# Co-administration of haloperidol does not alter anesthetic ketamine-induced go/no-go reversal learning impairments in rats

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# Abstract

Ketamine, an NDMA receptor antagonist drug, is used as a general anesthetic in humans and animals because of its wide margin of safety and limited respiratory effects. However, the long-term effects of anesthetic ketamine exposure on behavior are largely unknown. Previously, our lab has found that three exposures to anesthetic ketamine (100 mg/kg) in rats improves go/no-go reversal learning, a task used to model behavioral flexibility. In the present study we sought to block the alterations in go/no-go reversal learning caused by anesthetic ketamine exposure by co-administering ketamine with haloperidol, a dopamine D2 receptor antagonist and classical anti-psychotic. In the study rats received intraperitoneal injections of haloperidol (1 mg/kg or 10 mg/kg) or vehicle co-administered with ketamine or saline to determine whether haloperidol co-administration could protect against ketamine-induced alterations in go/no-go reversal learning. Twelve days following the final injection the rats began go/no-go discrimination and reversal learning training. Haloperidol alone had no effects on discrimination or reversal learning. In contrast to our previous findings, ketamine impaired go/no-go reversal learning and haloperidol did not block this effect. It is possible that methodological differences between the present study and previous studies from our lab may have contributed to this discrepant finding, although more research is needed to isolate the cause of the opposite effect. These data show that haloperidol is not protective against ketamine's effects when ketamine results in impaired go/no-go reversal learning. Other drugs, like clozapine or selective alpha-7 nicotinic agonist drugs, may be more promising candidates to block anesthetic ketamine's effects on go/no-go reversal learning.

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# Chapter 1 - Introduction

Ketamine, an NMDA receptor antagonist, is used as an amnestic, analgesic, and anesthetic in humans and animals (Marland et al., 2013; Stokes, Flecknell, & Richardson, 2009; White, Way, & Trevor, 1982). In addition to its anesthetic uses, ketamine is also used as a drug of abuse, date rape drug, and has shown some success as a rapid acting anti-depressant (Abdallah et al., 2016; Basheer, 2011; Liu, Lin, Wu, & Zhou, 2016). As an anesthetic in the United States, the use of ketamine in adults was discouraged because adults showed a higher rate of uncomfortable and stressful dissociative hallucinations, known as emergence symptoms, than children when recovering from anesthesia (Domino, 2010; Green et al., 1998). Children did not exhibit high rates of emergence symptoms so ketamine was initially limited to pediatric populations, although there seem to be fewer aversive emergence phenomena in adults than were previously reported (Green & Johnson, 1990; Green & Li, 2000). One of the first surgeons to use anesthetic ketamine reported 33% of adults experienced emergence phenomena (Domino, 2010) whereas systematic reviews report 0.8-20% of adults experience them (Elia & Tramèr, 2005; Strayer & Nelson, 2008). Ketamine has many benefits due to its rapid-acting effects, short half-life, wide margin of safety, and limited effects on respiratory function (Green et al., 1998; Green et al., 1999; Li & Vlisides, 2016; White et al., 1982). It can also be safely used as a general anesthetic by non-physicians, making it ideal for use in both pediatric and adult populations in countries with fewer medical resources (Kurdi, Theerth, & Deva, 2014). Indeed, ketamine is listed as a general anesthetic on the World Health Organization's list of essential medicines (World Health Organization, 2017). All of these attributes also make ketamine a safe option for outpatient procedures, burn units, and prehospital situations (e.g. natural disasters and ambulances) but the long-term effects are understudied (Kurdi et al., 2014; Li & Vlisides, 2016; Marland et al., 2013; Mulvey, Qadri, & Maqsood, 2006; White et al., 1982).

Due to the benefits and uses of anesthetic ketamine, it is important to study the long-term effects of anesthetic doses of ketamine on behavior. Even though ketamine is a safe anesthetic option for the duration of the acute exposure period it is unclear whether anesthetic ketamine exposure has any long-term effects. Lifetime subanesthetic, recreational use of ketamine is associated with decreased frontal cortex connectivity and gray matter, but these data are confounded because the participants were polydrug users making it difficult to attribute this effect to ketamine rather than other drugs (Liao et al., 2010, 2011). However, a similar pattern is seen in people that have only used ketamine, with increasing amounts of atrophy of gray and white matter of the prefrontal cortex, capsule striatum, and other areas observed as the years of abuse increase (Wang, Zheng, Xu, Lam, & Yew, 2013). Although long-term low dose recreational use of ketamine is not equivalent to more brief, high dose anesthetic ketamine exposure, this shows that ketamine can result in frontal cortex changes, so the long-term effects of anesthetic ketamine exposure on behavior should be examined (Liao et al., 2010, 2011; Wang et al., 2013).

## Ketamine's effects on reversal learning

Reversal learning, a frontal-cortex dependent task, is often used to model flexible decision-making in the laboratory. In reversal learning, one response earns a reinforcer while the other response does not, then the identity of the reinforced response is reversed, such that the previously reinforced response is no longer rewarded and vice versa. Many different drugs such as cocaine (Calu et al., 2007; McCracken & Grace, 2013; Schoenbaum, Saddoris, Ramus, Shaham, & Setlow, 2004), opioids (Seip-Cammack & Shapiro, 2014), methamphetamine (Cox et al., 2016; Izquierdo et al., 2010; but see Daberkow, Riedy, Kesner, & Keefe, 2008), and alcohol (Badanich, Becker, & Woodward, 2011; Badanich et al., 2016; but see DePoy et al., 2013; Fisher, Bright, Gallo, Pajser, & Pickens, 2017; Kroener et al., 2012; Ray, Hite, Gallo, & Pickens, 2018) impair reversal learning in rodents but the acute and long-term effects of ketamine exposure on choice reversal learning are mixed. During a choice reversal learning task, both levers are presented simultaneously during each trial and the animal is reinforced after they respond on the rewarded lever. Responses on the non-rewarded lever are not reinforced. One study observed choice reversal learning impairments following acute subanesthetic ketamine exposure (Terry, Plagenhoef, & Callahan, 2016), but others show no effect of acute subanesthetic ketamine exposure on choice reversal learning (Gastambide, Mitchell, Robbins, Tricklebank, & Gilmour, 2013; Kos, Nikiforuk, Rafa, & Popik, 2011; Nikiforuk, Gołembiowska, & Popik, 2010). Subchronic, subanesthetic ketamine injections in adult rats also show variable long-term effects on choice reversal learning. If assessed ten days following the last injection, 10 injections of 30 mg/kg ketamine given 2X per day for 5 days impaired choice reversal learning and resulted in a selective increase in perseverative errors compared to the saline control rats (Floresco, Zhang, & Enomoto, 2009). Conversely, 5 or 10 once-daily injections of 30 mg/kg ketamine did not impair choice reversal learning in a test 10 days post injection (Nikiforuk &

