BLOCKADE OF STRIATAL DOPAMINE D1 RECEPTORS REDUCES QUININE-RESISTANT ALCOHOL INTAKE

by

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A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Psychological Sciences

Indianapolis, Indiana

May 2019

THE PURDUE UNIVERSITY GRADUATE SCHOOL
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To my parents who have loved, supported, and guided me tirelessly for twenty-eight years.

ACKNOWLEDGMENTS

It takes a village.

My committee was instrumental to the development and execution of this project. I want to thank my mentor, Dr. Nicholas Grahame, for guiding me through this dissertation, as well as helping me to find my way as a critical and meticulous scientist. Thank you to Dr. Stephen Boehm for adopting me into your lab, sharing your equipment, and becoming a co-mentor this past year. I would like to thank Dr. Marian Logrip for her incredible support, from calming talks in your office to helping me slice brains late on a Friday night. Finally, thank you, Dr. Woody Hopf, for paving the quinine path and lending your extensive knowledge to my project. I would also like to thank my previous committee members, Drs. Cristine Czachowski and Charley Goodlett, who helped shaped the foundation of this dissertation.

I have leaned on the entire Addiction Neuroscience area for support in all endeavors, science and otherwise. I am incredibly appreciative to the entire faculty who have always embraced an open door policy and helped develop my critical thinking skills in journal club each week. I have been so lucky to spend the past five years studying alongside some of the most intelligent, enthusiastic, and helpful people I've ever met, my fellow graduate students. I cannot even begin to thank you all enough for your help learning new techniques, reading bottles, and understanding a paper we were supposed to read. Not only have you all been valuable colleagues, you've all become my friends. I would like to especially thank Lauren Millie and Claire Carron for being incredible lab mates and friends. You never complained when I burst into your office to ask you to read an email I was drafting and always listened when I wanted to complain (and usually knew to throw chocolate or beer at me, as well!). It has been awesome to see you both learn and grow during your time in our program and become such strong scientists; I am so grateful to you for how much you've taught me about science and about myself.

I have the most loving and supportive family that have been by my side for not only the past five years, but my entire life. Thank you, Mom and Dad, for answering the phone multiple times a day when I thought I just could not do it anymore (and thanks, Mom, for reminding me I had to stay, or else I'd lose my healthcare!) and always being a stable voice of reason. You both have given me so much and that always served as a strong reminder to just keep pushing. Thank

you, Kelli, for being a constant inspiration to keep working hard and to do it all with a smile on my face. I'm so proud of all you've accomplished.

Finally, I owe an enormous thank you to my incredible husband, Christopher, for absolutely everything over the past five years. You never blinked once when I said to you, "I've got a rough couple weeks coming up and I could use a little extra love and understanding", pretty much every other week for five years. You have always shown such an interest in my work, but also understood when I needed to talk about anything but mice and quinine. Thank you for never complaining about spending so much time in airports because my dream graduate program happened to be in a landlocked state. I am so blessed to have such an understanding and compassionate partner and I cannot wait to see what lies ahead for us.

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ABSTRACT

Author: Houck, Christa A. PhD
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Degree Received: May 2019
Title: Blockade of Striatal Dopamine D1 Receptors Reduces Quinine-Resistant Alcohol Intake
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Drinking despite aversive consequences, or compulsive drinking, is a criterion of alcohol use disorder and can be modeled in rodents by adding bitter quinine into alcohol. Previous studies have shown the development of quinine-resistant ethanol (EtOH) drinking following a drinking history, but used animals that achieved relatively low blood alcohol levels. Selectively bred crossed High Alcohol Preferring (cHAP) mice average over 250 mg/dl during a two-bottle choice procedure. Compulsive drinking is hypothesized to be D1-receptor mediated via the dorsolateral striatum (DLS). We hypothesized that 2 weeks of free-choice EtOH would lead to quinine resistance and intra-DLS infusion of a D1-antagonist, SCH23390, would attenuate quinine-resistant alcohol drinking with no effect on non-conflicted EtOH drinking. Infusion of SCH23390 into the DMS would not affect quinine-resistant drinking.

cHAP mice had guide cannulae placed in the DLS or DMS and had either two weeks (2W) of EtOH and water two-bottle choice or were EtOH naïve (0W). Mice were infused with either SCH23390 or saline immediately prior to one 10% EtOH and water test day and SCH23390 did not disturb alcohol drinking. The following day, we adulterated the EtOH with 0.32-g/L quinine (0.89 mM), and mice received the same microinjection. For animals cannulated in the DLS, 2W history group infused with saline drank more quinine-adulterated EtOH than the 0W saline mice. While SCH23390 infused 0W animals looked no different from saline treated mice, it attenuated quinine + EtOH intake in the 2W animals to the level of 0W

animals. Interestingly, DMS-cannulated mice demonstrated similar behavior, with SCH23390 reducing EtOH + quinine consumption, while leaving EtOH consumption undisturbed.

Quinine resistance following 2 weeks of free-choice EtOH consumption is attenuated by acute administration of a D1-antagonist in the DLS, suggesting that an alcohol history induces compulsivity and that dopamine contributes to this behavior. This is unique to compulsive drinking, as non-conflicted EtOH drinking was unaffected.

INTRODUCTION

An Introduction to Compulsivity & Alcohol Use Disorder

A compulsion is an action that occurs even when the outcome of that action becomes negative or maladaptive (Everitt and Robbins, 2005, Hopf and Lesscher, 2014). Compulsive behaviors are seen in a number of DSM-5 diagnoses, such as Tourette's disorder (tic disorder), autism spectrum disorder, and obsessive compulsive disorder (Dalley et al., 2011). Beyond these more commonly cited compulsive diagnoses, drug and alcohol addiction can also be viewed as a compulsive disorder (Torregrossa et al., 2008, Dalley et al., 2011).

One criterion for an alcohol use disorder (AUD) is continued alcohol use in spite of negative consequences (American Psychiatric Association, 2013). Compulsive drinking can be described as continued use in the face of social, legal, and physical consequences. Further, this shift to compulsive drinking appears to be relatively permanent; 52% of patients seeking treatment for AUD are unable to reestablish non-problem drinking (Ilgen et al., 2008) and must abstain completely from alcohol use. The idea that an AUD is fueled by a compulsion to drink is not a recent development (Edwards and Gross, 1976), yet insufficient research has been done to understand the underlying mechanisms of this disorder.

The Striatum & Dopamine May Contribute to Compulsivity

In order to target compulsive drinking, it is important to understand the neural mechanisms behind this behavior. While the exact mechanism of compulsivity is still unknown, the striatum has been implicated as a region of interest, a few studies have demonstrated its involvement in compulsivity. Administration of an NMDA antagonist into the nucleus

accumbens (NAc) core attenuated quinine-resistant alcohol intake (Seif et al., 2013). Compulsive cocaine seeking was attenuated following inactivation of the dorsolateral striatum (DLS) as well as the ventral striatum (Jonkman et al., 2012). Interestingly, this study found that ventral striatum, but not DLS, inactivation reduced unpunished cocaine seeking, lending itself to the idea that the non-conflicted and compulsive drug taking may have different neural mechanisms.

Classically, the striatum has been divided into two distinct sections, the ventral striatum, composed of the NAc core and shell, and the dorsal striatum, made up of the dorsomedial striatum (DMS) and DLS (Voorn et al., 2004). However, it is becoming clearer that the DMS looks more like the ventral striatum on a molecular level (Voorn et al., 2004) and may in fact underlie similar behaviors as the NAc as well (Yin et al., 2005a, Yin et al., 2005b, Thorn et al., 2010, Gremel and Costa, 2013).

Nucleus accumbens (NAc) has long been known to be a structure important in processing reward and associating outcomes with an action (Schultz, 1997) and this process is vital for ascribing a consequence, both negative and positive, to drinking. In addition, the striatum has also been shown to be involved in responding to aversive stimuli (Kravitz and Kreitzer, 2012), like quinine. Behavioral flexibility, or the ability to adapt to new stimuli within a learned behavior, has also implicated the striatum (Ragozzino, 2007) and may be impaired in chronic drinking subjects. It seems as if these behaviors all may be related to, or can contribute to, compulsive drinking and therefore the striatum may play a role in compulsive behaviors as well.

Another behavioral process that is likely tied closely to compulsivity is habit formation (Everitt and Robbins, 2005, Hopf and Lesscher, 2014). A habit can be defined as a response that is performed without regard for the outcome of that response. This behavior is originally

classified as goal-directed, or an action performed in order to obtain a known outcome. Over time and when the outcome becomes less predictable, this goal-directed behavior can shift to a habit. While not identical to compulsive behavior where a subject performs an action in spite of a negative consequence, a habit is performed without regard or thought for the outcome (Hopf and Lesscher, 2014). It is imperative to note that these behaviors are distinctly different, but they do appear to be closely related.

Fortunately, much is known about the brain regions responsible for the shift from goal-directed to habitual behavior. The ventral striatum, specifically the NAc core, contributes to the acquisition of goal-directed behavior (Corbit et al., 2001). Through a series of lesion experiments and pharmacological manipulation, the DMS was also shown to be responsible for goal-directed behavior (Yin et al., 2005a, Yin et al., 2005b) and the DLS necessary for habits (Yin et al., 2004). Thorn et al. (2010) showed that firing rates in the DMS were increased in the early stages of acquiring an instrumental behavior (a maze task). This was also seen in mice learning goal-directed instrumental behavior, but this firing later decreased in comparison to the DLS (Gremel and Costa, 2013), indicating that activity shifted from predominantly DMS to DLS. Further, the NAc and DLS were both found to be necessary for cocaine seeking after a long period (30 days) of training, likely creating a habit, as a non-specific dopamine antagonist administered into both of those regions contralaterally reduced cocaine seeking (Belin and Everitt, 2008). These structures have been shown to hold equal importance for goal-directed and habitual behaviors in humans: the DMS homologous to the caudate and the DLS homologous to the putamen (Valentin et al., 2007, Tanaka et al., 2008, Tricomi et al., 2009, Sjoerds et al., 2013), giving the findings of rodent studies some translational validity (McKim et al., 2016).

Understanding how the striatum's function changes during the shift from non-conflicted to compulsive drinking has been hypothesized in various resources (Everitt and Robbins, 2005, Hopf and Lesscher, 2014, Haber, 2016). To briefly summarize, dopamine is released from the ventral tegmental area (VTA) into the NAc during early alcohol consumption. Dopamine binds to D1 receptors on GABAergic medium spiny neurons in the NAc. A large population of these neurons project down to the substantia nigra (SN), which contains GABAergic interneurons that inhibit dopamine neurons that project up to the striatum. When DA acts in the NAc, GABA release on those interneurons serves to disinhibit DA neurons that project back up to the NAc, as well as the DMS. With repeated drinking, these DA projections are strengthened through the dorsal striatum in a feed-forward fashion. D1-receptors are expressed more densely in the ventral striatum (NAc core and shell) than the DMS and DLS (Gagnon et al., 2017). This dopamine spiral is illustrated in Figure 1.

While this process of strengthening projections from the SN to the DLS leading to habit formation is a natural occurrence that happens over time (Adams and Dickinson, 1981, Haber et al., 2000), previous studies have shown that a history of drugs of abuse may expedite habit formation (Corbit et al., 2012, Nelson and Killcross, 2006). The mechanism behind this hastened process is probably not due to an increase in D1-receptor expression, as a number of studies have indicated no change in D1-receptor expression in the dorsal striatum over a range of alcohol histories (Pellegrino and Druse, 1992, Hietala et al., 1990, Woods et al., 1995), even in as long as 14 weeks (Sari et al., 2006). Contributing to these findings, McBride et al. (1997) found no difference in D1-receptor densities in the striatum of high and low alcohol preferring rats (HADs and LADs, respectively), in the absence of alcohol administration. These previous

studies suggest that the acceleration of habit formation following drug exposure is likely not due to changes in D1-receptor expression in the striatum.

In addition to animal models implicating dopamine in the striatum in the shift from goal-directed to habitual behavior, human studies have confirmed these findings. Knowlton et al. (1996) showed that patients diagnosed with Parkinson's disease could not acquire habits, but had no impairments on a memory task. This deficit was likely due to deterioration of the SN, a key structure in the dopaminergic spiral in the striatum. In fact, one review suggests that the behavioral impairments seen in Parkinson's disease patients may be completely caused by failure to create habits and an overreliance on goal-directed behavior (Redgrave et al., 2010). In addition, postmortem brains of humans with alcohol use disorder show no difference in D1-receptor density in the dorsal striatum as compared to undiagnosed controls (Tupala and Tiihonen, 2005).

Understanding the intricacies of this dopamine circuit could be a key to treating problematic drinking. Previous literature has hypothesized that this circuit operates in one direction, from the ventral striatum to the dorsal striatum and does not work in reverse (Haber et al., 2000, Everitt and Robbins, 2016). While this irreversible shift reflects a majority of human patients with alcohol use disorder who are unable to resume non-problematic drinking, there is a subset of the population that is able to restore goal-directed drinking (Ilgen et al., 2008). While this idea has not been fully explored in the laboratory, one study was able to demonstrate the plasticity between goal-directed and habitual behavior by manipulating inputs into the striatum (Gremel et al., 2016).

It is important to keep in mind that habitual behavior is not the same as compulsivity. Compulsivity requires consideration of a negative outcome and acting in spite of that outcome.

Although it was hypothesized that these behaviors may directly lead to one another (goal-directed → habitual → compulsive; (Everitt and Robbins, 2005), recent work has indicated that compulsive drinking can arise extremely quickly (Lei et al., 2016), complicating this relationship. However, it is evident that these behaviors are somehow related. If compulsive behavior also relies on the striatum, then manipulating these structures into restoring goal-directed drinking may be an effective treatment for alcohol use disorder. I seek to reverse compulsive drinking via pharmacological manipulation of the striatonigrostriatal pathway.

Literature Review of Compulsivity Research

To help treat patients with an AUD who drink in this problematic manner, several animal models have been developed to explore the neurobiology of compulsive drinking. Hopf and Lesscher (2014) have previously detailed the various manners that compulsive drinking can be modeled in rodents. Frequently, punishing cocaine (Vanderschuren and Everitt, 2004, Jonkman et al., 2012) or alcohol (Seif et al., 2013) seeking by shocking an animal prior to self-administration has been used. Another model of compulsivity, conditioned taste aversion, pairs an unconditioned flavor with an aversive injection of lithium chloride and subsequently adds that flavor to alcohol. Animals have been shown to be less sensitive to a conditioned taste aversion following long histories of alcohol consumption (O'Tousa and Grahame, 2016). This is perhaps a weaker model of compulsive alcohol drinking as the consequence is not direct, but is instead a negative association with no actual consequence to consuming the flavor. In addition, with repeated test sessions, the animal can extinguish this conditioned aversion without repeated injections of lithium chloride.

One of the first studies to look at conflicted drinking developed a novel model to address this behavior. Wolffgramm (1991) gave rats access to alcohol for nine months to instill a long-

term drinking history and, following nine months of abstinence, gave rats the choice between water or alcohol mixed with quinine hydrochloride, a bitter salt with an unpleasant taste. Rats that had experience drinking alcohol in the past drank through the aversive taste, but alcohol naïve animals did not, indicating that the alcohol history induced a state of aversion-resistant drinking. Because the quinine test day occurred following nine months of abstinence, we can be confident that changes due to long-term drinking are persistent and may even be permanent, providing further evidence that treatments for compulsivity are necessary to explore, even after long periods of abstinence. This holds translational significance as patients with AUDs can resume compulsive drinking following a period of relapse.

Following Wolffgramm's pioneering study, other researchers began to use quinine-adulterated alcohol as a model of drinking in spite of negative consequences. A number of papers have used different rodent models to replicate Wolffgramm's findings and found similar results: an alcohol drinking history can lead to increased quinine + alcohol consumption compared to naïve controls (Seif et al., 2013, Lei et al., 2016, Lesscher et al., 2010, Fachin-Scheit et al., 2006). In fact, these findings extend beyond home cage self-administration to operant responding for alcohol and subsequently alcohol + quinine, as well (Hopf et al., 2010, Randall et al., 2017). However, the length of alcohol history has differed drastically among experiments, with some rodents requiring months of alcohol drinking (Seif et al., 2013, Hopf et al., 2010, Fachin-Scheit et al., 2006) and others only needing a single 24 hour period (Lei et al., 2016). In addition to a diversity of alcohol histories, the concentrations of quinine used throughout these experiments have also varied, ranging from as little as 0.001 g/L (Dess, 2000), up to 0.09 g/L (Lesscher et al., 2010) to still see quinine-resistant drinking. While the use of

quinine resistance as a model of compulsive drinking is a simple and accessible task, there is much disparity within the methods used.

