcohol access ($F(1,26) = 12.3$, $p = 0.002$), and a day \times access interaction ($F(13,338) = 1.9$, $p = 0.03$;

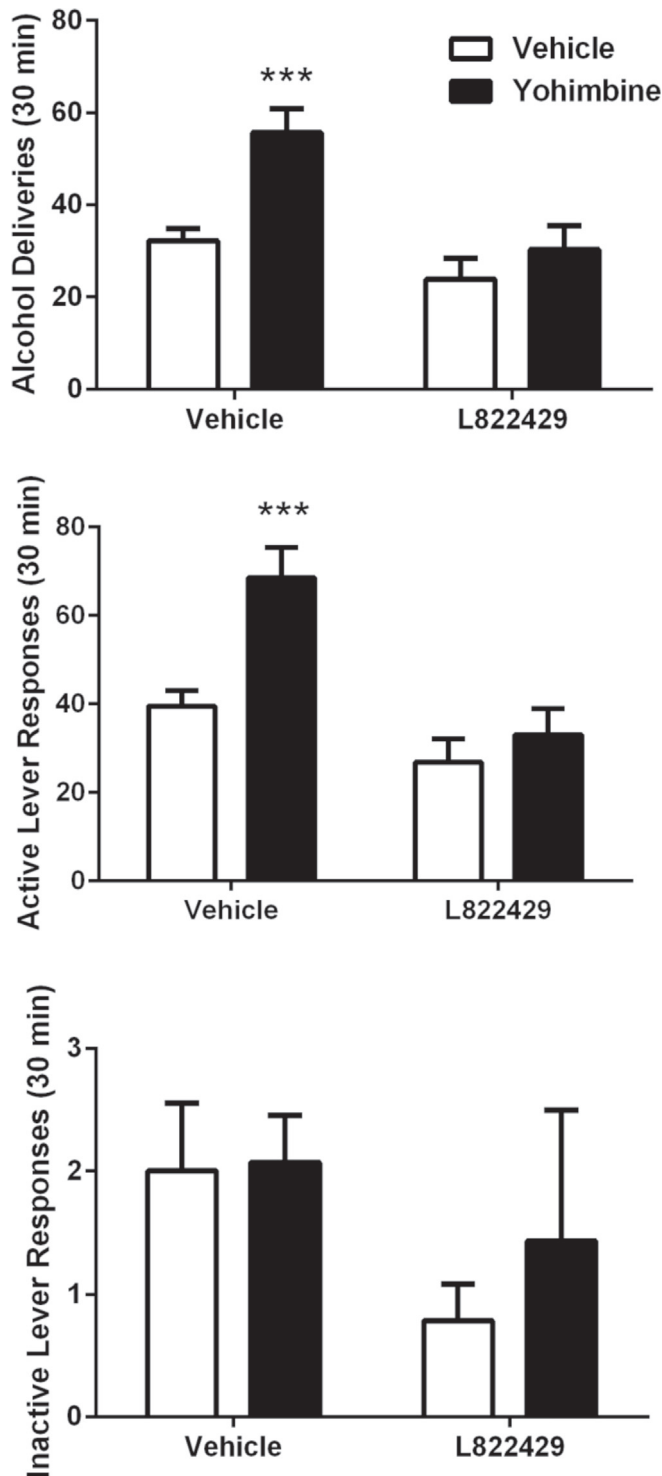


Fig. 1. L822429 attenuates yohimbine-induced escalation of alcohol self-administration. Alcohol deliveries received (A), active lever responses (B), and inactive lever responses (C) during operant self-administration for 10% ethanol. Yohimbine pretreatment significantly increased alcohol self-administration, which was attenuated by NK1R antagonism with L822429. *** $p < 0.001$ compared to all other groups.

Fig. 2A). Post-hoc Newman-Keuls tests indicated that alcohol consumption in the intermittent access group significantly differed from day 1 consumption on days 13 ($p = 0.02$) and 14 ($p = 0.01$). Alcohol consumption in the continuous access group did not differ from day 1 consumption on any day.