Popik, 2012). Previous research in our lab gave 3 once-daily injections of 100 mg/kg ketamine to examine the long-term effects of a subchronic, anesthetic dose on choice reversal learning assessed after a 19 day washout period. These data showed that our dosing regimen can cause impairments in choice reversal learning, such that prior ketamine exposure increases perseverative responding (Pickens, Aurand, Hunt, & Fisher, 2017), similar to the results of Floresco and colleagues' study (2009), although we have recently found that this effect may not always replicate (Pickens et al., in prep).

Anesthetic ketamine exposure produces a different pattern in a go/no-go reversal learning task, where only one lever is presented during each trial. In our version of the task, responding on one lever (go response) is reinforced, while withholding responding on the other lever (no-go response) is also reinforced. In a subsequent experimental phase, the lever contingencies are reversed. Additionally, in order to pass the discrimination or reversal phases, the rats have to meet a strict criterion: 26 correct trials in a row for three consecutive days. In the go/no-go reversal learning experiment, male and female rats were either given 3 once-daily intraperitoneal (i.p.) injections of a subanesthetic dose (50 mg/kg) or a higher anesthetic dose (100 mg/kg). We found no sex differences and the 100 mg/kg dose, but not the 50 mg/kg dose, affected the number of errors, such that the rats made fewer errors during the reversal phase compared to controls (Pickens et al., in prep). Since our ketamine exposure regimen (multiple injections of an anesthetic level) produced a persistent behavioral change observed more than 3 weeks after the ketamine exposure, it is likely our exposure regimen produced a neurotoxic effect.

To replicate the improved performance effect and elucidate the parameters of ketamine's effects on go/no-go reversal learning, a follow-up experiment assessed whether 3 injections (or fewer) are needed to produce this alteration in the go/no-go task. We have found that three anesthetic ketamine injections were needed to facilitate go/no-go reversal learning, replicating our previous experiment and suggesting our initial finding was not a Type 1 error (Pickens et al., in prep). In order to determine whether three anesthetic ketamine exposures produce neurotoxicity, we also quantified parvalbumin- (PV) expressing GABA interneurons in brain regions related to reversal learning. These interneurons are involved in regulating inhibitory tone and are sensitive to NMDA receptor antagonist exposure (Homayoun & Moghaddam, 2007; Seamans, 2008). We found a decreased number of PV+ neurons in the prelimbic cortex (PL) following three anesthetic doses of ketamine compared to saline controls, indicating that our dosing regimen is causing neurotoxicity, although we have found that this effect may not always replicate (Pickens et al., in prep).

Even though our ketamine exposure regimen reduces the number of errors made in go/no-go reversal learning compared to controls, we do not think this means that anesthetic ketamine exposure improves general learning abilities or cognitive flexibility. The improvements we have observed following anesthetic ketamine exposure indicate that this go/no-go reversal learning task may not be a pure assay of cognitive flexibility as what we believe to be inflexible behavior is actually aiding performance. Other studies have also found that some manipulations, including brain inactivations (Dalton, Wang, Phillips, & Floresco, 2016) or lesions (Graybeal et al., 2011; Riceberg & Shapiro, 2012; Boulougouris, Dalley, & Robbins, 2007), pharmacological manipulations (Costa, Tran, Turchi, & Averbeck, 2015), and behavioral manipulations (Dhawan, Tait, & Brown, 2019; van Horik & Emery, 2018; Graybeal et al., 2011), can facilitate reversal learning with different parameters than the ones used here. While there is not a consistent explanation for these paradoxical results, some theorize that the manipulations can cause the subjects to be less reliant on previous experience (Jang et al., 2015) or can cause more habitual responding (Graybeal et al., 2011). This could aid performance after the switch, as it prevents them from making responses based on the old contingencies to ensure that the contingencies have not switched again. In order to pass the discrimination and reversal phases in our go/no-go reversal learning task, the rat has to meet a strict criterion of 26 correct trials in a row for three consecutive days. As such, the strict criterion punishes exploration of other response options. Therefore, being more habitual in responding may aid performance in our go/no-go reversal learning task while being less exploratory or more habitual is maladaptive in other situations.

## Ketamine's neurotoxic effects

As an NMDA receptor antagonist, ketamine was previously investigated as a potential drug to protect against ischemia damage caused by overactive NMDA receptors (NMDAR). Neurotoxicity from hypoxic ischemia (Church, Zeman, & Lodge, 1988; Proescholdt, Heimann, & Kempski, 2001) and spinal cord injury (Tang, Yu, Li, & Sun, 2015) is attenuated following acute ketamine exposure. Although NMDAR antagonists can prevent neurotoxicity in some areas of the nervous system, later studies revealed that NMDA receptor antagonists, such as ketamine, phencyclidine (PCP), and dizocilpine (MK-801), induce neurotoxicity in other regions of the nervous system (Olney, Labruyere, & Price, 1989). NMDAR antagonist-induced neurotoxicity is thought to occur by hypofunction of NMDARs. Glutamate, through NMDARs, is involved in a feedback loop that helps regulate inhibitory tone by tonically stimulating inhibitory GABA interneurons. When the NMDARs are antagonized, the GABA interneurons cease firing which disrupts the inhibitory tone. This, in turn, disinhibits the post-synaptic excitatory pyramidal neurons and produces excitotoxicity (Farber, 2003; Homayoun & Moghaddam, 2007; Seamans, 2008).