Models of compulsivity have been used in other drugs of abuse, such as cocaine (Deroche-Gamonet et al., 2004, Vanderschuren and Everitt, 2004). Interestingly, the data so far indicate that compulsivity, as measured by quinine-resistant drinking and foot shock, only occurs for drugs of abuse and does not generalize to natural reinforcers, such as sucrose (Dess, 2000, Vanderschuren and Everitt, 2004) or saccharin (Houck et al., under review). This further suggests that compulsive substance use is a specific pathology and not simply a response to any reinforcing substance.

Using Selectively Bred Mice as a Model of Alcohol Use Disorder

Using quinine-resistance as a model of compulsivity is an effective and easy-to-produce method of studying AUD with strong face validity. In order to further improve this model to look even closer at the human condition, it is necessary to assess the amount of alcohol consumed by these rodents during the drinking history. While not all of the aforementioned studies measured blood alcohol levels (BALs) during the drinking history, the majority that did observed levels ranging between 50 – 60 mg/dL (Fachin-Scheit et al., 2006, Hopf et al., 2010, Seif et al., 2013, Wolffgramm, 1991), approaching, but not reaching, the NIAAA definition of an alcohol binge of 80 mg/dL. One exception to this is Lesscher et al. (2010) who had mice that reached 100 mg/dL. Interestingly, Mello and Mendelson (1970) measured BALs of humans with alcohol use disorder and found they can reach between 200 – 300 mg/dL while drinking. While it is clear that the previous studies have made contact with compulsive drinking when animals have reached lower BALs, it is necessary to investigate animals that drink to levels modeling those in human patients diagnosed with AUD.

Our lab has developed four lines of high alcohol preferring (HAP) mice, HAP1, 2, and 3, selectively bred from an Hs/Ibg progenitor strain (Oberlin et al., 2011, Grahame et al., 1999), and crossed HAP (cHAP), bred from crossing the HAP1 and HAP2 lines. Mice were bred for high alcohol preference and intake on a 24 hour, two bottle choice (2BC) task where they had access to both 10% alcohol and water. cHAP mice on average consume 23.85 g/kg/day resulting in BALs of over 250 mg/dl during 2BC (Matson and Grahame, 2013). These findings indicate that cHAPs can make contact with the high alcohol consumption and BALs seen in patients with alcohol use disorder and may provide a novel viewpoint of compulsive drinking.

Conclusion

Compulsive drinking, as a symptom of alcohol use disorder, is a problematic behavior that adversely affects many people and has been vastly understudied. There is modest evidence to suggest that the striatum is responsible for the shift between non-conflicted drinking and compulsions, but the exact mechanism is still unknown. Furthermore, no study has attempted to attenuate compulsive alcohol drinking in animals with an alcohol history without affecting non-conflicted alcohol consumption. Using cHAP mice selectively bred for high alcohol consumption and known to rapidly form habits (Houck and Grahame, 2018), I sought to induce compulsive drinking, defined as being willing to drink aversively flavored alcohol, following two weeks of unadulterated alcohol exposure, and reverse it with SCH23390, a dopamine D1-receptor antagonist.

Specific Aims

Aim 1: Disrupt compulsive-like drinking in cHAP mice by infusing a D1-receptor antagonist into the dorsolateral striatum (DLS).

Following a two-week drinking history that is sufficient to induce compulsive drinking in cHAP mice, we hypothesized that administering SCH23390, a D1-receptor antagonist, into the DLS, a structure implicated in aversion, inflexibility, and habitual behavior, would reduce quinine-resistant alcohol consumption. Considering long-term drinking likely strengthens the striatonigrostriatal pathway, leading to increased dopamine release in the DLS, inhibiting dopamine activity in the DLS should allow the goal-directed ventral striatum to act and reduce aversive drinking. Because non-conflicted drinking is a goal-directed behavior, intra-DLS SCH23390 should have no effect on the baseline, non-conflicted alcohol drinking.

Aim 2: Assess the role of the dorsomedial striatum (DMS) in compulsive cHAP mice.

Compulsive drinking in cHAP mice that arises following two weeks of alcohol consumption likely utilizes the DLS, but not the DMS, a vital structure implicated in goal-directed behavior. Therefore, infusion of the D1-receptor antagonist into the DMS should not affect conflicted drinking, as dopamine should still be released into the DLS, the implicated area for compulsive-like drinking. We hypothesize that SCH23390 in the DMS may impair unadulterated alcohol consumption, due to interference with goal-directed behavior.

Aim 3: Examine the effects of a D1 antagonist in the DLS on non-compulsive cHAP mice.

We hypothesize that blocking D1 receptors in alcohol naïve animals should have no effect on quinine + alcohol consumption, as the animals have not had sufficient alcohol exposure to be compulsive and therefore the DLS should not yet be recruited in this behavior.

METHODS

General Design

cHAP mice with guide cannulae aimed at the DLS (Pilot, Aims 1 & 3) or DMS (Aim 2) had either two weeks (2W) of EtOH and water 2BC (Pilot, Aims 1 & 2) or were EtOH naïve (0W, Aim 3). Mice were infused bilaterally with either SCH23390, a dopamine D1-receptor antagonist, or saline immediately prior to one 10% EtOH and water test day where baseline alcohol intakes were measured bihourly during the dark cycle. The following day, we gave mice the same microinjection and adulterated the EtOH with 0.32-g/L quinine (0.89 mM).

Subjects

142 cHAP mice (72 male) were divided among four separate experiments (SCH Dose Pilot, Aims 1 – 3). Mice were approximately 60 – 81 days old on the surgery date and about 67 – 88 days old for the start of the drinking. Within each experiment, mice were counterbalanced by sex, family, and surgery date into treatment groups. With the exception of the Pilot experiment, mice were also counterbalanced for infusion order; mice received an infusion either on the first baseline (BL) day and the second quinine (QA) day, or the first QA day and the second BL day (BL-QA or QA-BL). In the SCH Dose Pilot, mice received either a SAL (0.0 μg/0.25 μL), SCH Low (0.25 μg/0.25 μL), or SCH High (0.50 μg/0.25 μL) infusion. In Experiments 1 – 3, mice were divided into one of two groups: SCH or SAL. Mice were single housed following surgery in standard Plexiglas cages lined with pine bedding. The housing room followed a standard 12-hour reverse light cycle (lights off at 0700). All experiments were

performed in accordance with the Institutional Animal Care and Use Committee (IACUC) of IUPUI and NIH Guide for the Care and Use of Laboratory Animals.

Solutions

For surgical procedures, animals were anesthetized with isoflurane, ranging in concentration from 0.5 – 3.0% suspended in 2 liters per minute of oxygen during the surgery. Immediately following surgery, mice received a 10-mL/kg s.c. injection of 5 mg/mL carprofen for analgesia and anti-inflammation.

Two bottle choice procedures consisted of one 50 mL tube of 10% EtOH (v/v) and a 25 mL tube of tap water. For baseline testing, mice had access to 10 mL tubes filled with 10% EtOH and tap water. Quinine adulterated EtOH is a mixture of 10% EtOH and 0.32 g/L (0.887 mM) quinine hydrochloride (Fischer Scientific, Nazareth, PA).

For the Pilot experiment, SCH23390 (Tocris Bioscience, Bristol, UK) was dissolved in 0.9% sterile saline at a concentration of 0.25 μg/0.25 μL and 0.50 μg/0.25 μL, based on previous work that these doses have a motivational effect on heroin seeking (Bossert et al., 2009) without interfering with basic locomotor activity (Presti et al., 2003). Because that dose was too low to detect behaviorally relevant effects, Aims 1 – 3 used a concentration of 3.0 μg/0.25 μL. Saline infused animals received an equivolumetric infusion of saline. All drugs and Saline were infused at a rate of 125 nl/min. Microinjectors remained in place for 30 seconds following infusion and then removed slowly to prevent injury or interference with the infusion.

Surgery

Between 60 – 81 days of age, mice were single housed and surgically implanted with bilateral guide cannulae. Animals were anesthetized with isoflurane gas and placed into a Kopf

Stereotaxic Instrument (David Kopf Instruments, Tujunga, CA). 10 mm guide cannulae made of 25-gauge stainless steel tubing (Component Supply Company, Sparta, TN) were either placed bilaterally into the dorsolateral striatum (A/P: +0.38 mm, M/L: +/- 2.5 mm, D/V: -3.0 mm; Pilot, Aims 1 & 3) or the dorsomedial striatum (A/P: +0.50 mm, M/L: +/- 1.25 mm, D/V: -2.75 mm; Aim 2). Two surgical screws were also implanted posterior to the guide cannulae to secure the skullcap, affixed with dental cement (Stoelting Co., Wood Dale, IL). Wire stylets were placed into the cannulae to prevent occlusion and maintain patency. Following the surgery, mice received a s.c. injection of carprofen and moved to recover in an empty Plexiglas cage on a heating pad. Once animals regained consciousness, they were each transferred to a fresh cage and returned to the colony room where food and water is available *ad libitum*.

Procedures

SCH Dose Pilot

Animals were left alone to recover for 5 – 7 days following surgery before the 2BC procedure began. At the start of the two week drinking history, mice were given access to a 50 mL bottle of 10% EtOH and a 25 mL bottle of tap water. Fluid consumption and preference was measured three times a week (Monday, Wednesday, and Friday) and bottle side was alternated at each reading to prevent a side preference from forming.

To acclimate mice to restraint for microinjections, animals were scruffed and restrained following each bottle reading to ensure the stylets were still in the cannulae and replace them with sanitized new ones if necessary. Following 11 days of drinking, mice had microinjectors lowered into the guide cannulae as a mock infusion, but no drug was administered.

14 days following the start of the 2BC procedure, the first quinine test occurred. At the start of the dark cycle at 7 am, stylets were removed from the guide cannulae and microinjectors

(25G cuff and 33G injector) were slowly inserted into the guide cannulae. Microinjectors were connected to two 10-μL Hamilton syringes in a Cole-Parmer (74900-series) dual infusion pump via PE-20 tubing. Mice were given an infusion of SCH23390 (0.0, 0.25, or 0.50 μg/0.25μL/side) at a rate of 125 nL/min, resulting in a two minute long infusion. Microinjectors were left in place for 30 seconds following the infusion to allow drug diffusion and then were slowly removed and fresh stylets were replaced. Immediately following the infusion, mice were given access to two 10-mL tubes: one containing 10% EtOH + 0.32 g/L quinine and another containing tap water. Fluid intake was measured every two hours during the dark cycle, with a final reading 24 hours after the start of the quinine test session.

On the following day, mice received 24 hours of 2BC of 10% EtOH (no quinine) and water in order to re-establish baseline drinking. Previous data show that mice will resume normal drinking following a quinine test day (Houck et al., under review). Following this day of unadulterated EtOH, animals will have two more quinine test days, identical to that above, with one quinine-free day in between. These three test days served to determine the best SCH23390 dose for future studies, as well as assess the effects of repeated microinjections on the mice.

Aim 1: Does intra-DLS SCH23390 Disrupt Compulsive Drinking?

Identical to the Pilot procedure, mice had guide cannulae implanted into the DLS and were left to recover for 5 – 7 days before the 2-week 2BC procedure began. Similarly to the pilot, mice received access to 10% EtOH and water bottles and volume consumed was measured 3 times a week and sides were changed to avoid the establishment of a side preference. Mice were scruffed and restrained following each bottle reading to check the presence of stylets in the cannulae and habituate them for handling during microinjections. They received a mock microinjection after 11 days of drinking, but no infusion was given.

13 days following the start of the 2BC procedure, first baseline drinking (BL1) trajectory was measured. Mice were divided into one of two infusion order groups: BL-QA or QA-BL. When the lights went out at 7 am, **BL-QA** mice were infused with 3.0 μg/0.25 μL SCH23390 or SAL, per microinjection procedures outlined above. QA-BL mice received no infusion or handling. Following the infusions of the BL-QA mice, both infusion order groups were given access to two 10 mL tubes: one containing 10% EtOH and another containing tap water. Fluid intake was measured every two hours during the dark cycle.

At 7 am the following morning, the first quinine test (QA1) began. On this day, **QA-BL** mice received their SCH or SAL infusion and all subjects' 10% EtOH bottle was replaced with a 10% EtOH + 0.32 g/L quinine tube. Again, bihourly intake readings were performed to determine fluid intake and preference during the dark cycle.

Following QA1, all mice had an "OFF" day, where they received access to unadulterated 10% EtOH and water for 24 hours. No handling or microinjections occurred on this day and bihourly intakes were not recorded. After the rest day, the second baseline day (BL2) began. BL2 was identical to BL1, but on this day the **QA-BL** group received their assigned drug infusion prior to all mice having 2BC access with 10% EtOH and water all day. The experiment ended with a second quinine adulteration day (QA2) where the **BL-QA** mice were microinjected with their assigned drug before the 2BC of 10% EtOH + quinine and tap water. The infusion order groups not only allowed us to test for order effects from test days, but it also allowed for non-infusion BL and QA days to measure any potential carryover drug effects or stress responses caused by a microinjection prior to drinking.

Aim 2: Does intra-DMS SCH23390 Disrupt Compulsive Drinking?

This experiment utilized identical procedures to Experiment 1, but guide cannulae were implanted in the DMS. All other procedures were the same.

Aim 3: Does intra-DLS SCH23390 Disrupt Non-Compulsive/Goal-Directed Drinking?

Guide cannulae were placed in the DLS and mice recovered for 5 – 7 days. Following the recovery period, mice were scruffed and microinjectors lowered into the guide cannulae for a mock infusion, but no drug infusion was administered. On the following day at 7 am, these mice began BL1, identical to the two-week drinking history animals in Aims 1 & 2.

Histology

Following QA2, mice were cervically dislocated and decapitated in order to extract their brains. For the Pilot experiment only, mouse brains were preserved in 4% paraformaldehyde for 24 hours and then moved to a 30% sucrose in PBS solution. Once the brains sunk in this solution, they were moved to a -80°C freezer before slicing. Due to technical difficulties during staining of the Pilot tissue, Aims 1 – 3 utilized a different freezing technique wherein brains were extracted and immediately flash frozen in -20°C 2-methylbutane. After the brains were frozen, they were temporarily moved to a -20°C freezer to be sliced the following day.

To verify cannulae placement, the brain was sliced into 40-micron slices on a cryostat and mounted onto microslides (25 x 75 x 1 mm). Following mounting, they were stained with cresyl violet. Once the slices were mounted and stained, damage from the guide cannulae was compared against The Mouse Brain in Stereotaxic Coordinates (Paxinos & Franklin) to determine microinjector hits. Only bilateral hits were analyzed in the experiments; unilateral hits and misses were removed from the analyses. A “miss” occurred if the bottom of the cannula was

too dorsal (not fully through the corpus callosum), too ventral (no longer in the striatum), or too medial/lateral (crossed over the center of the dorsal striatum, based on the intended placement of DMS or DLS).

Statistical Analysis

Data were analyzed using SPSS (SPSS, Version 22, Chicago, IL) and results were graphed on Graphpad Prism (Graphpad Prism, v. 6.0, La Jolla, CA). Significance was set at $p < 0.05$.

Before overall analyses were conducted, ANOVAs measuring total intake on each infused baseline and quinine test day were performed to determine if there was an interaction of Drug*Infusion Order within each Cohort and History (DLS experiments only) group. In the absence of an interaction, data were collapsed by Order. To ensure that there was no interaction of Drug*Cohort, another ANOVA was conducted within each History group (DLS experiment only) for both the BL and QA days. Because there was no interaction, data were collapsed across Cohort. Finally, it was necessary to check for a Drug*Sex interaction on both test days. In the absence of a significant interaction, the data were collapsed by Sex. Therefore, all subsequent analyses were collapsed across Order (BL-QA/QA-BL), Cohort, and Sex.

For mice undergoing a two-week alcohol history, EtOH consumption was measured with repeated-measures ANOVA by Day and Drug treatment, to ensure no baseline differences in future drug treatment were present. Analysis of baseline and quinine test day EtOH and water consumption for infused animals were performed with repeated measures ANOVA by Time point (9 am, 11 am, 1 pm, 3 pm, 5 pm, 7 pm) to see if drinking trajectory changed as a function of Drug administration or History (DLS experiments only). In the absence of an interaction with time, an ANOVA was performed on the entire day's consumption. However, because there was

an *a priori* hypothesis that SCH23390 would only be active for approximately 2 hours following infusion based on previous receptor occupancy studies (Neisewander et al., 1998), individual ANOVAs were run at the 9 am and 11 am time points. All infusion test day analyses were also performed for the non-infused test days.

