INTRODUCTION
A recent clinical trial has shown that Deep Brain Stimulation of the nucleus accumbens (NAc) in severely alcohol-dependent subjects results in a “complete disappearance of [alcohol] craving” and a reduction in relapse [1]. These results, although promising, are very poorly understood from a mechanistic standpoint. Prior work in this area has shown that using DREADDs to increase NAc activity results in decreased ethanol (EtOH) self-administration, however, increasing NAc activity in this way doesn’t change the rewarding value of EtOH as measured by conditioned place preference. Therefore, we hypothesize that increased activity in the NAc could decrease alcohol-seeking behavior by increasing the aversive effects of alcohol, which is what the present study aims to investigate using a Conditioned Taste Aversion (CTA) experimental paradigm.

CTA is a specialized, highly robust form of Pavlovian conditioning wherein an animal associates a taste (conditioned stimulus [CS]) with the aversive effects of a toxic or poisonous substance (unconditioned stimulus [US]). In the first published CTA experiment, for example, a novel saccharin solution was paired with a dose of radiation, resulting in decreased consumption of the saccharin solution [2]. A CTA can also be induced by association of a novel taste with drugs of abuse such as alcohol, morphine, THC, cocaine, and amphetamine [3]. Though this seems at first somewhat counterintuitive, as these drugs are rewarding and reinforcing, it shows these drugs also have negative effects which can be measured in a CTA experimental paradigm. In the present study, the effects of alterations in activity of the NAc to the aversive effects of alcohol are examined using such a methodology.

To produce these alterations, we used a recently engineered class of mutagenized muscarinic receptors called DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). DREADDs, unlike endogenous muscarinic receptors, do not bind to acetylcholine and are instead activated by the otherwise pharmacologically inert drug clozapine n-oxide (CNO). There are two kinds of DREADDs used in this study; hM3Dq, a Gq-coupled GPCR whose activation increases intracellular calcium levels which increases neuronal firing, and hM4Di, which is Gi-coupled so its’ activation decreases cAMP and increases GIRK activity resulting in decreased neuronal firing.

In this study, one group of mice were stereotaxically injected (bilaterally into the NAc) with AAV expressing the mutated M3-receptor (hM3Dq) to increase NAc activity, another group with the mutated M4-receptor (hM4Di) to decrease NAc activity, and a final group was injected with GFP as a control (CNO should not have any effect on this group as it does not express a DREADD).

METHODS
Female C57BL/6J mice (n=11-13/group) were stereotaxically injected with AAV hM3Dq, hM4Di, or GFP bilaterally into the NAc, and subsequently underwent 2 weeks of recovery, followed by a week of limited water access (9-11AM daily). To conduct the actual experiment, the mice were then given 1 hour of access to a 0.15% Saccharin solution without CNO or EtOH injections, followed by a planned 10 days of 5 EtOH-saccharin pairings (“trial days”) interspersed with 5 limited-water access days (Fig. 1A). On trial days, the mice were injected with CNO (1g/kg) at 9:30AM, allowed access to the saccharin solution from 10-11AM, and then at 11AM control animals (4/treatment) were injected with saline, and the rest were injected with 2g/kg EtOH IP (intraperitoneally). On trial days, mice were given unmeasured access to water from 4-5 pm to prevent dehydration. On the days in between trials, mice were allowed unmeasured access to water from 9-11AM.

On the sixth day of the experiment (between the second and third trials), however, the mice were accidentally given the saccharin solution instead of water (without CNO injections), so the entire initial methodology had to be abandoned and a new methodology was constructed. In the revised experimental design (Fig. 1B), the mice were given two different Kool Aid solutions (0.1% Kool Aid, 0.15% saccharin in tap water) in grape and cherry flavors. On the first day of the experiment, the mice were given both solutions for 1 hour (10-11AM) of measured drinking. On days 2 and 4 of the experiment, all 35 mice were given IP injections of CNO (1g/kg) 30 minutes prior to 1 hour (10-11AM) of measured drinking of the cherry flavored solution, followed immediately by IP injections of EtOH (2.5g/kg). On days 3 and 5, all mice were given IP injections of CNO 30 minutes prior to 1 hour (10-11AM) of measured drinking of the grape solution, followed immediately by IP saline injections. On the
Figure 1: Experimental timeline. [A] Shows the timeline as originally designed, [B] shows the modifications made after the original methodology had to be abandoned.

Figure 2: Pre-(day 1) and Post-(day 6) experiment cherry solution preferences for the GFP, hM3Dq (Gq) and hM4Di (Gi) groups (n = 7-10/group). Preference is defined as cherry intake divided by total (grape + cherry) intake.

RESULTS
Two-way ANOVA was used to analyze the data (Fig. 2) where the factors were time (pre/post test) and treatment group (GFP/Gi/Gq). A main effect of time was observed indicating a significant EtOH-induced CTA occurred in all groups (F(2,32)=70.62, p < 0.0001). However, we did not observe a main effect of treatment group, thus no significant difference in CTA-expression was found between treatment groups (F(2,32)=0.08, p = 0.92).

DISCUSSION
The results seem to suggest that changing the activity of the NAc has no impact on expression of an EtOH-induced CTA, which is unexpected, considering the results of previous studies [see introduction] and the robust effects observed in Vogues et al.’s clinical trial. One possible explanation for this is that the NAc may be involved in alcohol craving, but the associations mediating a CTA occur in other brain regions. This seems unlikely, however, as the results of several taste studies indicate a strong role for the NAc in taste memory formation/retrieval and the association of taste unconditioned and conditioned stimuli. Another possible explanation is that the different groups could develop a CTA at different rates, as the data presented here does not take into account the acquisition or development of the taste aversion, but only looks at its’ final expression.

There are a myriad of other possible explanations for the results. We injected the DREADDs into both the shell and core of the NAc, but it’s possible there are subregion-specific effects of NAc activity (shell vs. core) on alcohol consumption. We assumed in our experimental design that the association between the CNO injection and saccharin consumption is negligible compared to the association of saccharin and EtOH injections, but this is not necessarily the case. It could be that CNO should be given with EtOH instead of before saccharin. It could also be important to give CNO 4 hours post-EtOH injection to “relieve” withdrawal symptoms, which are at a maximum at 6 hours after acute injection.

Ultimately, it’s unclear if the lack of significant difference observed between treatment groups is because changing activity in the NAc doesn’t alter acquisition of an EtOH-induced CTA, or because a mistake forced us to abandon the original methodology and attempt a recovery with an unexplored experimental design. Therefore, I propose we repeat the experiment again using the original methodology, perhaps with altered injection times.

REFERENCES

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