Neurotoxicity in the retrosplenial cortex (RSC), an area particularly vulnerable to NMDAR antagonist exposure (Olney et al., 1989; Allen & Iversen, 1990; Fix et al., 1993; Fix et al., 1995), can be blocked with pharmacological agents targeting GABA, acetylcholine, serotonin, dopamine, and norepinephrine systems (Farber et al., 1995; Farber, Foster, Duhan, & Olney, 1996; Farber, Hanslick, Kirby, McWilliams, & Olney, 1998; Farber, Kim, Dikranian, Jiang, & Heinkel, 2002; Kim, Price, Olney, & Farber, 1999; Olney et al., 1991; F. R. Sharp et al., 1992; Morimoto et al., 2002). This suggests that the mechanism by which NMDAR antagonists produce neuronal injury is complex and involves many different neurotransmitter systems. Of note, many different drugs of abuse, that all affect the dopaminergic system, produce long-term deficits in reversal learning (Calu et al., 2007; McCracken & Grace, 2013; Schoenbaum et al., 2004; Seip-Cammack & Shapiro, 2014; Cox et al., 2016; Izquierdo et al., 2010; Badanich et al., 2011; Badanich et al., 2016; but see Daberkow et al., 2008; DePoy et al., 2013; Fisher et al., 2017; Kroener et al., 2012). Acute subanesthetic and anesthetic ketamine (18-100 mg/kg) exposure also leads to the release of dopamine (Kokkinou, Ashok, & Howes, 2017; Masuzawa et al., 2003) and ketamine-induced neurotoxicity markers in the RSC can be blocked with dopamine antagonists at high doses (Farber et al., 1993; F. R. Sharp et al., 1992; Morimoto et al., 2002; Nakki, Nickolenko, Chang, Sagar, & Sharp, 1996). Therefore, the long-term effects of anesthetic ketamine on go/no-go reversal learning may occur due to its dopaminergic effects.

In order to better characterize the mechanism by which anesthetic ketamine may affect go/no-go reversal learning, I will co-administer either a low or high dose of haloperidol (that target different receptor types) with ketamine to potentially block ketamine's long-term effects. As mentioned previously, ketamine has many benefits, a wide margin of safety and is listed as an essential medicine by the World Health Organization (Green et al., 1999; Green et al., 1998; White et al., 1982; Li & Vlisides, 2016; Kurdi et al., 2014; Marland et al., 2013; Mulvey et al., 2006; World Health Organization, 2017). Given the benefits of ketamine as an anesthetic, it is worthwhile to research ways to make ketamine anesthesia safer. In addition, anesthetic ketamine administration is provided by licensed medical personnel in controlled environments. As such, finding an agent that could be safely co-administered at the time of ketamine exposure, that does not block anesthesia and helps prevent neurotoxicity, is valuable because it could be added to standard anesthetic practice with anesthetic ketamine use. Therefore, knowing the mechanism by which ketamine is producing these long-term effects would allow for targeted blockade when ketamine is used as an anesthetic.

**Haloperidol as a neuroprotective agent**

Haloperidol, a dopamine D2 receptor (D2R) antagonist, is a first-generation antipsychotic. Haloperidol protects against the production of ketamine- and MK-801-induced neurotoxic markers, like vacuolization and heat shock protein 70/72, in the RSC (Farber et al., 1993; Nakki et al., 1996; F. R. Sharp et al., 1992; Morimoto et al., 2002). However, the protective effects of haloperidol in the RSC may not be specific to action at D2Rs as the haloperidol dose (5.1 mg/kg or 19 mg/kg) needed to block 50% of MK-801 induced vacuolization is much higher than the dose needed to occupy 100% of D2Rs (Arnt & Skarsfeldt, 1998; Farber et al., 1993; Moison et al., 2003; Morimoto et al., 2002). Thus, the protective effects of haloperidol in the RSC are likely the result of its effects on sigma-2 receptors, affected only at higher doses of haloperidol, and not D2Rs, affected with lower doses of haloperidol (Farber et al., 1993; J. W. Sharp & Williams, 1996; Farber, 2003). Therefore, a low dose of haloperidol will likely not protect against ketamine-induced neurotoxicity in the RSC. While the RSC will likely sustain damage when a low dose of haloperidol is used, the RSC does not appear to be necessary for non-spatial reversal learning tasks (Aggleton, Neave, Nagle, & Sahgal, 1995) so a damaged RSC should not affect performance on our non-spatial go/no-go reversal learning task. Even though D2Rs may not be involved in neurotoxicity in the RSC, lower doses of haloperidol that block D2Rs may be sufficient to block ketamine's effects on reversal learning by protecting brain regions relevant to non-spatial reversal learning.

There are also applied reasons for using haloperidol to block ketamine's effects. Haloperidol is already FDA approved (U.S. Food and Drug Administration, n.d.), so it can be administered safely in human populations if it effectively blocks the long-term effects of ketamine. Haloperidol has also been successfully co-administered with ketamine in humans to alleviate prehospital agitation, showing that is haloperidol can be safely co-administered without disrupting ketamine's anesthetic effects in humans (Olives, Nystrom, Cole, Dodd, & Ho, 2016).

Therefore, I investigated the roles of sigma receptors and D2Rs in ketamine's neurotoxic effects using high and low doses of haloperidol. At high doses, haloperidol blocks sigma-2 receptors ($\sigma_2$), which are thought to be involved in neurotoxicity in the RSC and may block toxicity in brain areas relevant to our task (Farber et al., 2002; Farber, 2003). At low doses, haloperidol preferentially antagonizes D2Rs and sigma-1 receptors ($\sigma_1$) (Bowen, Moses, Tolentino, & Walker, 1990; Moison et al., 2003). In order to better characterize the role of D2 and sigma receptors in NMDAR antagonist neurotoxicity, along with ketamine I co-administered a high dose of haloperidol (10 mg/kg) or a low dose of haloperidol (1 mg/kg) to determine which set of receptors may be involved in ketamine's effects on go/no-go reversal learning. The high dose was chosen to block $\sigma_2$ receptors, although D2/$\sigma_1$ receptors would also be affected, and the low dose of haloperidol was chosen to preferentially antagonize D2/$\sigma_1$ receptors. While more selective drugs could have been used to determine the contribution of D2 and sigma receptors, the use of haloperidol allowed us to determine which of these receptors may be involved and may be worth investigating with more selectively in future studies.