Locomotor data were obtained from a home cage locomotor monitoring system (Opto M3, Columbus Instruments Inc., Columbus, OH, as previously described in Linsenbardt & Boehm, 2012) in 10-minute bins for 23 hours each day following the beginning of the dark cycle. For ease of analysis, the 10-minute bins were collapsed into half hour bins. An ANOVA was run to determine if there was an effect of Drug on locomotor activity throughout the entire dataset. A second ANOVA was conducted looking just at the first two hours following infusion, while SCH23390 was still likely binding to D1-receptors.

RESULTS

Attrition

Throughout the study, we lost a number of mice and they were therefore excluded from analyses (Table 1). Due to technical issues in the Pilot Experiment, histological verification of cannulae placement was not possible and therefore the placement data are not included in this document. Pilot results should be interpreted with caution. Aim 1, Cohorts 2 and 3 were not analyzed in this study due to experimenter errors and equipment malfunction (Cohort 2: wrong concentration of quinine used; Cohort 3: infusion pump failure). There are no locomotor data for Aim 1, Cohort 1 as the home cage locomotor system was not implemented until after the completion of that experiment. Therefore, all Aim 1 locomotor data is based solely on Cohort 4.

Following successful completion of the test days, all mice were sacrificed and brains were sliced and stained for histological verification of cannulae placement. In Aim 1, four SCH-treated mice and one SAL-treated mouse were dropped from the experiment (Figure 2). One SCH-treated mouse was dropped from Aim 2 (Figure 3). Two SCH-treated mice were dropped from Aim 3 (Figure 4).

Two-Bottle Choice History Intake (Pilot, Aim 1 & Aim 2)

Pilot Study

EtOH intake was measured at six time points throughout the 14-day drinking history, as demonstrated in Figure 5. Alcohol intake escalated across the two week drinking period ($F(5,170) = 22.89$, $p < 0.001$) and female mice drank considerably more than males ($F(1,34) =$

20.205, $p = 0.003$). However, Sex, Drug, or Cohort did not interact with Day (Fs < 0.921, ps > 0.468), suggesting that Drug group assignment was balanced.

Aim 1 & Aim 2

During the 2BC access, there was a strong effect of Time, demonstrating escalation of intake across the two weeks, $F(5,200) = 8.329$, $p < 0.001$ (Figure 6). However, neither future Drug infusion ($F(1,40) = 0.385$, $p = 0.539$), nor brain region cannulated (DLS/DMS, $F(1,40) = 1.651$, $p = 0.206$) affected intake, indicating that before drug manipulations began, both sets of 2W drinking groups were behaving equally.

On the final day of the two-week drinking history, DLS-cannulated mice averaged 22.20 g/kg per day and DMS-cannulated mice averaged 21.62 g/kg/day, indicating they were reaching extremely high doses of EtOH throughout the 2BC procedure (Figure 7). In addition, these intakes look similar to a previous experiment in our lab where cHAPs showed compulsive drinking after two weeks of 2BC (Houck et al., under review), therefore we are confident that the surgeries did not impair alcohol consumption.

Intra-DLS SCH23390 May Attenuate Quinine-Adulterated Drinking – Pilot Data

To determine an optimal dose of SCH23390 to use in later experiments, four cohorts of pilot mice were run to test two SCH doses, Lo = 0.25µg/0.25µL/side and Hi = 0.50 µg/0.25µL/side. Looking at the first QA day, Cohort did not interact with Drug, allowing us to collapse across Cohort ($F(6,39) = 2.045$, $p = 0.094$). We found a main effect of Sex ($F(1,39) = 5.629$, $p = 0.023$), but since there was no interaction with Drug ($F(2,39) = 0.471$, $p = 0.628$), we were able to collapse across sexes. Within each QA day, we performed a RMANOVA to look at the change in quinine + EtOH drinking across the day (Figure 8, left). Because Time never

interacted with Drug (Fs < 1.07, ps > 0.39), we collapsed across the whole day for each QA test (Figure 8, right).

We found no main effect of drug on any of the QA days (Fs < 1.2, ps > 0.31). However, due to the *a priori* hypothesis that SCH23390 would only be acting for the first two hours following infusion, we isolated the 9 am and 11 am time points. While there was a modest trend at 9 am on QA2 ($F(3,39) = 2.76$, $p = 0.079$), no other time points approached significance (Fs < 1.5, ps > 0.25). While there was no significant effect of either 0.25 or 0.50 μg/0.25μL/side SCH throughout the pilot, we saw a non-significant visual trend that SCH was attenuating quinine-resistant drinking. Based on this, we decided to proceed with the subsequent experiments at a much higher dose of SCH, 3.0μg/0.25μL/side) in hopes we would be able to produce a larger drug effect.

Because targeting dopamine is known to cause locomotor deficits (Neisewander et al., 1998), we utilized a home cage locomotor system to monitor mouse movement during the 2BC drinking. Considering there were no differences in the entire 23-hour period following infusion on any of the test days (Fs < 0.92, ps > 0.39, Figure 9), we decided to focus on the first two hours after infusion. On QA1, there was a significant main effect of Drug ($F(2,9) = 21.06$, $p < 0.001$), with the SAL treated mice moving more than both the SCH Lo ($t(6) = 3.612$, $p = 0.011$) and SCH Hi ($t(6) = 5.801$, $p = 0.001$) mice. Interestingly, there were no two-hour differences on QA2 or QA3 (Fs < 3.2, $p > 0.08$), implying that SCH only had a locomotor depressant effect on the first day of treatment. Because we chose to increase the dose of SCH in subsequent experiments, we monitored the drug's effect on locomotor behavior and counterbalance the drug treatment order (as detailed in Methods).

Intra-DLS SCH23390 Attenuates Quinine-Adulterated, But Not Quinine-Free Alcohol Drinking

Collapsing by Cohort, Order, and Sex

Before examining the effects of SCH on BL and QA drinking, it was necessary to first rule out any effects of Cohort (Cohort 1 or Cohort 2), Order (BL-QA/QA-BL), and Sex. We were able to collapse across Cohort due to the absence of any effects or interactions of Cohort (Fs < 1.67, ps > 0.20) on both BL and QA infusion days. While there was a main effect of Order ($F(1,35) = 4.359$, $p = 0.046$), Order did not interact with Drug or History group (Fs < 3.32, ps > 0.08), allowing us to collapse across Order. To collapse by Sex, we performed ANOVAs on total intake for each test day and found Sex did not interact with Drug or History (Fs < 1.40, ps > 0.24). Consequently, all subsequent analyses are collapsed by Order, Cohort, and Sex.

Baseline Drinking Test

Looking at the drinking trajectory across BL, there was a main effect of Time ($F(5,155) = 6.861$, $p < 0.001$), indicating that the pattern of alcohol intake changed throughout the dark cycle, as expected (Figure 10, left). However, because Time did not interact with either Drug or History in any way (Fs < 1.2, ps > 0.31), we were able to collapse across the entire day to assess differences in total EtOH intake. 2W mice drank more than 0W mice, as expected based on their prior drinking experience ($F(1,31) = 5.464$, $p = 0.026$), but SCH23390 had no effect ($p > 0.50$).

As previously stated, we had an *a priori* hypothesis that the SCH23390 would only be active for approximately two hours after infusion, we isolated the 9 am and 11 am time points (2 and 4 h after infusion, respectively) to determine the effects of a drinking history and the SCH infusion. We found that there was no effect of drug within either history group, indicating that SCH had no effect on non-conflicted EtOH drinking (ts < 0.80, ps > 0.437).

Quinine Drinking Test

When quinine was added to the EtOH bottle, we again found a main effect of Time ($F(5,155) = 4.035$, $p = 0.002$, showing a change in overall intake throughout the day (Figure 10, right). In addition to the main effect of Time, there was a Time*History interaction ($F(5,155) = 2.620$, $p = 0.026$), that suggested 2W animals drank more than 0W animals at certain time points but there were no drug effects overall (ps > 0.20). However, due to the aforementioned *a priori* hypothesis, we ran analyses on the 9 am and 11 am time points and found that the Drug*History interaction was just short of significance at 9 am ($F(1,35) = 2.153$, $p = 0.15$) and was not significant at 11 am ($F(1,35) = 1.195$, $p = 0.283$). However, when looking at Drug effects within each History group, we found the SCH reduced EtOH + quinine intake in 2W mice at 9 am only ($t(15) = 2.414$, $p = 0.029$). Furthermore, there was no difference between the 2W SCH mice and either of the 0W groups (ps > 0.17), suggesting that the administration of SCH reduced conflicted drinking to the levels of EtOH naïve animals. 0W SCH did not differ from 0W SAL ($t(16) = 0.515$, $p = 0.614$), indicating that the SCH effect was specific to the 2W compulsive animals.

Change from BL-QA

To ensure that drug differences seen on the quinine test day were not simply due to preexisting differences on the baseline drinking day, we evaluated the change in intake from the baseline to quinine day. While there was a significant decrease in intake from the EtOH to EtOH + quinine day at 9 am in the 2W SCH, 0W SCH, and 0W SAL groups (ts > 2.36, ps < 0.05), there was no decrease in the 2W SAL group ($t(9) = 0.762$, $p = 0.466$), demonstrating quinine-resistant drinking in this group alone.

Water & Total Fluid Intake Analyses

To determine nonspecific effects of SCH, we also analyzed water intake. Looking across the entire BL day, there was no main effect of Time or interactions of Time*History, Time*Drug, or Time*Drug*History (Fs < 1.6, ps > 0.16, data not shown), indicating that groups did not perform differently throughout each timepoint. Collapsing by Time and looking at the entire day as a whole, there was no Drug*History interaction, ($F(1, 34) = 0.227$, $p = 0.637$), but there was a main effect of History ($F(1,34) = 8.412$, $p = 0.007$), where alcohol naïve animals drank more water than those with a drinking history (Figure 11). However, because we were most interested in the 9 am and 11 am time points, we performed t-tests within each History group and found that Drug administration did not affect water intake (ts < 1.3, ps > 0.18, data not shown). Interestingly, total fluid intake was not different between groups receiving SCH or SAL (ts < 0.85, ps > 0.40, data not shown), so overall intake was unchanged.

Similarly, we were able to collapse across timepoints for the QA day (Fs < 2.1, ps > 0.05) and saw no Drug*History interaction on water intake for the whole day ($F(1,34) = 0.385$, $p = 0.540$, Figure 11). While we also saw no differences between drug treatment groups in water intake at the isolated 9 am and 11 am time points (ts < 1.2, ps > 0.248), there was also no change in total fluid intake (ts < 1.07, ps > 0.30, Figure 12). This suggests that avidity, defined as willingness to drink regardless of substance, was not affected by administration of SCH.

Locomotor Activity

Because SCH23390 is a dopamine antagonist, we were concerned about a reduction in locomotor behavior affecting animals' ability to drink, especially in light of the results of the Pilot experiment. Unfortunately, we were unable to utilize the home cage locomotor system for the first cohort of Aim 1, resulting in very variable locomotor results due to small sample sizes.

We observed strong effects of Drug infusion on both the BL and QA test days (Fs > 45.9, ps < 0.001, Figure 13), driven by the extreme variability of the small 2W SCH group. On the BL test, there was no effect of Drug within the first two hours ($F(3,12) = 1.15$, $p = 0.369$), but there was a strong effect on the QA day, likely due to the errant 2W SCH group $F(3,12) = 25.55$, $p < 0.01$ Unfortunately, these data cannot be adequately interpreted due to the small sample sizes and extreme variability in results.

When focusing on our only complete group, the 0W animals, we are able to get a clearer picture of SCH's effect, with a reduction in locomotor activity in SCH mice on both the BL ($t(6) = 3.65$, $p = 0.011$) and QA ($t(6) = 4.431$, $p = 0.004$, Figure 14). SCH23390 likely reduced locomotor activity.

However, to ensure that the changes in locomotor activity seen in the 0W SCH animals was not causing the reduction of EtOH + quinine intake in the 2W SCH mice, we plotted the locomotor activity against intake in the first two hours following the infusion (Figure 15). There was a very weak positive correlation ($r^2 = 0.062$) that did not differ from zero ($F(1,44) = 2.909$, $p = 0.095$). Therefore, we are confident that the SCH locomotor effects did not impact EtOH/EtOH + quinine intake.

Previous Intra-DLS SCH23390 Infusion Does Not Affect Later Quinine-Adulterated or Quinine-Free Alcohol Intake

In addition to a set of BL-QA days where mice received microinjections, they also had one set of BL-QA days where they did not receive an infusion. In order to determine any carryover effects from the drug, we sought to analyze intake and locomotor activity on these days. On both BL and QA days, Time did not interact with any variable (Fs < 1.4, ps > 0.24, data not shown), allowing us to collapse across the whole day and assess total EtOH or EtOH +

quinine intake. There was neither an effect of Drug nor History on total intake on either the BL or QA day (Fs < 2.6, ps > 0.12, Figure 16). Figure 17 shows that water intake did not differ between groups on BL (Fs < 0.50, ps > 0.49), but there was a strong Drug*History interaction on QA, with 2W SAL mice drinking significantly less water than the other groups (ts > 2.3, ps < 0.025).

Consistent with the infusion day locomotor data, the small 2W group sizes make the non-infusion locomotor data difficult to interpret. On both BL and QA, there is a strong effect of Drug (Fs > 23.5, ps < 0.001), unfortunately driven by the variable behavior of the 2W SCH group (Figure 18). However, excluding the 2W animals, there was no effect of Drug on 0W mice (ts < 0.66, ps > 0.51), suggesting that SCH had no carryover effects to a non-infusion day.

Intra-DMS SCH23390 Attenuates Quinine-Adulterated, But Not Quinine-Free Alcohol Drinking

Collapsing by Cohort, Order, and Sex

In order to analyze the data in aggregate, we needed to first ensure there were no effects of Order (BL-QA/QA-BL), Cohort, or Sex. We looked at Total EtOH/EtOH + Quinine intake on both the BL and QA days split by Cohort and found no Drug*Order interactions (Fs < 0.67, ps > 0.41), permitting us to collapse between Order. To collapse across Cohorts, we found no Drug*Cohort interaction (Fs < 1.7 , ps > 0.20) on the total EtOH/EtOH + Quinine intake on each test day. Finally, to assess Sex effects between groups, we ran an additional ANOVA and saw a main effect of Sex on the BL day only ($F(1,28) = 8.34$, $p = 0.008$). However, because there was no Drug*Sex interaction on either day (Fs < 0.21, ps > 0.64), the increased consumption in the females affected both Drug groups equally. Because we had no significant interactions with Drug

treatment on either the BL or QA days, all of the following analyses are collapsed across Order, Cohort, and Sex.

Baseline Drinking Test

Across the BL day, we found a main effect of Time ($F(5,130) = 7.0$, $p < 0.001$), illustrating the change in EtOH intake throughout the day, from a high rate of consumption at the start of the dark cycle to a lower one as the light cycle approached (Figure 19, left). This pattern was not affected by Drug infusion ($F(5,130) = 1,14$, $p = 0.342$), so we were able to collapse and analyze total EtOH intake. ($t(26) = 0.407$, $p = 0.687$, demonstrating that SCH had no effect on unadulterated EtOH consumption throughout the whole day.

However, because SCH's effects likely would not last all day long and could subsequently be underpowered to cause an interaction, we examined just the 9 am and 11 am timepoints. Even at these discrete times, we found that SCH had no effect on intake (ts < 0.85, ps > 0.41), further demonstrating that SCH did not affect non-conflicted EtOH intake.

Quinine Drinking Test

On the QA test day in Figure 19 (right), there was a main effect of Time ($F(5,130) = 2.45$, $p = 0.037$), indicating that the pattern of drinking started high and gradually reduced over time, as we've previously seen (Houck et al., under review). There was a trend toward a Time*Drug interaction ($F(5,130) = 2.152$, $p = 0.063$), suggesting that the pattern of intake changed across the day as a result of differential drug infusion. However, because it was not significant, we collapsed across Time and assessed full day drinking, where any hint of a Drug effect was lost, $t(26) = 0.814$, $p = 0.423$. Focusing at 9 am where SCH would most likely affect behavior, there was a strong effect of Drug ($t(26) = 3.175$, $p = 0.004$), indicating SCH's

attenuation of QR drinking. However, at 11 am, this effect had worn off ($t(26) = 0.843$, $p = 0.407$), demonstrating SCH's short term effects.