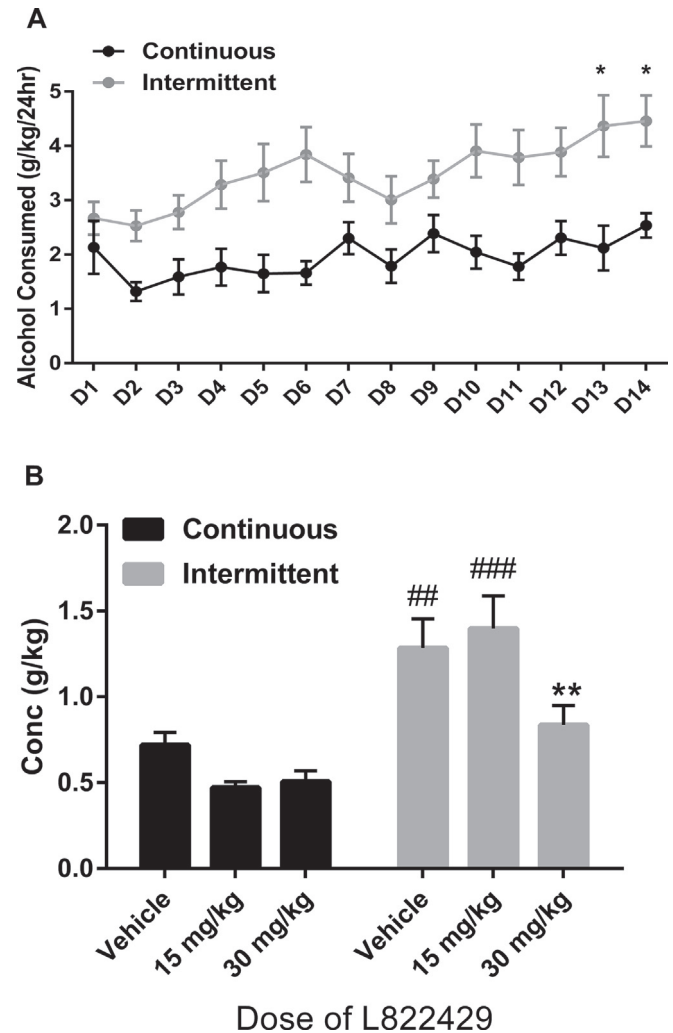


Fig. 2. L822429 attenuates escalated alcohol consumption induced by intermittent access. (A) Intermittent access to alcohol induces a significant escalation in alcohol consumption compared to continuous access (main effect of access conditions, * $p < 0.05$ compared to Day 1 consumption in continuous group). (B) Alcohol consumption in the first 2 h of access. NK1R antagonism attenuates escalated alcohol consumption induced by intermittent access to alcohol. ## $p < 0.01$, ### $p < 0.001$ compared to continuous access for that dose of L822429, ** $p < 0.01$ compared to vehicle treated intermittent access.

We found that L822429 decreased escalated alcohol consumption in rats with intermittent access to alcohol, but not rats with continuous access to alcohol, when consumption was measured 2 h after alcohol presentation (Fig. 2B). This 2 h measurement time-point was included to account for the fact that the limited half-life of L822429 does not allow for full 24 h NK1R antagonism (Singewald et al., 2008). Specifically, two-way ANOVA analysis of the concentration (g/kg) of alcohol consumed showed a main effect of access ($F(1,26) = 19.9$, $p = 0.0001$), a main effect of dose ($F(2,52) = 5.6$, $p = 0.007$), and an access \times dose interaction effect ($F(2,52) = 4.1$, $p = 0.02$). Newman-Keuls posthoc tests indicated that there was a statistically significant decrease in consumption following 30 mg/kg L822429 treatment in rats with intermittent access compared to consumption following vehicle treatment in this group ($p = 0.004$). Drinking in the continuous access rats was not affected by either dose of L822429 (15 mg/kg, $p = 0.23$; 30 mg/kg, $p = 0.16$). Escalated intake was evidenced by an increase in consumption in intermittent access rats following vehicle treatment ($p = 0.008$) compared to vehicle-treated rats with continuous

access. Alcohol consumption in rats with intermittent alcohol access that were treated with 30 mg/kg L822429 did not differ from alcohol intake in the continuous access group after vehicle treatment ($p = 0.52$), indicating that L822429 treatment returned alcohol consumption levels in intermittent access rats to the level of consumption in continuous access controls.