**Hypotheses**

The goal of this study was to determine whether co-administration of haloperidol dose-dependently protects against anesthetic ketamine-induced changes in go/no-go reversal learning, which would be an indirect measure of whether haloperidol blocks or reduces neurotoxicity from anesthetic ketamine exposure. I hypothesized that neither ketamine nor haloperidol would affect initial discrimination learning, such that all groups would perform similarly. I hypothesized that ketamine alone would improve reversal learning, such that the group that only receives ketamine will make fewer errors in reversal learning compared to the saline only group. I also hypothesized that co-administering a low or high dose of haloperidol with ketamine would block ketamine's effects on reversal learning, such that the groups that receive haloperidol and ketamine would perform similarly to the saline only group and make more reversal learning errors than the ketamine only group. Finally, I hypothesized that co-administering a low or high dose of haloperidol in the absence of ketamine would not affect reversal learning performance. This pattern of results would indicate that D2/$\sigma_1$ receptors are the likely mechanism of haloperidol's protective effects.

# Chapter 2 - Methods

## Subjects

Male naïve Long Evans rats (n = 71) from Charles River laboratories (Kingston, NY or Raleigh, NC), 150-175 g upon arrival in the facility, were used for all experiments. All animals were individually housed and maintained on a 12-hour reverse light-dark cycle (lights off at 7:30 AM) in a temperature and humidity controlled room.

The animals were on free feed until a week after they received injections. They were then food-restricted to 85% of their free-feeding weight by daily feedings with a minimum of 5 g of food chow per day. Once rats reached their 85% target weights, the target body weight was increased by 1 g/day for the remainder of the experiment, such that the rats' target weights gradually increased by 7 g/week. The rats were fed to maintain them at their target weights and water was available *ad libitum*. All procedures and animal care were in accordance with the Kansas State University Institutional Animal Care and Use Committee guidelines, the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and United States federal law.

## Behavioral apparatus

Experiments were conducted in 12 standard self-administration chambers (Med Associates, St. Albans, VT). Each chamber is equipped with a pellet dispenser that delivers a 45-mg precision pellet (Catalogue #1811155, TestDiet, Richmond, IN). The chambers have two retractable levers on either side of the food cup at approximately one third of the total height of the chamber, with a white cuelight located above each lever. A red houselight is mounted on the top-center of the back wall. A speaker for delivering auditory stimuli is located on the left side of the back wall of the chambers, on the opposite wall from the food cup. A Dell Optiplex computer, equipped with Med-PC for Windows, controls the equipment and records lever-presses.

## Drugs

Ketamine (VetOne, Boise, ID) was diluted in 0.9% sterile saline and given at a dose of 100 mg/kg (i.p., in 2 ml/kg of sterile saline). Haloperidol (Mylan, Rockford, IL) was diluted in 0.25% sterile acetic acid and given at doses of 1 or 10 mg/kg (i.p., in 2 ml/kg of sterile acetic acid and sterile saline).

The high dose of haloperidol was chosen based on research suggesting 10 mg/kg of haloperidol blocks ~75% of MK-801 induced vacuolization in the RSC (Farber et al., 1993). The low dose of haloperidol was chosen because 1 mg/kg blocks methamphetamine (METH) neurotoxicity and occupies ~90% of D2 receptors (Bowyer et al., 1994; Natesan et al., 2006). The low dose of haloperidol should theoretically also occupy a similar proportion of $\sigma_1$ receptors and D2Rs because haloperidol shares a similar affinity for D2 and $\sigma_1$ receptors (Bowen et al., 1990; Moison et al., 2003).

### Ketamine injections

Once the rats reached 250 g they received haloperidol or vehicle co-administered with intraperitoneal (i.p) injections of 100 mg/kg ketamine (Ket) or sterile saline (Sal) injections once daily for three consecutive days. The rats were monitored for 90 min following the injections in bedding-less cages with a 60W lightbulb positioned above them to maintain body heat. Gross observation indicated that rats injected with haloperidol showed a dose-dependent cataleptic response (i.e. posture rigidity and lack of motor initiation) at both the low and high dose of haloperidol. Following the observation period, the rats were returned to their cages and monitored daily prior to the start of behavioral training.

In a 2 X 3 between-subjects design, the rats (n = 71; 6 groups: 2 Exposure doses X 3 Treatment doses; 11-12/group; Table 1) received 3 i.p injections (2 ml/kg) of either a 1 mg/kg low or 10 mg/kg high dose of haloperidol (Hal) or 2 ml/kg vehicle (Veh) co-administered once daily 20 minutes prior to the ketamine or saline injections described above (Table 1). There were six groups: Veh+Sal, 1 mg/kg Hal+Sal, 10 mg/kg Hal+Sal, Veh+Ket, 1 mg/kg Hal+Ket, and 10 mg/kg Hal+Ket. For example, on each of the 3 days, the 10 mg/kg Hal+Ket group received an injection of 10 mg/kg haloperidol 20 minutes before receiving an injection of 100 mg/kg ketamine.

The number of animals was determined by conducting power analyses using G*Power 3.1.9.3 (Universität Düsseldorf). Using preliminary data from an experiment using similar experimental procedures, the effect size f was predicted to be 0.49. In order to achieve a power level of 0.95 with an alpha level of 0.05 for 6 groups, 12 animals per group were recommended.

### Go/no-go discrimination and reversal training

Twelve days following the final pair of injections the rats began behavioral training with one 40-min magazine training session where one pellet was delivered every 125 seconds to familiarize the animals with the reward and for them to associate the magazine with reward. There was no performance criterion during magazine training but anecdotally most rats eat all or the majority of the pellets. The following day the rats began go/no-go discrimination training. We used a biased discrimination procedure, where the rats were trained against the most common side bias, in order to make the initial discrimination harder. Because our rats, on average, show a slight side bias towards the right lever (Pickens et al., 2017), the left lever was the "go" lever throughout discrimination, and lever presses earned a reinforcer. In contrast, the right lever was the "no-go" lever and withholding a lever press earned a reinforcer. This is similar to Floresco and colleagues (2009), except they determine each individual's side bias and train against it (e.g. if the rat has a left side bias then lever presses to the right lever will be reinforced during discrimination training). During their side bias procedure, rats are first trained to lever press both levers within 10 s. Then on each trial, rats are presented with both levers. Each individual's side bias is then determined based on the ratio between the initial lever presses made to the right versus left lever on each trial. Importantly, they use a choice reversal learning task where both levers are presented simultaneously during each trial and there is a correct (lever presses are rewarded) and an incorrect (lever presses are not rewarded) lever. We chose not to use Floresco et al.'s (2009) side bias methodology in order to avoid giving our rats an initial "go" bias. In our task, the first time rats are exposed to levers is during the first discrimination session. Inevitably, rats first make omission errors and earn reinforcement for correctly withholding a response on the no-go lever because they have not learned to lever press. As the session progresses, the rats associate the cuelights and levers with food and make sign-tracking responses, meaning they approach and press the lever (Flagel, Akil, & Robinson, 2009). Due to this sign-tracking response, they quickly learn to lever press. They then start earning rewards for pressing the go lever and learn to not press the no-go lever. Using Floresco and colleagues' procedure (2009) would lead rats to be prone to lever-pressing prior to the go/no-go training, which would greatly impede their ability to learn the no-go response during the actual task. It would likely take a long time for the rats to not press the lever and realize they are reinforced for not pressing one of the levers. Further, rats make more commission errors (incorrectly pressing the no-go lever) than omission errors (incorrectly failing to press the go lever) in the current version of the task, suggesting rats have a response bias even without lever press training (Fisher et al., 2017; Pickens et al., in prep).