Change from BL-QA

To ensure that drug differences seen on the quinine test day were not simply due to preexisting differences on the baseline drinking day, we evaluated the change in intake from the baseline to quinine day. We did find a significant Day (BL/QA)*Time interaction ($F(5,130) = 3.108$, $p = 0.011$) as well as a main effect of Day ($F(5,130) = 42.162$, $p < 0.001$), but no interaction with Drug was present ($F(5,130) = 1.71$, $p = 0.137$), indicating that drinking was reduced overall between the BL and QA test days, but the diurnal intake pattern was unaffected and SCH did not differentially affect intake. Because SCH's effects were likely on a limited timeframe, we continued to focus only on the change at the 9 am and 11 am time points. We found a significant reduction in intake between the BL and QA days in both the SCH ($t(13) = 5.517$, $p < 0.001$) and SAL ($t(13) = 4.833$, $p < 0.001$) treated animals at 9 am, as well as 11 am (ts > 2.5, ps < 0.03). These findings suggest that although SCH reduced EtOH + quinine intake on the QA test exclusively, both Drug groups were sensitive to quinine adulteration.

Water & Total Fluid Intake Analyses

To ensure that the effects seen in SCH-treated mice were not due to changes in avidity and were not specific to EtOH + quinine, we examined water intake as well as total fluid consumption. While there were no effects of Time on the BL day (Fs < 1.29, ps > 0.27, Figure 20 left), there was a Time*Drug interaction on the QA test ($F(5,130) = 3.167$, $p = 0.01$, Figure 20, right), complementing the trend toward a Time*Drug interaction on EtOH + quinine intake. There was no difference in water intake between groups on the BL day ($t(13) = 0.553$, $p =$

0.585), but there was a very strong trend toward increased water consumption in the SCH mice on the QA day ($t(13) = , p = 0.054$). There were no differences at 11 am (ts < 0.843, ps > 0.407). To fully examine the 9 am effect on QA test, we found no significant changes in total fluid intake between drug groups, $t(26) = 1.401$, $p = 0.173$, further proving that SCH's effects were specific to EtOH + quinine intake (Figure 21).

Locomotor Activity

While the small sample sizes in the DLS experiment made locomotor activity difficult to interpret, the effects of SCH were captured more completely in the DMS experiment. While there was no difference between Drug groups during the full 23 hour analysis on the BL or QA tests (ts < 1.4, ps > 0.16, Figure 22), we did see a strong decrease in the first two hours of locomotor activity on the BL ($t(6) = 4.132$, $p = 0.006$) and QA ($t(6) = 8.676$, $p < 0.001$). However, because total fluid intake was unaffected by Drug group and that SCH reduced locomotor activity even when EtOH drinking was not affected, we are confident that the animals were still able to access the sipper tubes and that the reduction in QA drinking in the SCH treated 2W animals was due to a specific effect on conflicted drinking and not locomotor activity.

Because SCH demonstrated a strong locomotor depressant effect, we regressed EtOH/EtOH+ quinine intake onto locomotor activity in the first two hours of the dark cycle to see if locomotor changes drove the change in intake (Figure 23). Locomotor activity counts in the first two hours following the microinjection did not predict EtOH or EtOH + quinine intake ($r^2 = 0.001$) and the nonsignificant regression line bolstered this ($F(1,54) = 0.068$, $p = 0.795$). These findings suggest that locomotor changes due to SCH infusion were not the cause in the reduction of EtOH + quinine intake.

Previous Intra-DMS SCH23390 Infusion Does Not Affect Later Quinine-Adulterated or Quinine-Free Alcohol Intake

To examine any carryover effects surrounding infusion days, we analyzed EtOH/EtOH quinine (Figure 24) and water intake (Figure 25) on the BL and QA test days that mice did not receive a microinjection. There was a strong main effect of test Day ($F(1,130) = 37.43$, $p < 0.001$), as well as a strong Day*Time interaction ($F(5,130) = 10.211$, $p < 0.001$), demonstrating quinine's ability to reduce intake and change drinking patterns. However, Drug had no effect on any variable (Fs < 1.6, ps > 0.22), suggesting no carryover effects from a previous infusion. SCH's effects were not permanent, and only affected behavior when the drug was on board. In addition, water intake did not change between assigned Drug on BL ($t(13) = 0.322$, $p = 0.75$) or QA ($t(13) = 1.113$, $p = 0.276$).

We also assessed locomotor activity on the non-infusion days to ensure that SCH's locomotor depressant effects seen on infusion days did not carry over. The lack of significant change in locomotor activity between SCH and SAL animals on either the BL or QA test days (ts < 0.17, ps > 0.86, Figure 26) confirms our assessment that SCH's effects only occur when the drug is on board. Even though the drug was not administered and therefore not acting in a two-hour window, we still examined the first two hours of the dark cycle and again saw no effect of the previous (or future) Drug treatment (ts < 0.18, ps > 0.86).

DISCUSSION

General Discussion

This study sought to further elucidate the mechanism of compulsive drinking and was, to our knowledge, the first to attempt to reverse this problematic behavior via the dorsal striatum. After a two-week drinking history, cHAP mice became quinine resistant and we were able to reverse this effect with administration of a D1-antagonist in both the DLS and DMS. The antagonist had no effect on unadulterated alcohol drinking and also did not have strong effects on non-infusion days. These findings suggest that following a drinking history, dopamine signaling in the dorsal striatum plays an important role in promoting compulsive alcohol drinking behavior.

Understanding the DLS in Compulsive Drinking

In Aims 1 & 3, we hypothesized that mice that were cannulated in the DLS and given a two-week drinking history would show quinine-resistant alcohol consumption under control conditions, but attenuated quinine-resistance with administration of SCH. Because alcohol naïve animals would have not yet developed compulsive drinking, hypothesized to rely on the DLS, we expected they would be sensitive to quinine adulteration with no effect of SCH. The results of these two aims strongly support our hypotheses that SCH would exclusively attenuate compulsive alcohol intake without affecting non-conflicted drinking.

The most notable finding of these studies was the strong attenuation of EtOH + quinine intake in the 2W SCH animals to the level of alcohol naïve 0W animals at 9 am. While there was no increase in water drinking of the 2W SCH at 9 am (Figure 11) to complement the reduced

EtOH + quinine, there was no change in total fluid intake (Figure 12, right). In addition, we are confident that the effect of SCH was specific to the quinine-resistant drinking, as it did not affect unadulterated alcohol intake (Figure 10, left). Due to incomplete locomotor data from Aim 1, we cannot confidently detail the effect of SCH on movement, but findings from the 0W animals suggest that there was a locomotor depressant effect (Figure 13). However, the absence of change in BL drinking as well as total fluid intake between drug groups (Figure 14) and the lack of association between intake and locomotor activity (Figure 15) suggests that changes due to SCH are not caused by a decrease in locomotor activity.

The results of these experiments uniquely implicate dopamine signaling in the DLS in compulsive drinking, but not unadulterated alcohol drinking. To fully understand what this may mean for addiction research, it is important to understand how the DLS becomes recruited in problematic drinking behaviors. Our findings parallel those of Jonkman et al. (2012) who also found the DLS to be crucial for compulsive cocaine seeking, but did not contribute to unpunished cocaine seeking, even after the operant task had likely become habitual due to extended training. The current findings are able to expand on Jonkman and colleagues to name dopamine as a neurotransmitter of interest.

The implication of the DLS in compulsivity further contributes to the idea that compulsions are related to habits, or an action that is performed without regard for the value of the outcome (Everitt & Robbins, 2005). Because there has been significantly more work published on the neurobiology of habit formation, we based these studies on habit work hypothesizing that the underlying neurobiology may be related. These findings show a strong overlap between the habit and compulsivity fields, highlighting their similarities. Most notably, the present study's conclusions parallel a study by Nelson and Killcross (2013) where systemic

D1-antagonism attenuated a habit following an amphetamine history. While this habit study did not directly punish the instrumental response, as the lithium chloride devaluation procedure was not used on the test day, it is evident that responding despite a change in the value of the reinforcer looks similar to responding in the face of a negative consequence. A similar finding was seen in rats who had developed a habit for sweetened condensed milk; habitual responding for the reinforcer following satiation devaluation was reversed by intra-DLS SCH23390 (Furlong et al., 2014). Therefore, acting without regard for the value of the outcome may be considered to include a negatively valued outcome as well, blurring the line between habit and compulsions.

Because these behaviors are so similar, we can hope to extrapolate these compulsivity findings accordingly. While it was once hypothesized that the shift from goal-directed actions to habits and subsequent compulsions was irreversible via a spiraling dopaminergic pathway through the striatum (Everitt & Robbins, 2005; Haber et al., 2000), some recent studies (Gremel & Costa, 2013) are beginning to suggest otherwise. The ability to reverse a problematic behavior once thought to be relatively permanent is a valuable avenue of research to continue pursuing.

Understanding the DMS in Compulsive Drinking

In Aim 2, we targeted the DMS following two weeks of alcohol drinking and hypothesized we would see no effect of the D1-antagonist, as the DMS likely does not contribute to compulsive drinking. We also predicted a potential decrease in unadulterated alcohol consumption as a result of SCH, as the DMS has been shown to be responsible for goal directed behaviors (Gremel and Costa, 2013, Yin et al., 2005a, Yin et al., 2005b). Unexpectedly, we found that blocking dopamine signaling in the DMS led to an attenuation of EtOH + quinine intake and we did not see an effect of SCH23390 on goal-directed drinking on the baseline day.

Similar to the DLS experiments in Aims 1 & 3, the strong effect of SCH occurred within the first two hours of the QA test day (Figure 18, left). When EtOH + quinine intake was dramatically reduced in the SCH-treated mice, there was a robust increase in water consumption (Figure 20), suggesting that SCH-treated mice equally replaced the quinine-adulterated EtOH with water. SCH's specific effects on quinine-resistant drinking further is supported by no significant change in total fluid intake (Figure 21) and no effect of SCH on BL consumption (Figure 18, right). While there was a dramatic reduction in locomotor activity on both the BL and QA days (Figure 22), the absence of changes in overall avidity suggest that mice were still able to drink in spite of locomotor effects.

However, there was a much more notable finding in the Aim 2 results that blurs the interpretation of these results. While SCH did reduce EtOH + quinine intake at 9 am as compared to SAL control animals, both drug treatment groups showed a significant reduction in intake as compared to the BL day. This was not seen in the DLS 2W SAL mice. Even though there was a marked effect of SCH, it is unclear if these animals had actually developed compulsive drinking after their two-week drinking history. We specifically utilized a two-week drinking history in order to target the earlier stages of compulsive drinking, based on previous work in our lab (Houck et al., under review), hoping that this would be a more malleable behavior to manipulate. Because the addition of quinine did not completely attenuate drinking, we are confident that the mice had enough of a history to begin to develop compulsive drinking; there was enough EtOH + quinine intake to detect a strong reduction with the administration of SCH. Although we cannot call the 2W SAL animals compulsive, they still demonstrated quinine-resistant drinking and the results can be interpreted as such.

Another factor to consider when interpreting these findings is the absence of a 0W DMS

group. While the original goal of this aim was another control group, that D1-antagoism in general will interfere with compulsive drinking and is not specific to the DLS, this null result was not found. Therefore, this aim lacked its own control group and there is no non-manipulated 0W group to compare the 2W groups. Future studies will address this and determine if intra-DMS SCH affects EtOH + quinine intake in alcohol naïve animals that should not have developed compulsive drinking. However at this point, it is imperative to note that there is no appropriate control group to which we can compare the 2W SAL and SCH animals and therefore all conclusions should be made with caution.

There are a number of reasons why a dopamine antagonist in the DMS may lead to an attenuation of quinine-resistant drinking, similarly to the DLS experiment. Primarily, the DMS is not exclusively responsible for goal-directed drinking. The NAc is necessary for the acquisition of goal-directed behaviors (Corbit et al, 2001) and could have contributed to the quinine sensitivity. Similarly, Seif et al. (2013) found that blocking NMDA receptors in the NAc attenuated quinine-resistant, but not unadulterated, alcohol drinking. Seif and colleagues hypothesized that the difference between habit and compulsion is the acknowledgement of the negative outcome and that may require the use of cortical inputs. In their study, this was the PFC-NAc input. One potential explanation for the unexpected findings in the present study is that blocking dopamine receptors in the DMS interfered with cortical inputs into the DMS to result in similar behavioral effects. An additional theory is that habit and compulsions are not as related as the DLS experiments may have led us to believe.

Another reason for our unexpected findings could be the short alcohol history we chose. Following two weeks of alcohol drinking, we hypothesized that the automatic/compulsive behaviors of the DLS would be developed and the mice would be unaffected by blocking

dopamine signaling in the DMS, as the behavior would be reliant on DLS-based mechanisms. However, SCH23390 still had an effect, suggesting that perhaps the two-week drinking history leads to an incomplete development of compulsive drinking, still plastic and able to be manipulated by changes in the ventral striatum. This is supported by the pattern of drinking during the two-week drinking history; although there was a statistically significant escalation of EtOH intake over two-week history (Figure 6), the effect size was modest. Perhaps the absence of strong escalation of intake over two weeks contributed to an incomplete transition to compulsivity. This also supports the finding that the 2W SAL animals were not completely insensitive to the addition of quinine when cannulated in the DMS.

Targeting D1-receptors in the DMS was intended to manipulate the spiraling dopaminergic pathway implicated in habit expression, but a recent study provides an additional function of these receptors. Nguyen et al. (2019) found that a microinjection of SCH23390 into the DMS, but not the DLS, increased avoidance of an aversive arm in a maze, suggesting increased anxiety toward negative stimuli. Therefore, it is possible that SCH23390 in the present study did not interfere with compulsivity, as hypothesized in the DLS study, but instead heightened sensitivity to the aversive quinine. Future studies could look at behavior in the elevated plus maze in conjunction with quinine drinking to determine if D1-antagonism in the DMS truly interferes with compulsive drinking, or is just an amplification of anxiety toward aversive consequences.

Finally, SCH working in the DLS in addition to the DMS could have contributed to the similarities between the DLS and DMS studies. Although we chose a small, but concentrated infusion volume, it is possible that SCH23390 could have leaked into the DLS, as there are no defined boundaries in the region. Even though we excluded mice with placements too lateral to

be considered DMS, it is possible that the drug may have diffused laterally into the DLS. Further, there are no distinct boundaries between the two structures; instead there is an increasing gradient of MSNs moving laterally that correspond to more automatic behaviors (Voorn et al. 2004). Future studies may be able to better target the DMS in order to get more precise results.

Implications and Future Directions

The implications of this study are great and will add to the scarce studies examining compulsive alcohol intake, a detrimental phenotype seen in patients with AUD. To our knowledge, it is the first study to manipulate the dorsal striatum to affect compulsive drinking. Because the effects were specific to the quinine-adulterated alcohol and not generalized to unadulterated alcohol or total fluid, we are confident that the DLS plays a significant role in compulsive drinking. This supports the idea that compulsivity is closely related to habit formation processes, a behavior that has been heavily studied.

In addition, these findings have an even stronger impact on the field. When coupled with the unexpected findings from Seif et al. (2013), really give a new perspective on the neurobiology of compulsive drinking and how it may relate to habit. The habit literature implicates the NAc and DMS in goal-directed behaviors, but both of these studies found that reducing glutamatergic or dopaminergic activity, respectively, resulted in reduced quinine-resistant drinking. It is imperative to better understand how the striatum changes between habitual and compulsive drinking to cause these changes and, if Seif's proposed cortical mechanism is correct, how other structures may influence the striatum.

As a study with rich translational value, specifically targeting conflicted drinking is extremely important in developing potential therapeutics. Compulsive drinking can only be

measured when there is a conflict, such as fear of legal, social, or physical consequences. To be able to restore the ability to stop drinking in problematic situations to a majority of patients who currently are unable (Ilgen et al., 2008) could significantly improve quality of life, as an intervention can restore social, non-problematic drinking to this population. Pharmacological interventions attenuating specifically conflicted drinking without affecting non-problematic consumption may prove to be a successful treatment for patients with alcohol use disorder, especially due to non-compliance with current treatments (Fuller and Gordis, 2004).

The findings of this study provide a strong foundation for a number of future studies. Primarily, it is evident that more experiments need to be run to better understand the neural mechanisms underlying compulsive drinking. While work from habit studies has been very helpful in informing directions of these experiments, they are likely distinct processes with a number of commonalities. The striatum has been routinely shown to be important for the expression of compulsive drinking, but it is a large and diverse structure. The striatonigrostriatal pathway is one large component of the striatum and these findings implicate dopamine directly, but it is possible to manipulate dopamine transmission in other, more indirect ways.

This study hypothesized that the DLS, following two weeks of alcohol consumption, would have increased activity. This does not mean that dopamine release ceases in the ventral striatum, but instead the SN-DLS pathway is strengthened such that its function may override the more goal-directed function of the ventral striatum. Thus, we can potentially manipulate compulsivity in one of two ways: reduce activity in the DLS (as done in this study) or increase activity in the ventral striatum in an attempt to overpower the DLS.