Two way ANOVA analysis of 24 h consumption revealed a main effect of access condition (continuous versus intermittent, $F(1,26) = 27.68$, $p < 0.0001$), but no main effect of L822429 treatment ($F(2,52) = 1.4$, $p = 0.26$) and no access \times treatment interaction ($F(2,52) = 1.5$, $p = 0.22$).

3.3. *Tacr1* expression is increased by intermittent alcohol access in the dorsal striatum and NAC

We investigated the expression of *Tacr1* (gene for NK1R) in several brain regions involved in addiction including the PFC, dorsal striatum, AMG, NAC, and the LS in rats ($n = 6$ – 8) exposed to continuous alcohol access, intermittent alcohol access, or water only (subset of animals in L822429 experiment above). One-way ANOVA revealed a main effect of alcohol access on *Tacr1* expression in the dorsal striatum ($F(2,16) = 5.8$, $p = 0.01$). Newman-Keuls post-hoc tests indicated that expression was significantly increased in the intermittent access group compared to both continuous access ($p = 0.02$) and water only controls ($p = 0.03$, Fig. 3A). One-way ANOVA revealed a main effect of alcohol access on *Tacr1* expression in the NAC ($F(2,16) = 10.40$, $p = 0.001$). Newman-Keuls post-hoc tests indicated that expression was significantly increased in the intermittent access group compared to both continuous access ($p = 0.001$) and water only controls ($p = 0.008$, Fig. 3B). These results indicate that expression of the NK1R in the striatum is selectively upregulated in rats with intermittent access to alcohol as compared to rats with continuous alcohol access or access to water only.

One-way ANOVA indicated a main effect of alcohol access on *Tacr1* expression in the LS ($F(2,17) = 4.4$, $p = 0.03$, Fig. 3C). Newman-Keuls post-hoc tests revealed that expression was significantly increased in the intermittent access group compared to water only controls ($p = 0.03$). There was also a trend level increase in *Tacr1* expression in the continuous access group when compared to the water only group ($p = 0.09$), and no difference between the continuous and intermittent access groups ($p = 0.27$). These results suggest that alcohol access, either under continuous or intermittent schedules, induces increased *Tacr1* expression in the LS.

One-way ANOVA revealed a main effect of alcohol access on *Tacr1* expression in the PFC ($F(2,17) = 3.7$, $p = 0.046$, Fig. 3D). Newman-Keuls post-hoc tests indicated that expression was significantly increased in the continuous access group compared to the water only group ($p = 0.03$), but not in the intermittent access group when compared to the water only group ($p = 0.14$). Expression in intermittent access and continuous access rats did not significantly differ ($p = 0.21$). These results suggest that the NK1R is upregulated in the PFC following continuous access to alcohol, but not intermittent access to alcohol. However, the fact that continuous access was not different than intermittent access detracts from the specificity of these results.

One-way ANOVA revealed a main effect of alcohol access on *Tacr1* expression in the AMG ($F(2,13) = 5.1$, $p = 0.02$, Fig. 3E). Newman-Keuls post-hoc tests indicated that expression was significantly decreased in the intermittent access group relative to both the continuous access group ($p = 0.02$) and water only group ($p = 0.047$).

3.4. Intracranial infusion of L822429 into the dorsal striatum decreases alcohol consumption in rats with intermittent access to alcohol

Based on the results of qPCR analysis, we hypothesized that infusions of a NK1R antagonist into the dorsal striatum and NAC would block the escalated alcohol consumption observed in rats with intermittent access, because these two regions showed selective increases in *Tacr1* expression following intermittent alcohol access only. After inducing escalation by exposing rats to intermittent access to alcohol ($n = 15$ for dorsal striatum, $n = 13$ for NAC), we infused L822429 into these specific regions. One-way ANOVA was used to compare baseline consumption to alcohol consumption following infusion of either vehicle or L822429 (Fig. 4). One way ANOVA revealed a main effect of L822429 treatment in rats that were given an intra-dorsal striatum infusion of L822429 ($F(2,28) = 4.0$, $p = 0.03$; Fig. 4A). Fisher LSD post-hoc tests indicated that L822429 induced a significant decrease in alcohol intake in the L822429 treated group when compared to baseline ($p = 0.01$) but not in the vehicle treated group when compared to baseline ($p = 0.44$). One way ANOVA revealed no effect of L822429 infusion when given into the NAC ($F(2,24) = 2.6$, $p = 0.1$, Fig. 4B). Cannula placements for all rats included in the experiment are shown in Fig. 4C.