During discrimination sessions, the rats were presented with either the left or right lever during a trial in alternating fashion. Each trial began with a 2-sec illumination of a cuelight above one of the two levers. Following the 2-sec period, the lever below the cuelight extended and was available for 6 sec or until the rat pressed the lever. The trial ended with the lever retracting and cuelight ending once a lever press was made or 6 sec elapsed.

Correct responses (lever-press on the go lever or withhold a lever-press on the no-go lever) were reinforced with the delivery of a food pellet and the beginning of the intertrial interval (ITI) (5-11 sec). During the ITI, the houselight remained on but no cuelights or levers were available. Incorrect responses (lever-press on the no-go lever [commission error] or incorrect failure to press the go lever [omission error]) resulted in a 1-sec tone and a 15-sec timeout period, during which the houselight was extinguished and no levers or cuelights were available. Following the timeout period, the ITI began. In order to pass discrimination, the rats had to make 26 correct responses in a row for three consecutive days.

The session had three possible end points. First, the session would end when a rat made 26 correct responses in a row, which was our criterion rats had to meet to pass a session (Fisher et al., 2017). Second, the session could also end when the rat made an error after 48 minutes had elapsed, resulting in a failure to pass the session. This was to ensure the session had an endpoint that was not dependent on the rat performing well in the task to prevent long sessions. The session ended on an error and not just at 48 minutes to make sure we did not artificially cause the rat to fail the session by ending the session in the middle of a string of correct responses. Third, the session could also end if an error was made any time after a rat made a total of 120 correct responses during the session. We chose this endpoint to make sure the rats did not become sated on the pellets and lose motivation to perform the task. Unpublished data from our lab shows that, on a variable-interval-30 schedule, rats will maintain steady response rates up until they receive about 120 pellets and then responding starts to decrease. Allowing the rats to continue the task while they are sated could inflate omission errors (failure to press the go-lever) and correct no-go responses if the rats lose motivation to lever press. In addition, we wanted the endpoint to be an error response, so we did not prematurely end a session if a rat was in the middle of a string of correct responses. Therefore, the session ended either when the rat made 26 correct responses in a row, an error was made after 48 minutes, or an error was made following 120 correct responses.

Once the rats passed discrimination training, they advanced to the reversal learning phase. During the first reversal, the discrimination contingencies were reversed, such that the go lever became the no-go lever (reinforced for withholding lever presses) and the no-go lever became the go lever (reinforced for lever presses). Once the rats reached criterion (26 correct responses in a row) for three consecutive days during the reversal one phase, the contingencies were reversed back to the original discrimination contingencies. In order to pass the second reversal, the rats were required to reach criterion (26 correct responses in a row) for three consecutive days. As such, the reversal assessments include one instance where the rats go against any individual side bias and one where they go toward any individual side bias to account for performance deficits/facilitation based on individual side biases. In addition, some manipulations, like OFC lesions, can cause an initial impairment during the first reversal while causing an improvement in subsequent reversals (Boulougouris et al., 2007; Schoenbaum, Nugent, Saddoris, & Setlow, 2002). Therefore, performance when reversing back to the original discrimination contingencies could reveal additional information about underlying neural mechanisms.

**Statistical analyses**

The primary dependent variable was the errors to criterion made in each phase (discrimination or reversal). For all analyses, the errors to criterion were square root transformed to correct for normality and sphericity violations (for non-transformed data see Appendix A1). As with our previous study (Pickens et al., in prep), a square root transformation was chosen because the dataset contained zeros, making the dataset unfit for a log transformation without adding a constant to every value. In addition, the dependent variable is count data and a square root transformation is traditionally recommended for count data (Bartlett, 1947) and has been used in another more recent reversal learning experiment (Boulougouris et al., 2007). The errors for discrimination and reversal learning were subdivided into commission errors (lever-press on the no-go lever) or omission errors (failure to press the go lever) and learning errors (errors made before the rat made 26 consecutive correct responses for the first time) or maintenance errors (errors made after the rat made 26 consecutive correct responses for the first time) (Pickens et al., in prep; Fisher et al., 2017). We decided to subdivide the errors into commission and omission errors and into learning and maintenance errors for several reasons. Commission and omission errors represent different forms of errors. Commission errors, incorrectly pressing the no-go lever, represent a failure to correctly inhibit a response, whereas omission errors, failing to press the go lever, represent a failure to correctly initiate a response. Further, we have previously observed a relationship between commission errors, but not omission errors, and the amount of alcohol voluntarily consumed during early adulthood (Fisher et al., 2017). This dissociation suggests that there are different mechanisms underlying these error types. We subdivided the errors into learning and maintenance errors for similar reasons. Errors made when the rat is first learning the new set of contingencies (learning errors) represent errors made while the rat is initially learning to inhibit a previous pattern of stimulus-response associations and forming new stimulus-response associations to correctly perform the task. Maintenance errors represent the consolidation/maintenance of this new learning. Our lab has previously found that three anesthetic ketamine exposures causes lower learning errors compared to controls, with no group differences in maintenance errors, however this effect may not replicate (Pickens et al., in prep). Similarly, we have found correlations between alcohol consumption and maintenance errors (Fisher et al., 2017) and between PV+ neurons and maintenance errors, but not learning errors, in saline- and ketamine-exposed animals (Pickens et al., in prep). Beyond research in our own lab, Pastuzyn et al (2012) have shown that disrupting expression of *Arc*, an early immediate gene important for synaptic plasticity and learning, in the dorsomedial striatum does not affect initial reversal learning but does impair the consolidation/maintenance of that learning when tested on subsequent days after the original reversal criterion was reached. These findings suggest the learning and maintenance phases of reversal learning are supported by different mechanisms. In order to parse out how ketamine exposure affects reversal learning and the underlying cognitive mechanisms, we divided errors by their error type (commission and omission) and the error phase they occurred in (learning and maintenance).