Even further, there are various neurotransmitter systems that regulate the activity of the striatum. For example, manipulating glutamatergic inputs into the striatum (i.e. OFC, PFC,

hippocampus, amygdala) may prove to be useful targets without motor side effects seen with dopamine manipulation. NMDA receptors in the NAc are necessary for compulsive drinking (Seif et al., 2013), suggesting that glutamate also plays a role and can affect behavioral outputs. Gremel et al. (2016) were able to manipulate OFC-DMS projections via the endocannabinoid system to reverse habit formation. Expanding to other brain regions and neurotransmitter systems will help to characterize the mechanism of compulsive drinking

Limitations & Conclusions

While considering the findings of this study, it is important to keep some limitations in mind. Quinine resistance is a strong model of drinking in spite of negative consequences, (Hopf and Lesscher, 2014) but does not fully encapsulate the consequences seen in human patients with alcohol use disorder. As previously mentioned, these negative effects can occur either before, during, or following consumption of alcohol. Our model's aversive effects took place during the consumption phase, which can occur in human subjects (i.e. drinking a non-preferred beverage or consuming mouthwash in place of ethanol) but are not as common as post-consummatory consequences. Frequently humans with AUDs experience adverse outcomes, such as legal, social, or physical consequences, after they drink. Future studies may attempt to model and assess the potential differences as a result when these consequences occur and if they affect the generalizability of these results.

An inherent problem with interfering with D1-receptor functioning in the striatum is the risk of interrupting locomotor behavior. Originally, we chose lower doses of SCH23390 to avoid these undesired side effects, but they were insufficient to produce effects on alcohol drinking. For this reason, we used a higher dose knowing the potential side effects, but relied on locomotor data to ensure that the SCH23390 did not completely prohibit mice from accessing the sipper

tubes which would have affected these results. The home cage locomotor system was valuable in the DMS experiments, where group sizes were adequate to assess the depressant effect of SCH, but there were insufficient mice in the 2W DLS groups to allow us to examine SCH effects. However, a 0W group would be valuable to bolster those conclusions. While we were able to use water and total fluid intake to validate that the SCH23390 did not immobilize the animals completely, future studies are needed to better map the time course and magnitude of SCH's effects.

Especially in Aim 2, we considered that damage caused by insertion of the cannulae into the DMS may decrease alcohol self-administration during the 2BC drinking history by interfering with a structure involved in goal-directed behavior. Reduced alcohol intake could have impacted the strength of the 2BC drinking history and slowed the shift to compulsive drinking. However, DMS-cannulated mice were drinking at equal levels to DLS-cannulated mice in Aim 1 (Figure 7), as well as non-cannulated cHAP mice (Matson & Grahame, 2013; Houck et al., under review). In addition, we were able to detect quinine-resistant drinking in the SAL-treated mice, strengthening the argument that surgery did not completely ablate the function of the DMS. We believe that because the NAc was preserved in these animals, they were able to learn non-conflicted drinking without impairments and the cannulation did not interfere with the development and strengthening of the spiraling dopaminergic pathway to the DLS throughout the two-week drinking history.

Another factor to consider is the use of cHAP mice. These mice are selectively bred for high alcohol consumption and serve as an animal model of genetic contributions to alcohol use disorder, which was a significant contributing factor as to why we chose to use them in these experiments. However, these mice are not a commonly used line and therefore all experiments

performed here were based on work in other rodent species. Although doses of SCH and concentrations of quinine were optimized to this underused species, these mice may differ from previously used rodent models and so additional research assessing inconsistencies between strains and species would be useful in continuing evaluation of these results. In addition, due to their unique breeding history, there may be structural and functional differences within their brains that may contribute to the findings seen here. Future work should be continued with these animals, as they uniquely and almost exclusively make contact with the high voluntary alcohol consumption and subsequent BALs seen in humans with AUDs.

In summary, the current findings implicate D1-receptors in the DLS and DMS in compulsive, but not non-conflicted, alcohol intake. This work elucidating the role of the dorsal striatum in compulsive drinking serves to help the addiction research community better understand the neural mechanisms underlying alcohol in spite of negative consequences. Future studies should examine other neurotransmitter systems within the striatum that may also contribute to restoring goal-directed drinking, as well as more precisely determine the roles of different brain structures in the acquisition of compulsivity. These important studies may eventually help provide therapeutic benefit to patients with AUDs and restore non-disruptive drinking behaviors.

TABLE

Table 1. Attrition.

This table details the number of animals lost during the experiments. Although data are included from the Pilot experiment, cannula placements were unable to be verified. The entirety of Aim 1, Cohorts 2 & 3 were not included due to experimental errors.

Experiment	Group	Number of Mice at Start	Number of Mice Dropped	Reason
SCH Pilot	SAL	13	13	All brains were lost before histological cannulae placement verification.
	SCH Lo	14	14	
	SCH Hi	13	13	
Aim 1 – 2W DLS	SAL	11	1	Died on QA1 (no infusion)
	SCH	11	4	Missed cannulae placements
	Cohorts 2 & 3	30	30	Wrong quinine concentration/infusion pump failure
Aim 2 – 2W DMS	SAL	14	0	
	SCH	15	1	Missed cannulae placements
Aim 3 – 0W DLS	SAL	10	0	
	SCH	11	2	Missed cannulae placements

FIGURES

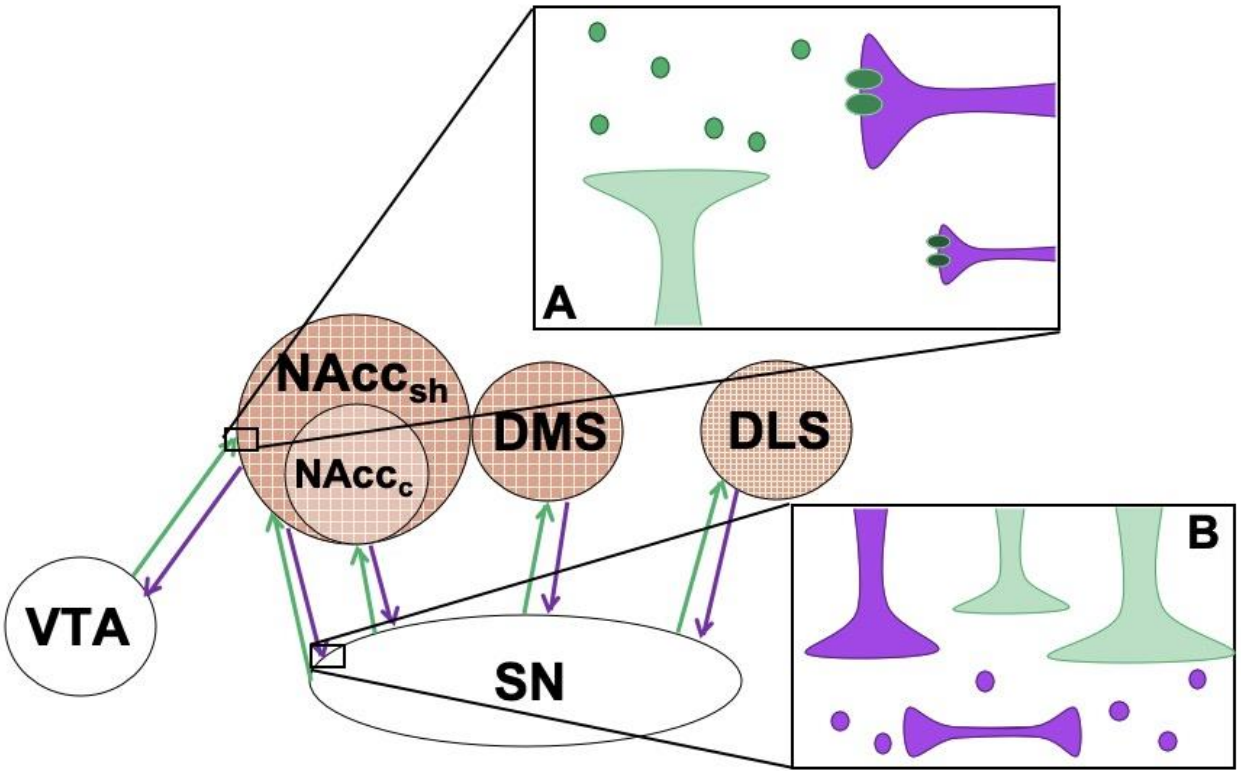


Figure 1. The Spiraling Striatonigrostriatal Dopamine System

The red regions are the striatum: area of interest, with the pattern indicating the density of MSNs. Green arrows and neurons are dopaminergic pathways, with light green receptors being D1 receptors and dark green receptors indicating D2 receptors. Purple are GABAergic MSNs. Magnification of squared spaces shows a more detailed view of the region at the synaptic level. **(A)** illustrates dopamine release from either the VTA (as pictured) or the SN (other green arrows in diagram). DA acts at the D1-receptor pictured on a GABAergic MSN. **(B)** shows GABA release in the SN from the NAc (as pictured) or dorsal striatum (other purple arrows in diagram). With continued DA release, this "feed-forward" spiral strengthens DA projections in the dorsal striatum. Figure is not drawn to scale. ($NAcc_{sh}$: nucleus accumbens shell, $NAcc_{core}$: nucleus accumbens core, DMS: dorsomedial striatum, DLS: dorsolateral striatum, VTA: ventral tegmental area, SN: substantia nigra). Adapted from Haber et al., 2000.

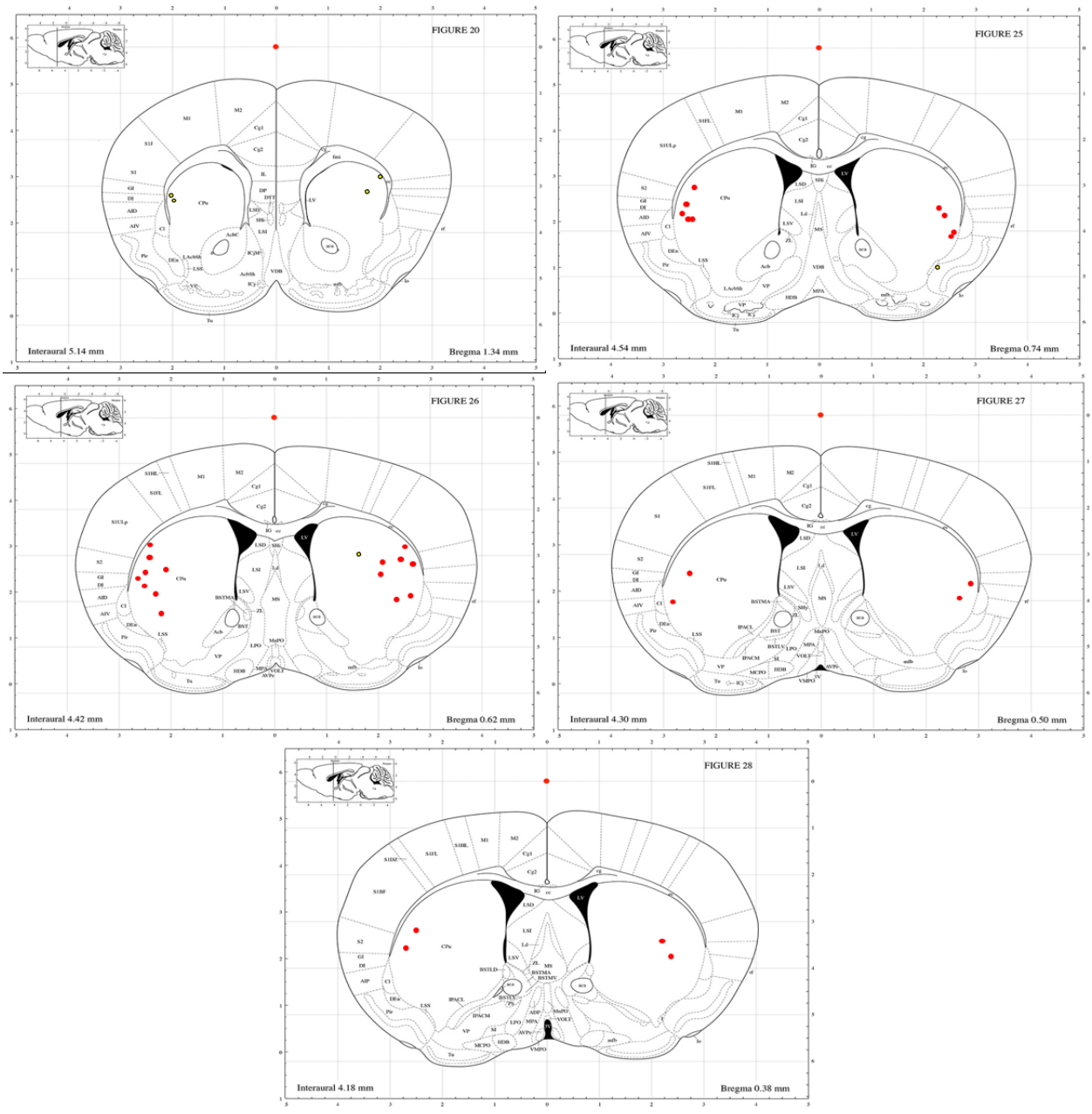


Figure 2. Aim 1 Hits

Intended coordinates were A/P: +0.38 mm, M/L: +/- 2.5 mm, D/V: -3.0 mm. Red dots indicate hits and yellow indicate misses. A mouse was excluded from the experiment if it did not have a bilateral hit (bilateral misses and unilateral hits were excluded). Adapted from Paxinos & Franklin Mouse Atlas.

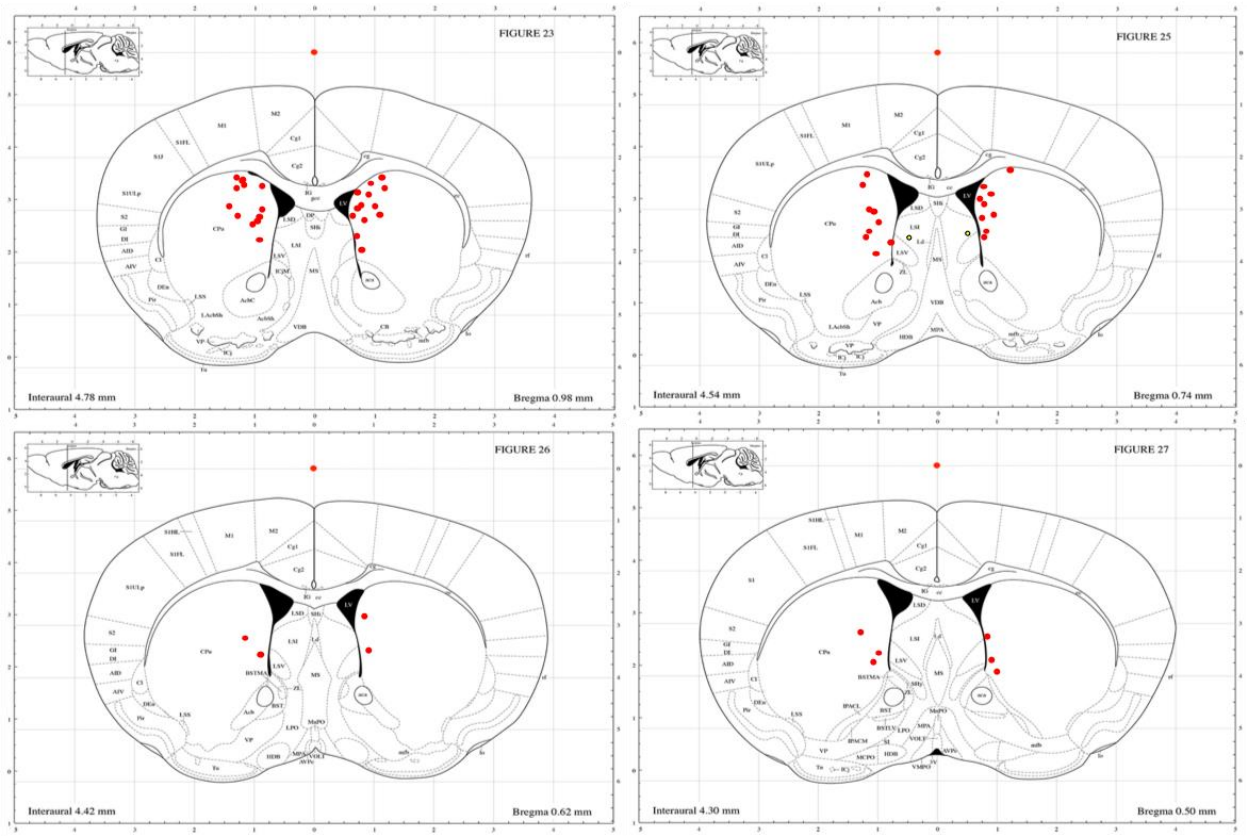


Figure 3. Aim 2 Hits

Intended coordinates were A/P: +0.50 mm, M/L: +/- 1.25 mm, D/V: -2.75 mm. Red dots indicate hits and yellow indicate misses. A mouse was excluded from the experiment if it did not have a bilateral hit (bilateral misses and unilateral hits were excluded). Adapted from Paxinos & Franklin Mouse Atlas.