4. Discussion

The present study investigated the effect of NK1R antagonism on escalated alcohol consumption observed in rats given yohimbine injections prior to self-administration of alcohol as well as rats exposed to intermittent access to alcohol. We found that systemic injection of the NK1R antagonist L822429 attenuates increased self-administration induced by both of these experimental manipulations. Next, we investigated expression of the NK1R in several brain regions involved in addiction and found that there is an increase in NK1R expression in the dorsal striatum and NAC specifically in rats with intermittent access to alcohol. We then targeted these regions with intracranial infusions of L822429 and found that infusions into the dorsal striatum, but not the NAC, decreased alcohol consumption in rats with intermittent access to alcohol.

Yohimbine, an antagonist of adrenergic autoreceptors, is an anxiogenic agent well known in addiction research for serving as an effective trigger of stress-induced reinstatement of drug and alcohol seeking. In addition to this property, yohimbine can also induce an acute escalation in alcohol intake in operant self-administration models (Le et al., 2005, 2013). We replicate this finding in our experiments, and show that this phenomenon is attenuated by pretreatment with the NK1R antagonist, L822429. This agrees well with our previous work showing that the NK1R mediates escalated alcohol self-administration in alcohol preferring rats (Schank et al., 2013) as well as yohimbine-induced reinstatement in outbred Wistar rats (Schank et al., 2014). While yohimbine induces escalation of alcohol intake, this effect is short-lived and is expressed for a single alcohol self-administration session. We next wanted to determine if the NK1R mediates escalated alcohol intake that is induced by intermittent access schedules in the two bottle choice paradigm, which is a long lasting effect. We found that this escalated intake is attenuated by NK1R antagonism, suggesting that NK1R activation mediates escalated alcohol intake that is induced by genetic selection (P rats), acute pharmacological induction (yohimbine), and manipulation of alcohol availability (intermittent access).

As described above, escalation induced by intermittent access is long lasting and presumably results from the recruitment and