For discrimination learning, a mixed factorial ANOVA was run to examine the effects of the between subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, 10 mg/kg Hal) and the within-subjects variables Error Type (Commission and Omission) and Error Phase (Learning and Maintenance) on the errors to criterion. For reversal learning, a mixed factorial ANOVA was run to examine the effects of the between subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, 10 mg/kg Hal) and the within-subjects variables Reversal Test (Reversal One – reverse away from discrimination contingencies and Reversal Two – reverse toward discrimination contingencies), Error Type (Commission and Omission), and Error Phase (Learning and Maintenance) on the errors to criterion.

We ran a secondary analysis using the number of trials to criterion as the dependent variable. Like with errors to criterion, trials to criterion were square root transformed to correct for normality violations as in Boulougouris and colleagues (2007). The same form of analyses as in the errors to criterion analyses was used for this measure. The trials to criterion were divided into trials to the first pass (learning trials to criterion) and subsequent trials until the rat passed the phase (maintenance trials to criterion).

For the discrimination learning trials to criterion analysis, a mixed factorial ANOVA was run to examine the effects of the between subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, 10 mg/kg Hal) and the within-subjects variable Criterion Phase (Learning and Maintenance). For reversal learning, a mixed factorial ANOVA was run to examine the effects of the between subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, 10 mg/kg Hal) and the within subjects variables Criterion Phase (Learning and Maintenance) and Reversal Test (Reversal One and Reversal Two). As the variable trials to criterion does not differentiate response types, we are not able to differentiate between commission or omission error types.

The analyses of errors to criterion and trials to criterion yielded different patterns of results, such that ketamine-exposed rats made more learning errors than controls but ketamine-exposed rats did not differ from controls in the number of trials needed to reach criterion during the learning phase. The different pattern suggested that ketamine exposure may have altered the pattern of errors the rats made during the learning phase. Because our criterion is 26 correct responses in a row, there are patterns of behavior that can produce more trials to criterion with the rat making fewer errors than a different rat that made more errors in fewer trials. Rats could initially make large amount of errors and then switch to making correct responses until reaching criterion, which would produce data with high errors to criterion but low trials to criterion. Alternatively, rats could intermittently make errors until criterion was reached which would produce data with low errors to criterion but high trials to criterion or some mix of the two. For example, making 30 errors with no correct responses between them and then having a string of 26 correct in a row would only add 30 trials to the minimum 26 trials to complete a session (with 30 errors). Conversely, having 5 strings with 20 correct in a row followed by an error in each string and then having 26 correct in a row would add 105 trials to the minimum of 26 trials to complete a session (with only 5 errors).

In order to determine whether ketamine produced different patterns of errors, we ran a third exploratory analysis with reversal learning errors to criterion (square root transformed) and divided them into three error classes. Errors made before the rats passed reversal learning the first time (i.e. learning phase) were subdivided into two categories: Perseverative and New Learning errors. The criteria for these error classifications were based on previous choice reversal learning research (Brigman et al., 2008; Brigman et al., 2010; Pickens et al., 2017) and determined before analyzing the data. We divided the sessions into 12-trial blocks (or the remaining trials at the end of the session) and then categorized each block based on whether the rats made incorrect responses on more or less than 50% of the trials. Errors were classified as Perseverative when rats performed at or worse than 50% during each block (e.g. all errors made during a block with six or fewer correct responses out of 12 trials). Perseverative errors suggest the rats are still performing as though the contingencies of the previous phase are still in place (e.g. Discrimination contingencies during Reversal 1 or Reversal 1 contingencies during Reversal 2). These errors are thought to be indicative of habitual, inflexible responding (Nilsson, 2015). We classified New Learning errors as errors made when the rats performed above 50% within each block (e.g. all errors made during a block with seven or more correct responses out of 12 trials). New Learning errors suggest that the rats now know the current contingencies are different than before and the incorrect responses are the result of learning the new contingency. All errors made after the rat passed the first time but before they reached criterion (3 days in a row) were classified as maintenance errors.

A mixed factorial ANOVA was run to examine the effects of the between subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, 10 mg/kg Hal) and the within-subjects variables Reversal Test (Reversal One – reverse away from discrimination contingencies and Reversal Two – reverse toward discrimination contingencies), Error Type (Commission and Omission), and Error Class (Perseverative, New Learning, and Maintenance) on the errors to criterion. The measure of trials to criterion will be more sensitive to new learning errors as these errors are spaced among a greater number of correct responses and will add more overall trials before the rat reaches criterion (26 correct responses). Whereas trials to criterion will be less sensitive to increases in perseverative errors as these errors are massed and therefore have a lower impact on overall trials to criterion. If the ketamine-exposed rats made more Perseverative errors or fewer New Learning errors than controls, it would suggest that the dissociation between the initial errors to criterion and trials to criterion analyses are the result of the groups making different patterns of errors, which produced approximately the same number of trials to criterion.

All data were analyzed using Statistica 13.3 (TIBCO, Palo Alto, CA, USA). The alpha level was set at 0.05 for all ANOVAs. Significant interactions with Exposure Group or Treatment Group were probed using Bonferroni-corrected simple effects.