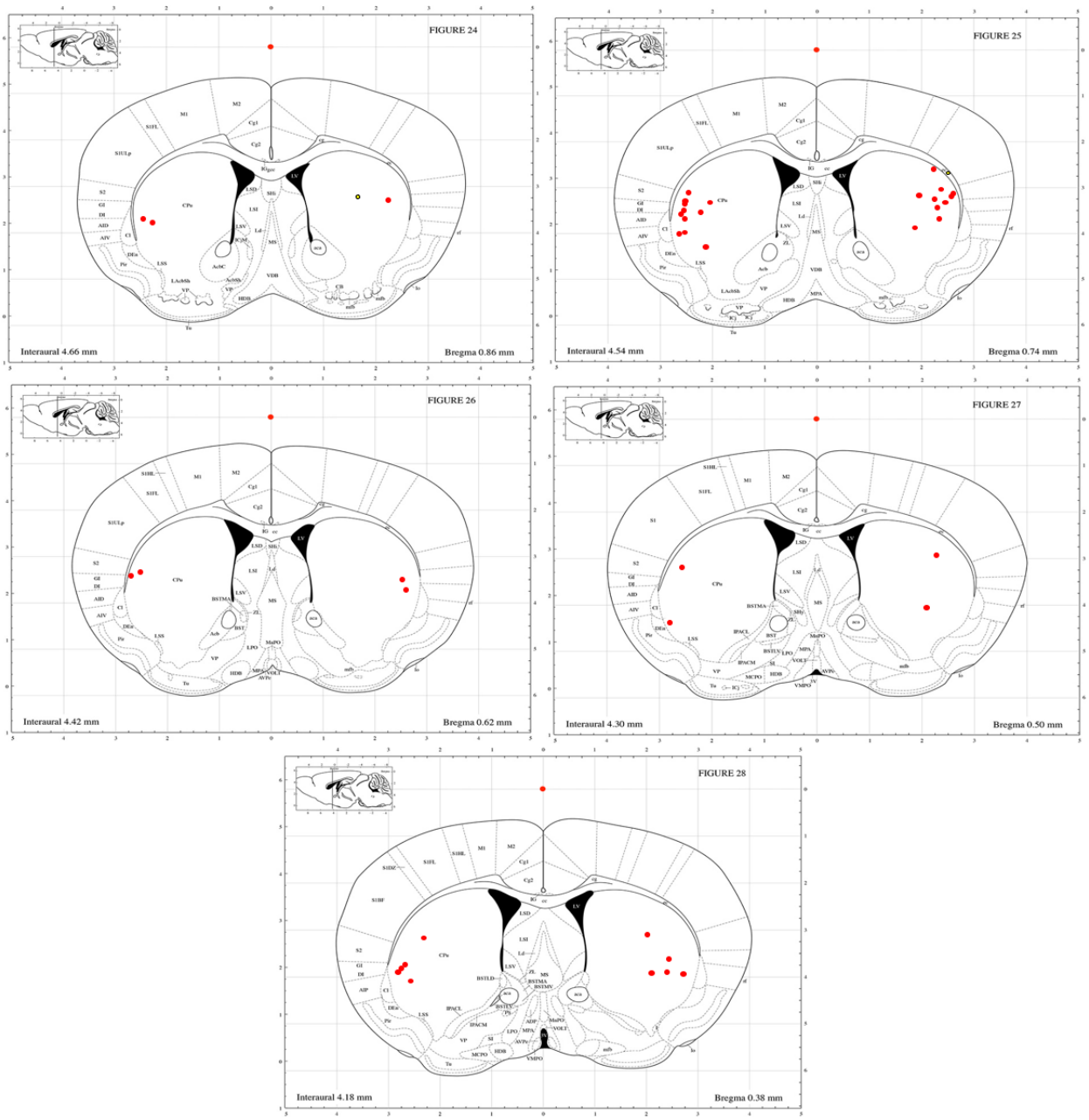


Figure 4. Aim 3 Hits

Intended coordinates were A/P: +0.38 mm, M/L: +/- 2.5 mm, D/V: -3.0 mm. Red dots indicate hits and yellow indicate misses. A mouse was excluded from the experiment if it did not have a bilateral hit (bilateral misses and unilateral hits were excluded). Adapted from Paxinos & Franklin Mouse Atlas.

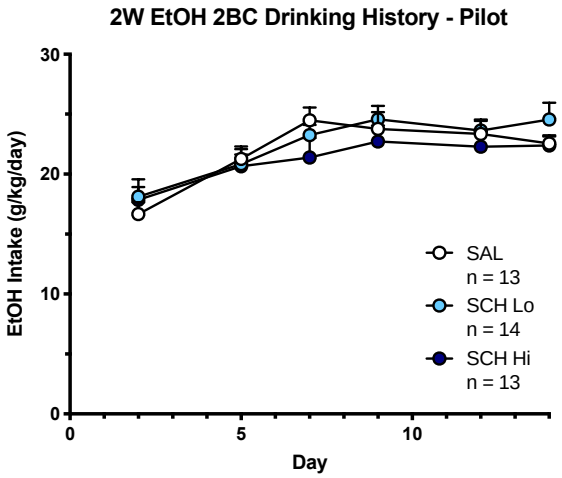


Figure 5. EtOH Intake During 2 Week 2BC Access – Pilot Experiment

Average daily intakes for each Drug treatment group (Drug is a pseudovariable). Main effect of Time ($p < 0.001$) demonstrating escalation across the two-week period. (SCH Lo = 0.25µg/0.25µL/side; SCH Hi = 0.50 µg/0.25µL/side)

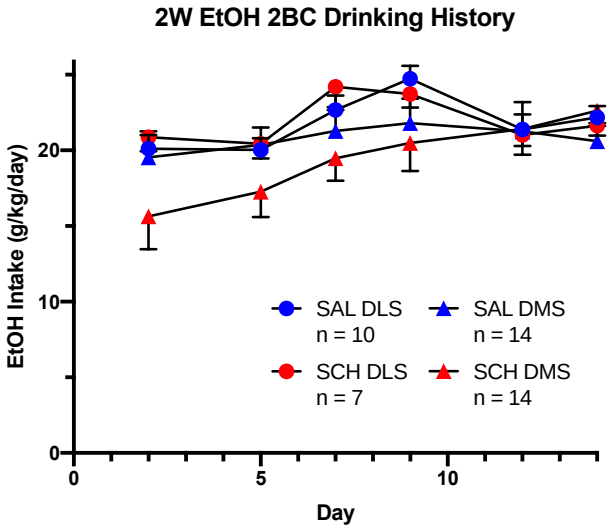


Figure 6. EtOH Intake During 2 Week 2BC Access – Aims 1 & 2
Average daily intakes for each Drug treatment group (Drug is a pseudovariable). Main effect of Time ($p < 0.001$) demonstrating escalation across the two-week period.

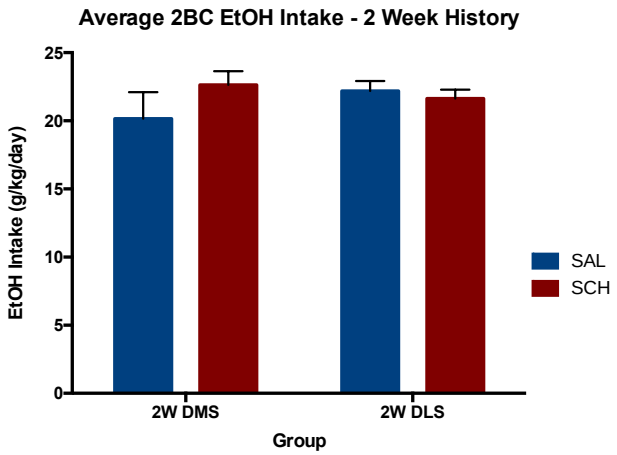


Figure 7. Day 14 EtOH Intake by Future Drug Treatment

There were no baseline differences between future drug treatment or brain region cannulated (*p*s > 0.2).

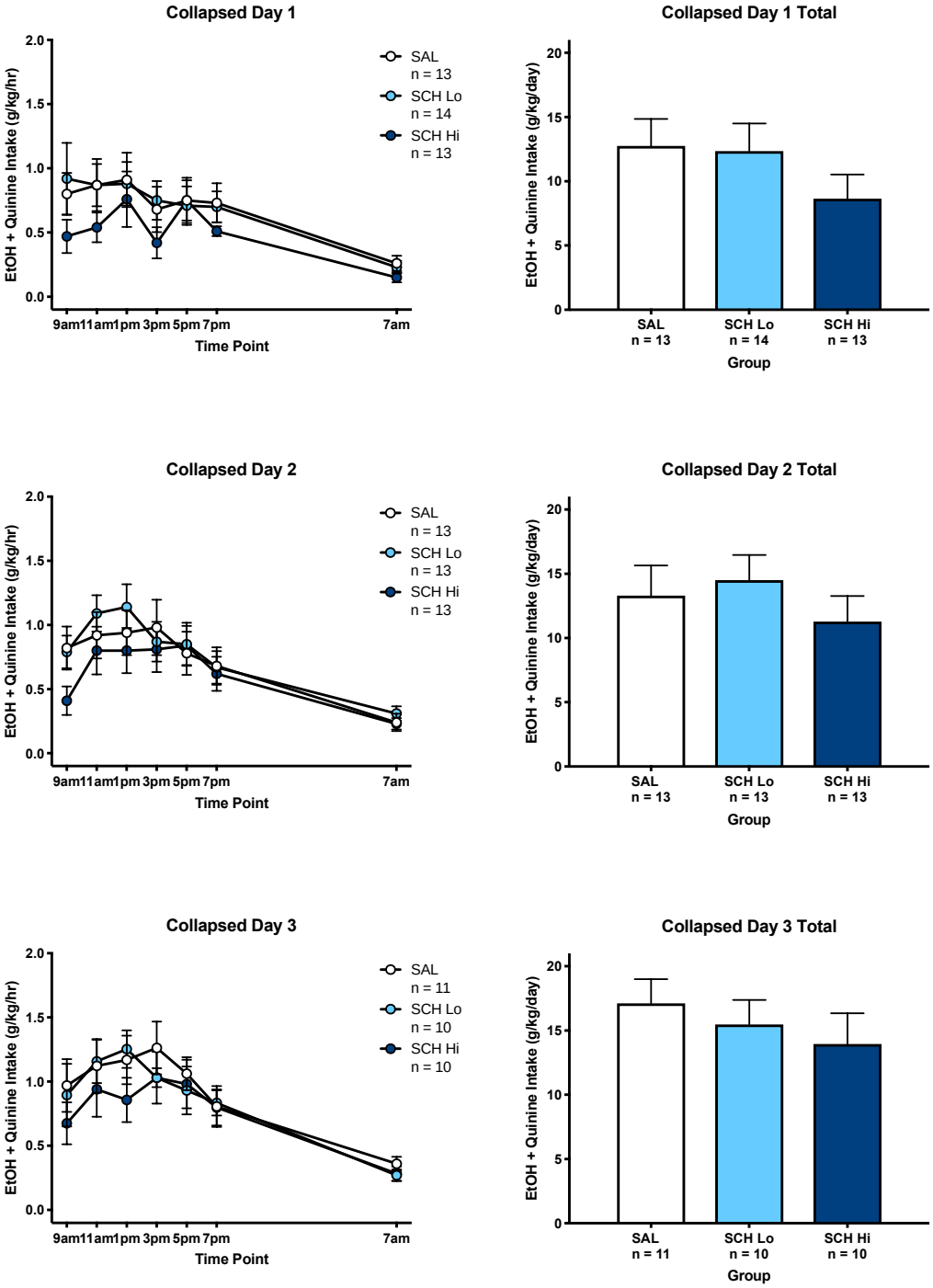


Figure 8. Pilot Days QA1 – QA3 – Total EtOH + Quinine Intake

Left column shows the bihourly intakes throughout the dark cycle. There was no effect of Drug or interaction with Time, so the right column shows the total EtOH + Quinine intake in the 24 hour test day.

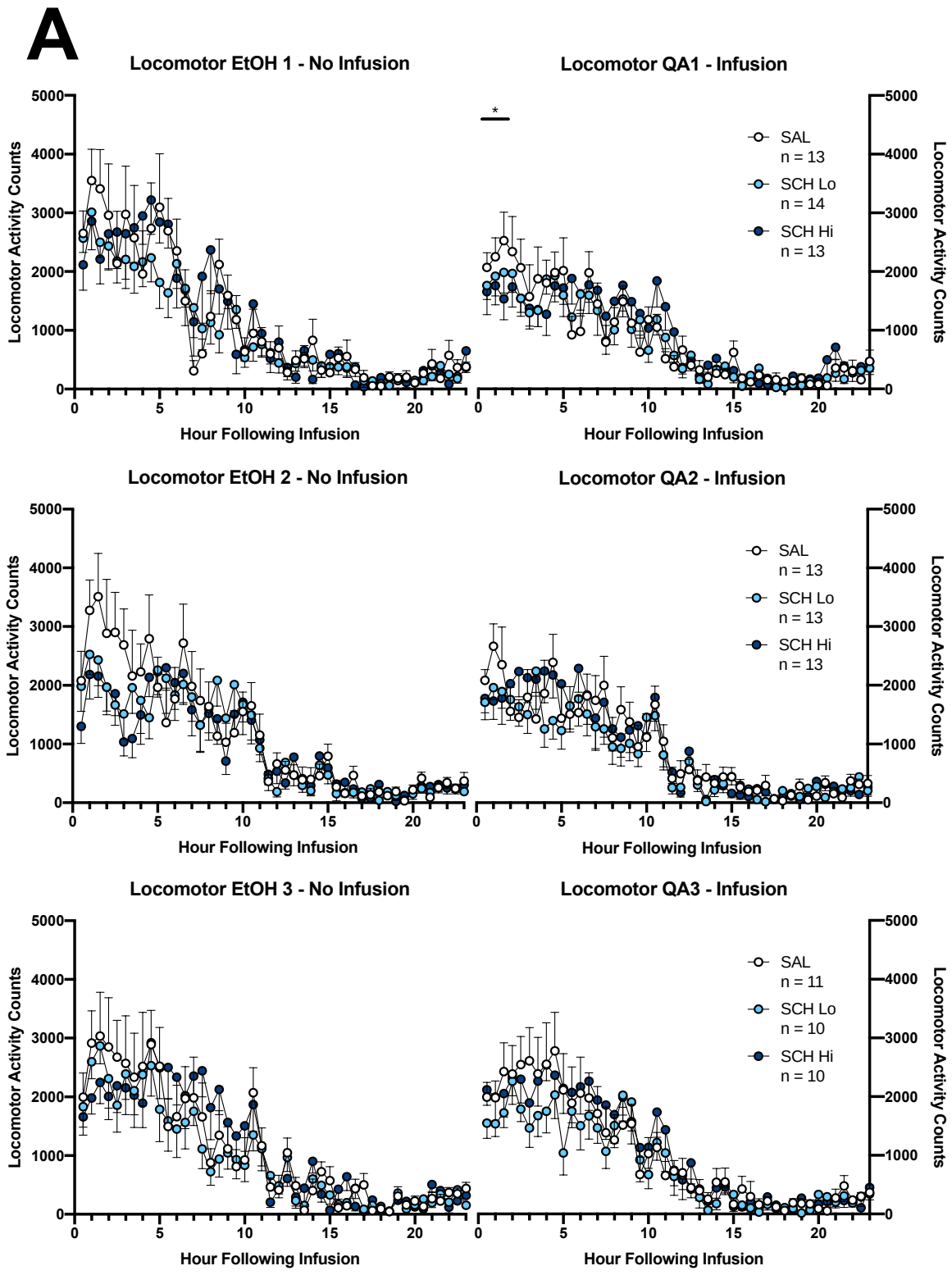
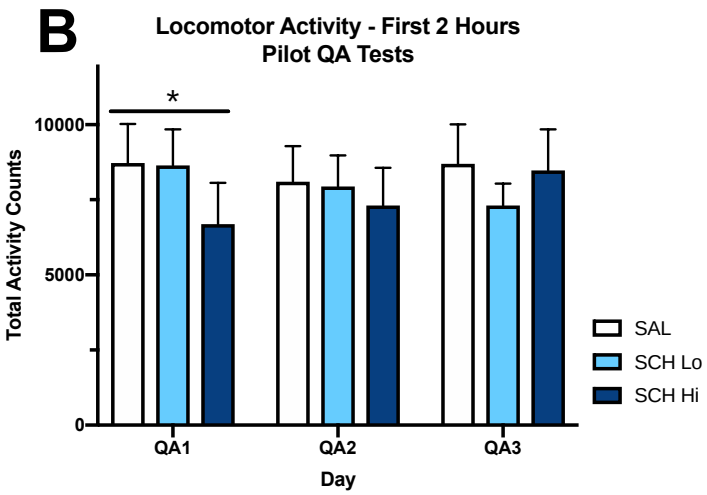


Figure 9. Pilot Experiment DLS-Cannulated Mice Locomotor Activity During No Infusion EtOH (left) and Infused QA (right) on QA 1 – 3 (top to bottom)

(A) There were no significant Drug effects across the entire 24-h period. Yellow indicates first 2 hours following drug infusion. (B) There was a main effect of Drug on the first two hours of QA1 only (*$p < 0.001$).