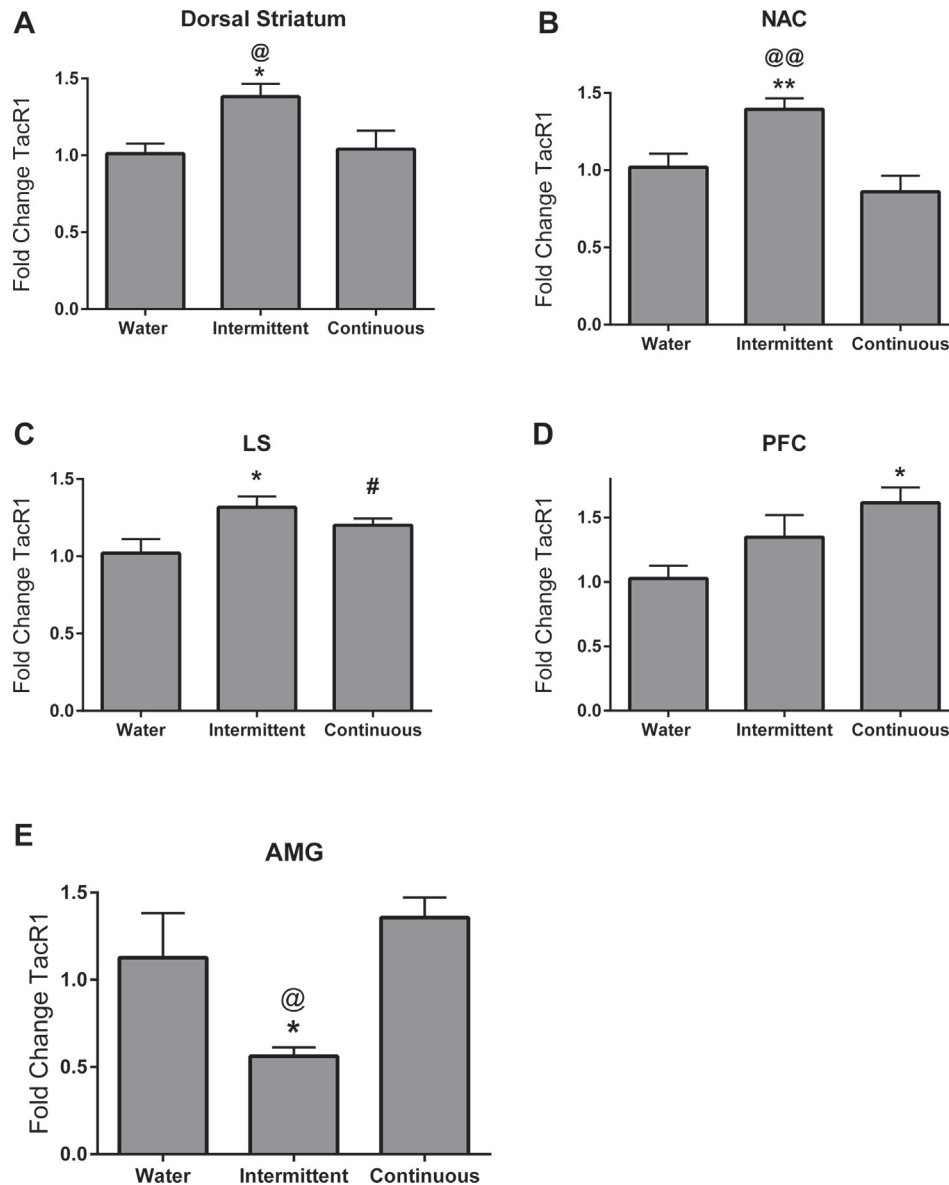


Fig. 3. Intermittent access to alcohol increases TACR1 expression in the NAC and Dorsal Striatum. TACR1 expression in the (A) NAC (B) Dorsal Striatum (C) LS (D) PFC (E) AMG. TACR1 is selectively increased following intermittent access to alcohol in the NAC and Dorsal Striatum. TACR1 expression in the LS is increased in both alcohol groups compared to water only controls. TACR1 expression in the PFC is selectively increased in the continuous access group relative to water controls. TACR1 expression in the AMG is significantly decreased in the intermittent access group compared to both continuous access and water controls. * $p < 0.05$, ** $p < 0.01$ compared to water only group, @ $p < 0.05$, @@ $p < 0.01$ compared to continuous access group. # $p < 0.1$ compared to water only group.

upregulation of stress systems in the brain, akin to the effects of extended access on intravenous self-administration of opiates and psychostimulants (Koob, 2009; Koob et al., 2014). The escalated alcohol intake observed in the P rat is driven by NK1Rs in the CeA, but it is unclear if a similar region mediates the NK1R effect on escalation induced by intermittent access. Thus, we examined NK1R expression in a set of brain regions involved in stress and drug seeking following exposure to intermittent access to alcohol as compared to rats exposed to continuous alcohol access or water only. We found that while AMG expression of the NK1R was actually decreased following intermittent access to alcohol, there was a selective increase in NK1R expression in the intermittent access group in the NAC and dorsal striatum. Therefore, we targeted these striatal regions for intracranial infusion of the NK1R antagonist (see below). The decrease in expression in the AMG was unexpected, based on the upregulation observed in P rats, and the

mechanism of this alteration is unclear. However, it should be noted that experiment was performed in alcohol naïve animals, whereas the data presented here were from alcohol experienced animals. In contrast, NK1R expression was specifically elevated in the continuous access group in the PFC. This was also somewhat surprising, but may fit with a role of frontal cortical regions in behavioral regulation (Barker et al., 2017; Gourley and Taylor, 2016). However, this is purely speculative and would need to be examined in well controlled studies. For example, it is unknown if increased Tacr1 expression would relate to an increase or decrease in cortical function. Furthermore, the fact that no significant difference in expression was observed between continuous access and intermittent access groups decreases the likelihood that this is a specific mediator of this behavior. Interestingly, NK1R expression was increased in both intermittent access and continuous access groups in the LS, suggesting that TacR1 expression in this region is affected