# Chapter 3 - Results

## Discrimination Learning

**Errors to criterion**. There was no effect of ketamine or haloperidol exposure on errors to criterion during discrimination learning (see Figure 1A). A mixed-factor ANOVA with the between-subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, or 10 mg/kg Hal) and the within-subjects variables Error Phase and Error Type found no main effect of Exposure Group ($F(1, 65)=1.02$, $p=.32$, $\eta^2_p=.015$), Treatment Group ($F(2, 65)=.64$, $p=.53$, $\eta^2_p=.019$) or any interactions of Exposure Group ($F$'s$(1, 65)=.68$-$2.43$, $p$'s$>.10$, $\eta^2_p$'s$=.01$-$.036$) or Treatment Group ($F$'s$(2, 65)=.62$-$1.83$, $p$'s$>.10$, $\eta^2_p$'s $=.019$-$.053$). This lack of effect indicates that ketamine exposure, haloperidol exposure or haloperidol co-administered with ketamine did not affect the acquisition of the go/no-go task. This finding was in support of our hypothesis that ketamine or haloperidol exposure would not affect initial discrimination learning. There were significant main effects of Error Phase ($F(1, 65)=240.35$, $p<.001$, $\eta^2_p=.79$), Error Type ($F(1, 65)=19.98$, $p<.001$, $\eta^2_p=.24$), and a significant Error Phase*Error Type interaction ($F(1, 65)=16.59$, $p<.001$, $\eta^2_p=.20$). No other effects or interactions were statistically significant (all $p>.05$).

**Trials to criterion**. There was also no effect of ketamine or haloperidol exposure on trials to criterion during discrimination learning (see Figure 2A). A mixed-factor ANOVA with the between-subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, or 10 mg/kg Hal) and the within-subjects variable Criterion Phase found no main effect of Exposure Group ($F(1, 65)=.19$, $p=.66$, $\eta^2_p<.01$), Treatment Group ($F(2, 65)=.80$, $p=.46$, $\eta^2_p=.024$), or any interactions including Exposure Group ($F(1, 65)=1.61$, $p=.21$, $\eta^2_p=.024$) or Treatment Group ($F$'s$(2, 65)=.34$-$.99$, $p$'s$>.10$, $\eta^2_p$'s $=.01$-$.03$). Similar to the analysis of the errors to criterion, ketamine or haloperidol exposure did not affect the number of trials needed to reach criterion during discrimination learning. These findings are in agreement with the errors to criterion analysis of discrimination learning hypotheses that ketamine or haloperidol exposure would not affect initial discrimination learning. There was a significant main effect of Criterion Phase ($F(1, 65)=30.04$, $p<.001$, $\eta^2_p=.32$). No other effects or interactions were statistically significant (all $p>.05$).

## Reversal Learning

**Errors to criterion**. The Ketamine groups made significantly more errors during the learning phase of reversal learning compared to the Saline groups. In order to determine how low or high doses of haloperidol co-administered with ketamine affect go/no-go reversal learning, a mixed-factor ANOVA was run with the between-subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, or 10 mg/kg Hal) and the within-subjects variables Error Phase, Error Type, and Reversal Phase (see Figure 1B). There was a significant main effect of Exposure Group ($F(1, 65)=4.69$, $p=.034$, $\eta^2_p=.067$) and significant interactions of Exposure Group*Error Phase ($F(1, 65)=6.89$, $p=.011$, $\eta^2_p=.096$), Exposure Group*Treatment Group*Error Type ($F(2, 65)=4.50$, $p=.014$, $\eta^2_p=.12$), and Exposure Group*Treatment Group*Error Phase*Error Type ($F(2, 65)=3.48$, $p=.037$, $\eta^2_p=.097$). While the Exposure*Error Phase interaction was qualified by a 3-way and 4-way interaction (discussed below), we also reported this effect as 2-way interactions are more stable than 3- and 4-way interactions. Probing the Exposure Group*Error Phase interaction revealed that the Ketamine groups made significantly more learning errors than the Saline groups ($F(1, 65)=12.95$, $p<.05$), but the groups did not differ on maintenance errors ($F(1, 65)=.02$, $p>.05$) (see Figure 1C). This finding was in contrast to our current hypothesis and our previous finding that ketamine selectively decreased learning errors (Pickens et al., in prep). The 3-way Exposure Group*Treatment Group*Error Phase interaction was qualified by the 4-way interaction of Exposure Group*Treatment Group*Error Phase*Error Type. Simple effects of the 4-way interaction indicated that the Veh+Ketamine group made more omission errors ($F(2, 65)=25.13$, $p<.05$) but fewer commission errors ($F(2, 65)=8.78$, $p<.05$) during the learning phase than the1 mg/kg Hal+Ketamine group (see Figure 1D). While these interactions were statistically significant, 4-way interactions are typically unstable, therefore these high level interactions should be treated with extreme caution until they are replicated. Thus, we failed to support our hypotheses that (1) the Veh+Ketamine group would make fewer learning errors than the Veh+Saline group and that (2) both the 1 mg/kg Hal+Ketamine and the 10 mg/kg Hal+Ketamine rats would make more learning errors than the Veh+Ketamine group. Simple effects revealed no differences between the groups that received low or high doses of haloperidol co-administered with saline and the group that received the vehicle co-administered with saline, providing support for our hypothesis that haloperidol alone would not impair reversal learning. There was no main effect of Treatment

Group ($F$(2, 65)=.32, $p$=.76, $\eta^2_p$=.010), or any other interactions including Exposure Group ($F$'s(1, 65)=.14-1.37, $p$'s= .25-.71, $\eta^2_p$'s=.002-.020) or Treatment Group ($F$'s(2, 65)=.036-2.14, $p$'s=.13-.96, $\eta^2_p$'s=.001-.061).

There were also significant main effects of Error Phase ($F$(1, 65)=426.55, $p$<.001, $\eta^2_p$=.87), Error Type ($F$(1, 65)=307.62, $p$<.001, $\eta^2_p$=.83), Reversal Phase ($F$(1, 65)=66.62, $p$<.001, $\eta^2_p$=.24), and significant interactions of Reversal Phase*Error Phase ($F$(1, 65)=26.90, $p$<.001, $\eta^2_p$=.29) and Error Phase*Error Type ($F$(1, 65)=33.60, $p$<.001, $\eta^2_p$=.34). No other effects or interactions were statistically significant (all $p$>.05).