Figure 9 continued



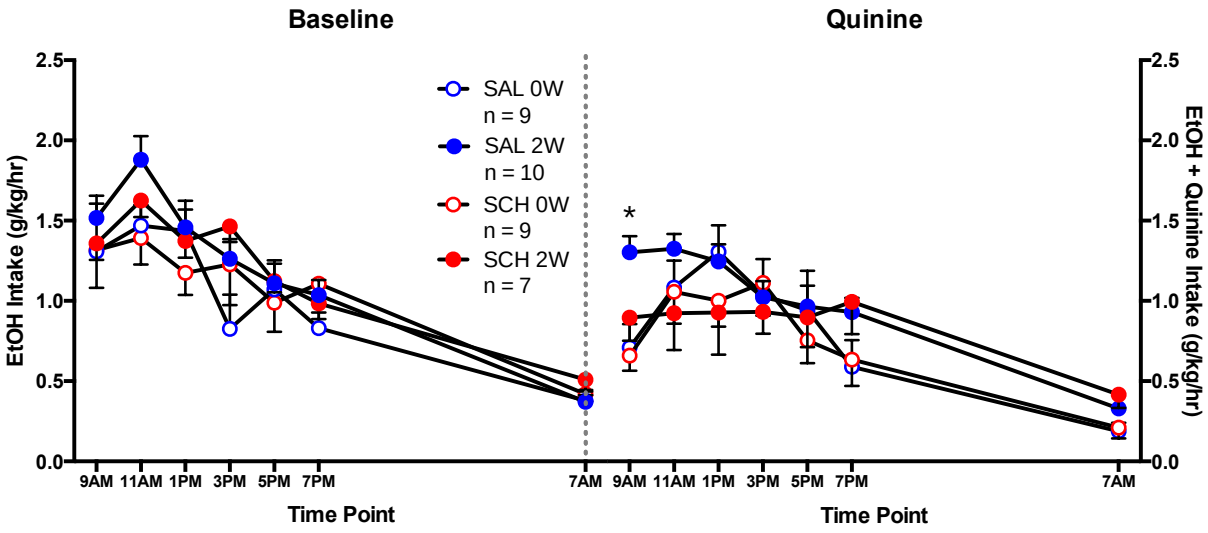


Figure 10. DLS-Cannulated Mice Total EtOH (BL, left) and EtOH + Quinine Intake (QA, right) on Infusion Test Days

There was no effect of Drug or History or interaction with Time on either test day. Main effect of Time for both days. Significant difference between 2W SAL and all other groups at 9 am on QA, (*$p < 0.03$.).

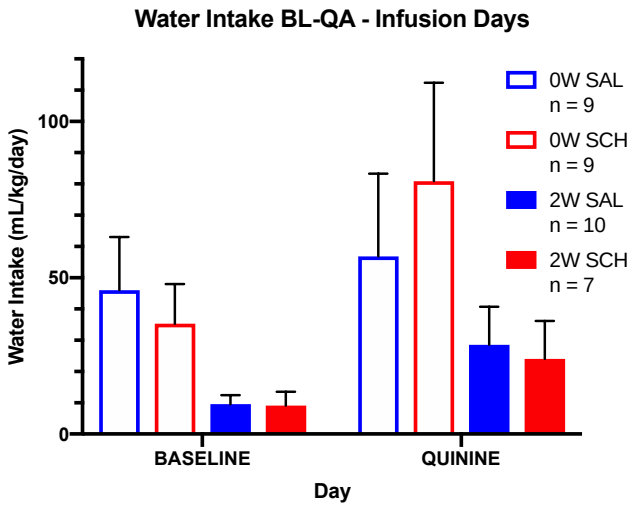


Figure 11. DLS-Cannulated Mice Total Water Intake on BL (left) and QA (right) on Infusion Test Days

There was no effect of Drug on either day.

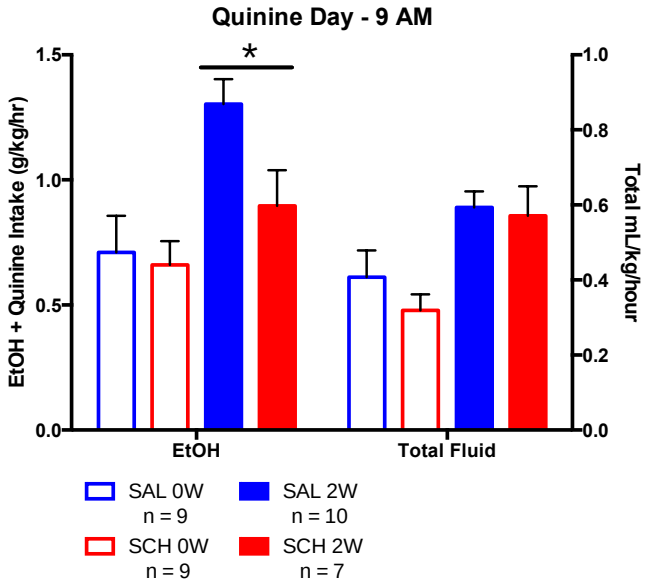


Figure 12. DLS-Cannulated Mice EtOH+ Quinine (left) and Total Fluid (right) Intake on QA Infusion Test Day

While there was an increase in EtOH + quinine intake in the 2W SAL mice (*$p < 0.03$), there was no difference in total fluid intake.

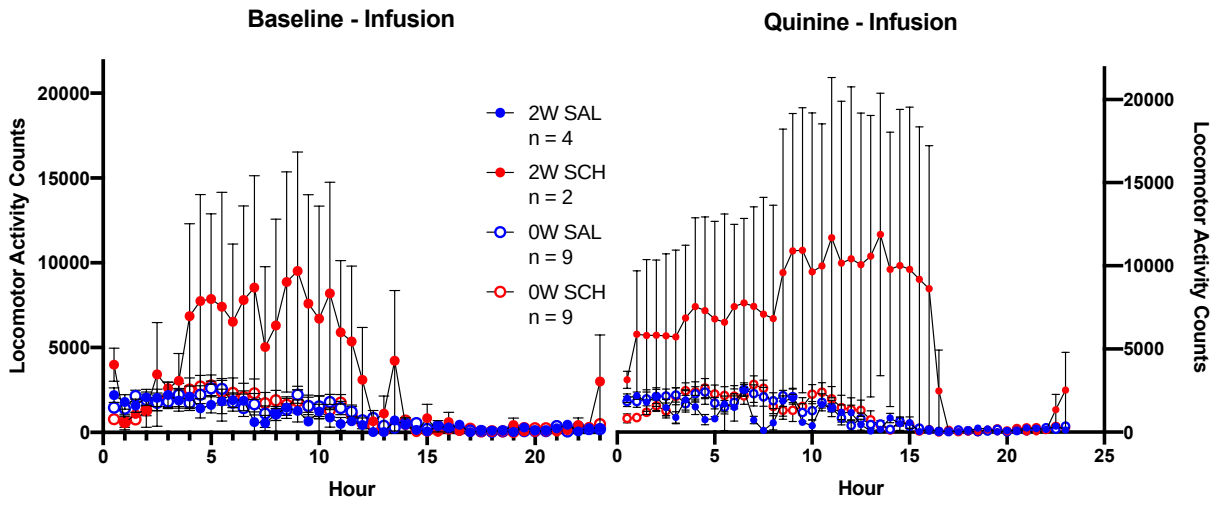


Figure 13. DLS-Cannulated Mice Locomotor Activity during BL (left) and QA (right) on Infusion Test Days

There was no effect of Drug on locomotor activity, but this is likely due to a small 2W SCH group size.

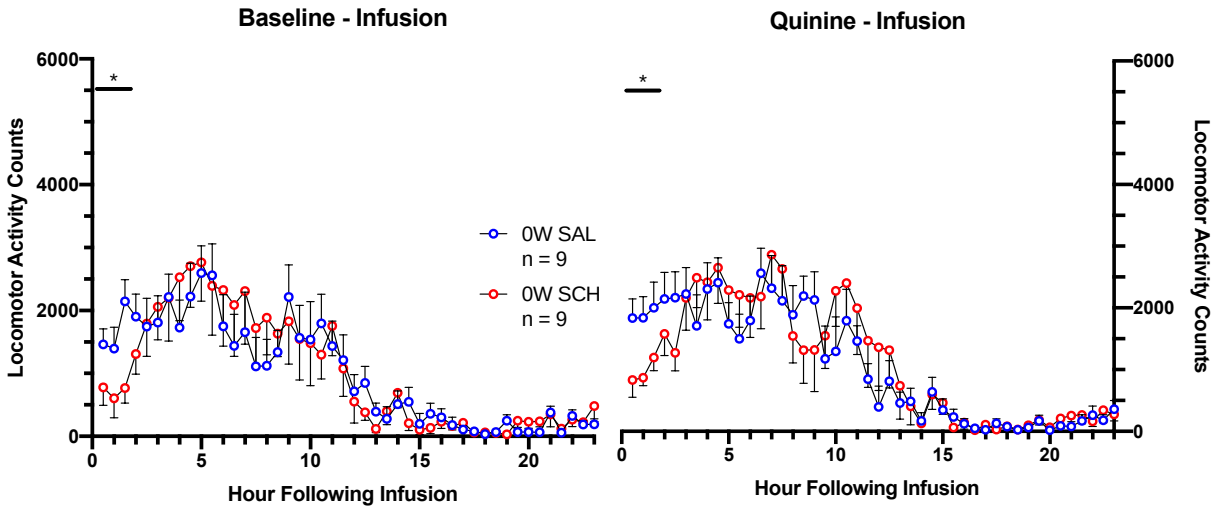


Figure 14. DLS-Cannulated Mice Locomotor Activity during BL (left) and QA (right) on Infusion Test Days–0W ONLY

Analysis of the infusion of SCH in 0W animals only showed that SCH significantly reduced locomotor activity in the first two hours of both the BL and QA test days (*ps < 0.003).

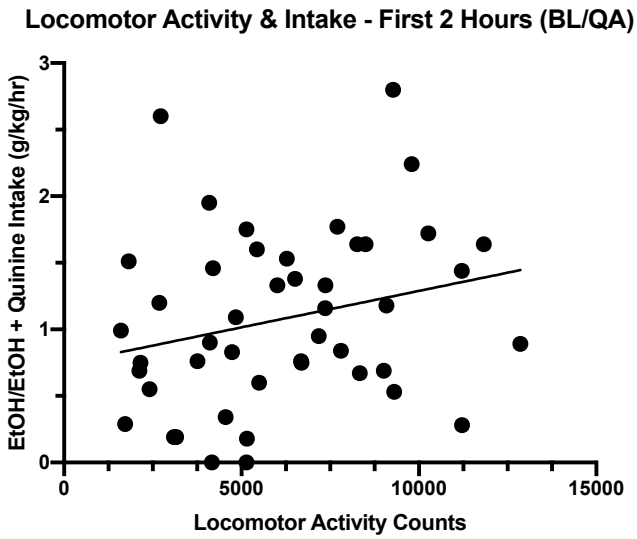


Figure 15. Locomotor Activity Does Not Predict EtOH/EtOH + Quinine Intake
In the first two hours of both the BL and QA day, locomotor activity did not predict EtOH (BL) or EtOH + quinine (QA) intake on infusion days.

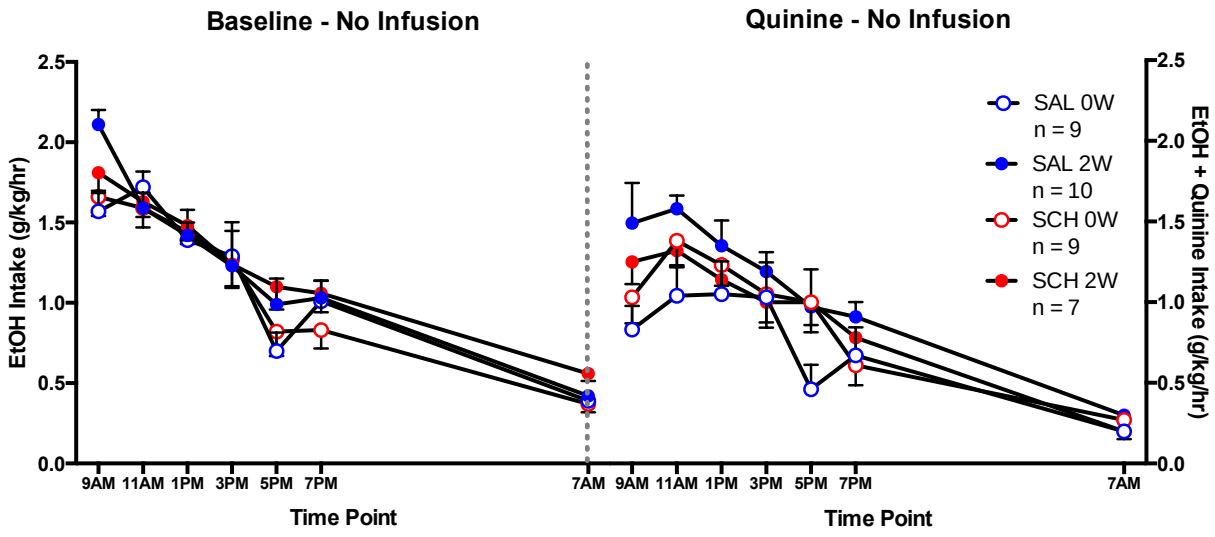


Figure 16. DLS-Cannulated Mice Total EtOH (BL, left) and EtOH + Quinine Intake (QA, right) for Non-Infusion Test Days

There was no effect of Drug or interaction with Time on either test day. Main effect of Time for both days.

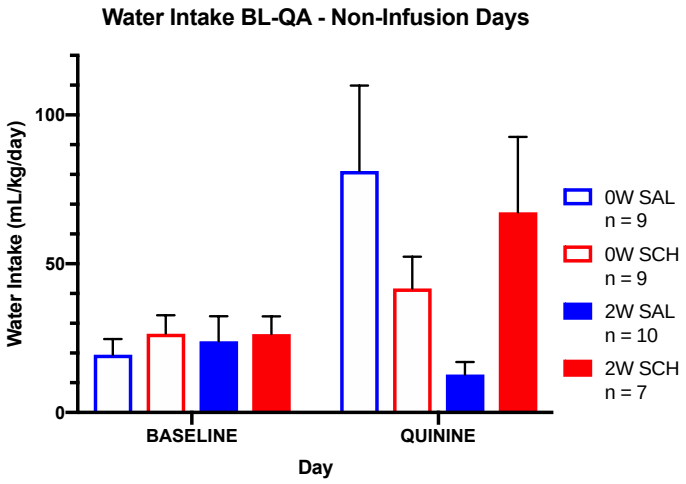


Figure 17. DLS-Cannulated Mice Total Water Intake on BL (left) and QA (right) on Non-Infusion Test Days

There were no effects on the BL test day, but a Drug*History interaction on the QA test day.