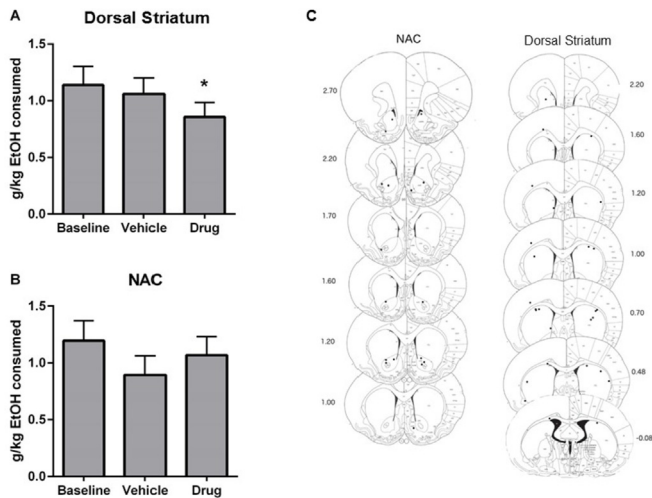


Fig. 4. NK1R antagonist infusion in the dorsal striatum attenuates escalated consumption in rats with intermittent access to alcohol. (A) Alcohol consumption 2 h after NK1R antagonist infusion into the dorsal striatum. L822429 infusion into the dorsal striatum significantly decreased consumption, * $p < 0.05$ compared to baseline. (B) Alcohol consumption 2 h after NK1R antagonist infusion into the NAC. (C) Cannula placements for rats included in intracranial infusion experiment.

by alcohol intake in general, and this does not depend on the specific access schedule.

Given the alterations in expression outlined above, we decided to target the NAC and dorsal striatum in experiments using intracranial infusion of the NK1R antagonist. In these experiments, we targeted the border between the NAC core and shell, to impact both of these subregions of the NAC. We found that, while NAC infusion had no effect, dorsal striatum infusion of the NK1R antagonist attenuated alcohol consumption in rats with intermittent access. While we targeted the more lateral regions of the dorsal striatum and infused a relatively small volume of the antagonist, we cannot rule out the possibility that drug may have diffused to more ventral and medial regions of the striatum during drug treatment. Importantly, both the dorsolateral and dorsomedial striatum have been shown to play a role in regulating alcohol intake (Ben Hamida et al., 2013; Corbit et al., 2012, 2014; Darcq et al., 2016; Even-Chen et al., 2017; Laguesse et al., 2018; Wang et al., 2010), and future experiments will dissect the relative importance of these dorsal striatal subregions in NK1R-mediated effects. Taken together, the results suggest that upregulation of the NK1R contributes to escalation in alcohol intake that is induced by intermittent access, but the critical region differs from the region that we have found to mediate escalated consumption in P rats, which is driven by NK1Rs in the CeA. While not entirely analogous, this is reminiscent of our findings concerning the effect of social interaction behavior and social defeat stress on alcohol intake in mice (Nelson et al., 2017). In these experiments we found that NK1R expression in the AMG was positively associated with innate alcohol preference, and inversely associated with innate social interaction behavior. In contrast, NK1R expression in the NAC was upregulated in mice susceptible to social defeat, which also consume increased amounts of alcohol. In other words, we have observed that in genetic models of escalated alcohol consumption (genetic selection for high alcohol preference, inherent alcohol consumption phenotype), NK1Rs in the AMG are critical, whereas in models of induction of high alcohol consumption (social defeat, intermittent access) striatal NK1Rs play a more prominent role.

In conclusion, we found that multiple models of escalated consumption depend on NK1R signaling, in agreement with our

earlier work in alcohol preferring rats. In models of intermittent access, the escalation that is induced is associated with increased NK1R expression in the striatum, and intra-dorsal striatum infusion of NK1R antagonist can attenuate this behavior. While we now have considerable data on the brain regions where NK1Rs are upregulated and function to mediate specific alcohol seeking behaviors, we do not know from where the SP-expressing projections that target these receptors originate. Future experiments will examine specific SP pathways in the brain, and determine their specific role in alcohol seeking.

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