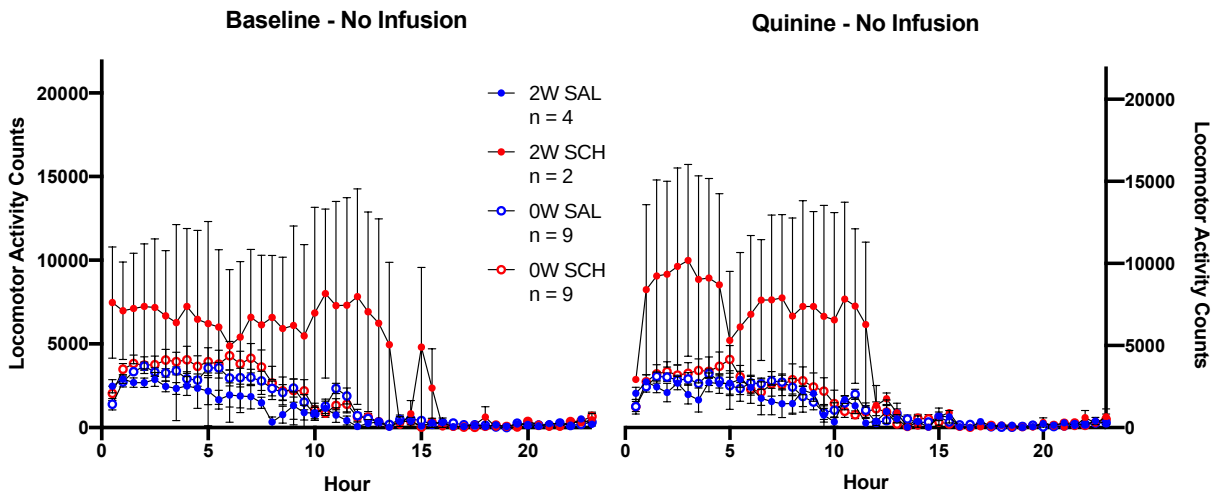


Figure 18. DLS-Cannulated Mice Locomotor Activity during BL (left) and QA (right) on Non-Infusion Test Day

There was no effect of Drug, as no infusions were administered on these days.

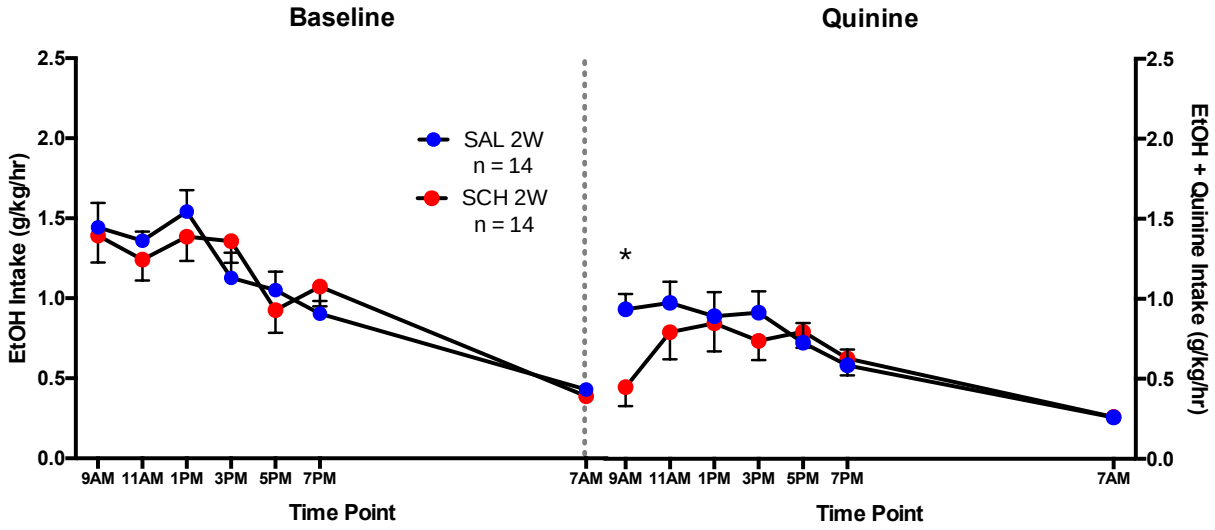


Figure 19. DMS-Cannulated Mice EtOH (BL, left) and EtOH + Quinine Intake (QA, right) on Infusion Test Days

There was no effect of Drug or interaction with Time on either test day. Main effect of Time for both days. Significant difference between SAL and SCH at 9 am on QA,*$p <$ 0.003

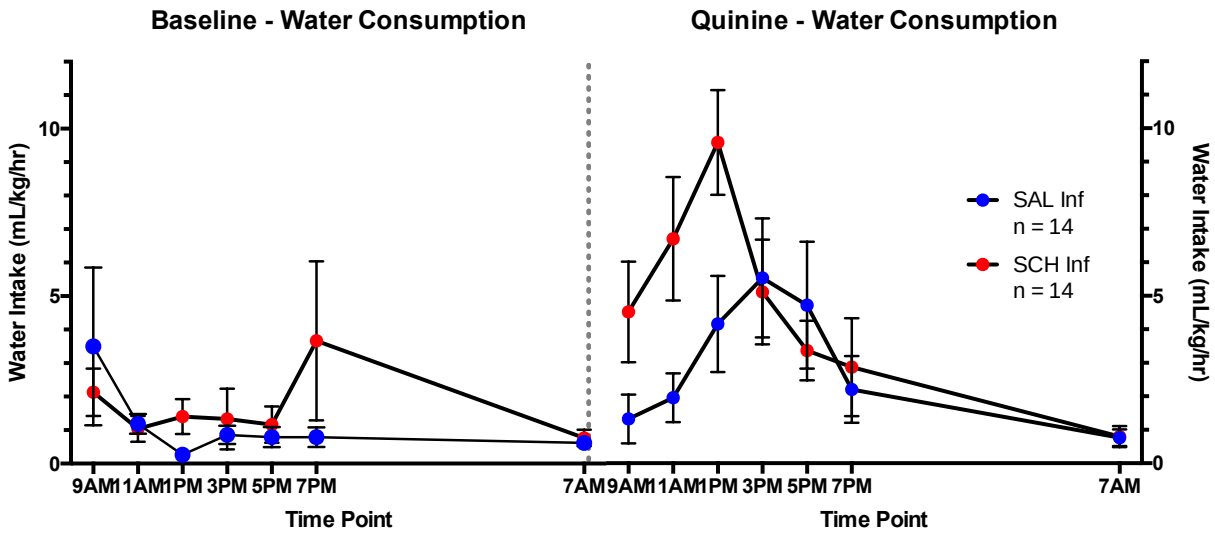


Figure 20. DMS-Cannulated Mice Total Water Intake Infusion Test Days (BL, left; QA, right) There was a main effect of Time on the BL test day as well as a Time*Drug interaction on the QA test day, as demonstrated by SCH mice drinking more water, $p = 0.01$.

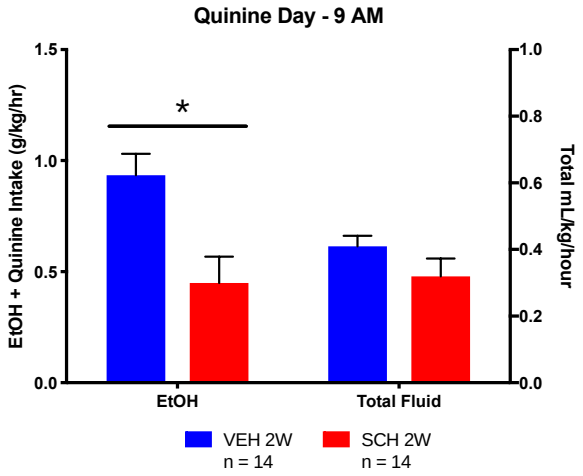


Figure 21. DMS-Cannulated Mice EtOH+ Quinine (left) and Total Fluid (right) Intake on QA Infusion Test Day

While there was an increase in EtOH + quinine intake in the SAL and SCH mice (*$p <$ 0.03), there was no difference in total fluid intake.

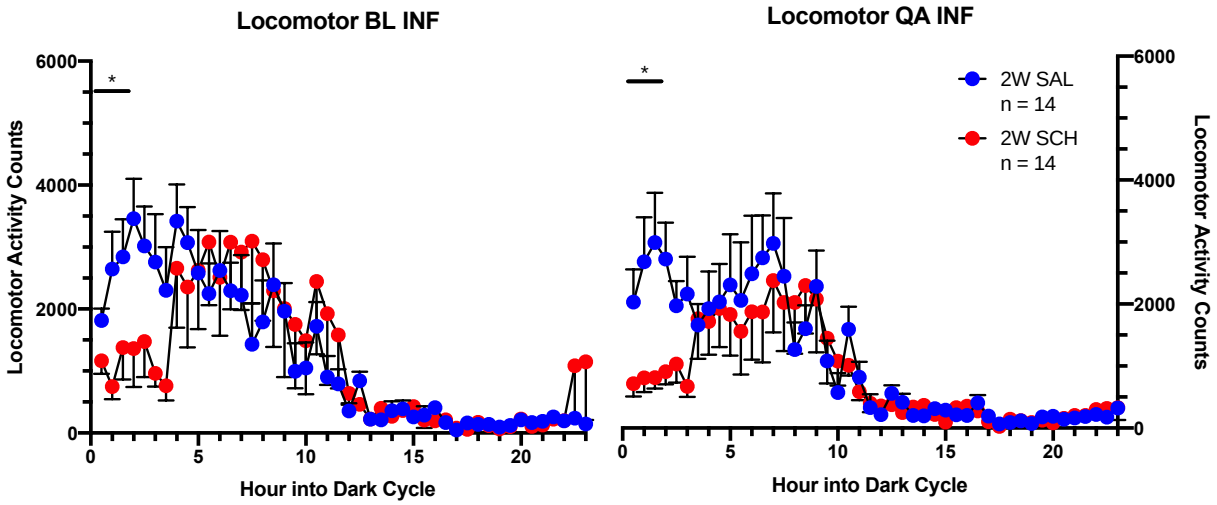


Figure 22. DMS-Cannulated Mice Locomotor Activity during BL (left) and QA (right) on Infusion Test Days

SCH significantly reduced locomotor activity in the first two hours on both test days (*ps < 0.006).

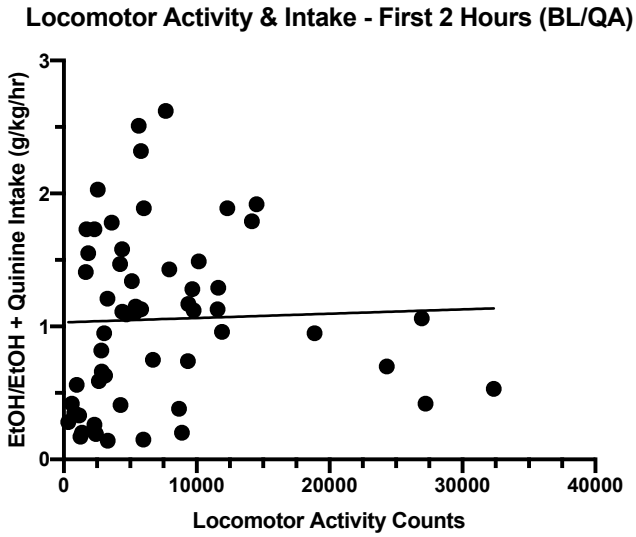


Figure 23. Locomotor Activity Does Not Predict EtOH/EtOH + Quinine Intake
In the first two hours of both the BL and QA day, locomotor activity did not predict EtOH (BL) or EtOH + quinine (QA) intake on infusion days.

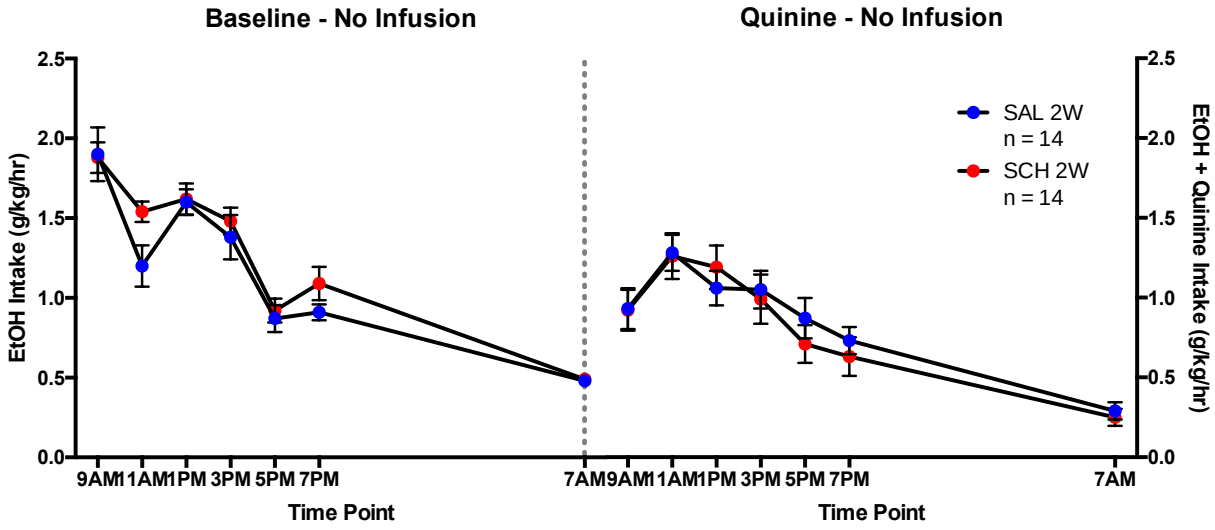


Figure 24. DMS-Cannulated Mice EtOH (BL, left) and EtOH + Quinine Intake (QA, right) on Non-Infusion Test Days

There was no effect of Drug or interaction with Time on either test day. Main effect of Time for both days.

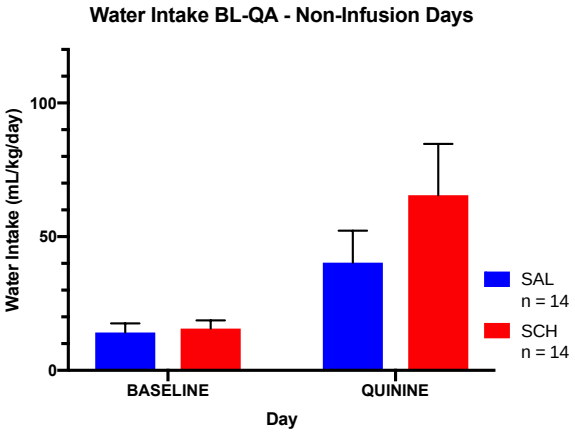


Figure 25. DMS-Cannulated Mice Total Water Intake on BL (left) and QA (right) on Non-Infusion Test Days

There was no effect of Drug on either day ($p > 0.05$).

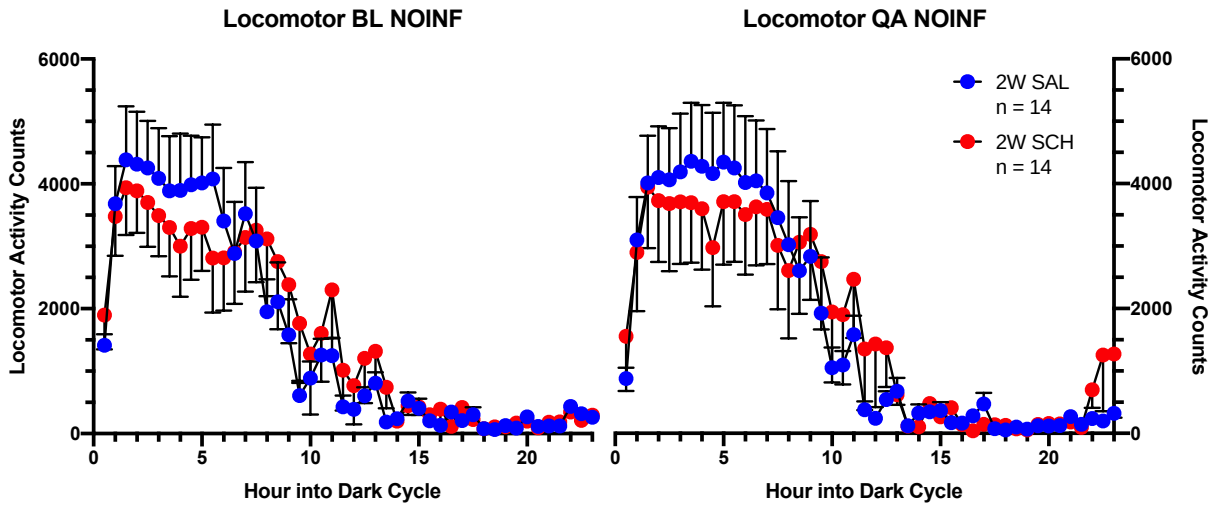


Figure 26. DMS-Cannulated Mice Locomotor Activity during BL (left) and QA (right) on Non-Infusion Test Days

No Drug group differences ($p > 0.05$).

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