**Trials to criterion**. There were no significant effects of ketamine or haloperidol exposure on trials to criterion during reversal learning but there was a marginal effect of ketamine exposure increasing the overall trials to criterion in reversal learning compared to saline exposure (see Figure 2B). A mixed-factor ANOVA with the between-subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, or 10 mg/kg Hal) and the within-subjects variables Criterion Phase and Reversal Phase found a marginally significant main effect of Exposure Group ($F$(1, 65)=3.02, $p$=.087, $\eta^2_p$=.044). The Ketamine groups ($M$=247.66 untransformed trials to criterion, $SEM$=18.07, 95% CI [211.57, 283.75]) needed marginally more trials to reach criterion than the Saline groups ($M$=209.13 untransformed trials to criterion, $SEM$=17.80, 95% CI [173.57, 244.68]). Unlike the analysis of the errors to criterion, there was no interaction of Exposure Group and Criterion Phase ($F$(1, 65)=2.18, $p$=.14, $\eta^2_p$=.032). This suggests that while the Ketamine groups made more overall learning errors, but not maintenance errors, than the Saline groups, the Saline and Ketamine groups completed the learning and maintenance phases in about the same number of trials as one another. There was no main effect Treatment Group ($F$(2, 65)=.28, $p$=.75, $\eta^2_p$=.008), or any interactions including Exposure Group ($F$'s(1, 65)=.021-2.18, $p$'s>.10, $\eta^2_p$ =.0003-.032) or Treatment Group ($F$'s(2, 65)=.053-1.81, $p$'s>.10, $\eta^2_p$'s =.001-.056). These findings were in agreement with the errors to criterion hypotheses and analysis showing that haloperidol did not affect reversal learning in the absence of ketamine exposure. While against our original hypothesis, the lack of haloperidol to alter ketamine's effects in reversal learning was consistent with the errors to criterion analysis. The trials to criterion analysis also failed to support our hypothesis that ketamine exposure would improve go/no-go reversal learning.

There were also significant main effects of Criterion Phase ($F(1, 65)=71.79$, $p<.001$, $\eta^2_p=.52$), Reversal Phase ($F(1, 65)=7.72$, $p<.01$, $\eta^2_p=.11$), and a significant interaction of Criterion Phase*Reversal Phase ($F(1, 65)=8.27$, $p<.01$, $\eta^2_p=.11$). No other effects or interactions were statistically significant (all $p>.05$).

**Error class**. The Ketamine groups made significantly more perseverative errors than the Saline groups, but did not differ on the number of new learning or maintenance errors (Figure 3B). To determine whether the discrepancy between the errors to criterion and trials to criterion analyses were due to differences in the temporal pattern of incorrect responses, a mixed-factor ANOVA was run with the between-subjects variables Exposure Group (Saline or Ketamine) and Treatment Group (Veh, 1 mg/kg Hal, or 10 mg/kg Hal) and the within-subjects variables Error Class, Error Type, and Reversal Phase (see Figure 3). There was a significant main effect of Exposure Group ($F(1, 65)=4.39$, $p=.040$, $\eta^2_p=.063$), significant interaction of Exposure Group*Error Class ($F(2, 130)=25.69$, $p=.002$, $\eta^2_p=.089$), and a marginally significant interaction of Treatment Group*Error Class ($F(2, 130)=2.32$, $p=.060$, $\eta^2_p=.067$). Probing the Exposure Group*Error Class interaction revealed that the Ketamine groups made significantly more perseverative errors than the Saline groups, ($F(2, 130)=21.92$, $p<.05$), but the groups did not differ on new learning errors, ($F(2, 130)=.64$, $p>.05$), or maintenance errors, ($F(2, 130)=.034$, $p>.05$) (see Figure 3B).

In addition, there was a marginally significant interaction of Treatment Group*Error Class ($F(4, 130)=2.32$, $p=.06$, $\eta^2_p=.066$) and Exposure Group*Treatment Group*Error Type ($F(2, 65)=5.73$, $p=.005$, $\eta^2_p=.15$), which were qualified by significant interactions of Exposure Group*Treatment Group*Error Class*Error Type ($F(4, 130)=3.28$, $p=.013$, $\eta^2_p=.092$) (see Figure 3D), and Exposure Group*Treatment Group*Error Class*Reversal Phase ($F(4, 130)=2.47$, $p=.048$, $\eta^2_p=.071$) (see Figure 3E). Due to the exploratory nature of the analysis and the instability of 4-way interactions, these interactions were not probed due to lack of power. If the same interactions are observed for any replication, they will be probed.

There was no main effect of Treatment Group ($F(2, 65)=.15$, $p=.86$, $\eta^2_p=.063$) or any other interactions with Exposure Group ($F$'s(1-2, 65-130)=.15-1.00, $p$'s=.16-.37, $\eta^2_p$'s=.002-.015) or Treatment Groups ($F$'s(2-4, 65-130)=.14-1.88, $p$'s=.16-.87, $\eta^2_p$'s=.004-.055).

There were also significant main effects of Error Class ($F(2, 130)=209.13$, $p<.001$, $\eta^2_p=.76$), Error Type ($F(1, 65)=324.48$, $p<.001$, $\eta^2_p=.83$), Reversal Phase ($F(1, 65)=24.50$,

$p<.001$, $\eta^2_p=.27$), and significant interactions of Reversal Phase*Error Class ($F(2, 130)=12.59$, $p<.001$, $\eta^2_p=.16$), Error Class*Error Type ($F(2, 65)=8.02$, $p<.001$, $\eta^2_p=.11$), and Reversal Phase*Error Class* Error Type ($F(2, 130)=5.12$, $p=.007$, $\eta^2_p=.073$). No other effects or interactions were statistically significant (all $p>.05$).

# Chapter 4 - Discussion

The present study investigated the effects of haloperidol co-administered with anesthetic ketamine on go/no-go reversal learning. We found no evidence that haloperidol blocks the effects of ketamine. However, we unexpectedly found that anesthetic ketamine exposure, regardless of haloperidol treatment, impaired go/no-go performance. The effect of ketamine is surprising given that our previous experiments have found that anesthetic ketamine exposure improves go/no-go reversal learning (Pickens et al., in prep). The opposing results may be due to methodological differences between the current and previous experiments. We plan on adding the current experiment and any follow-up experiments investigating the methodological differences to the manuscript that is currently in preparation to provide readers with a more complete picture of the unreliable long-term effects of anesthetic ketamine on go/no-go reversal learning. In addition to the current experiment and any follow-ups, this manuscript we will report (1) the original two go/no-go reversal learning experiments showing improved performance, (2) behavioral data from a modified version of the go/no-go reversal learning task in which ketamine rats showed improved performance, (3) the reduction in PV+ neurons in PL and the altered behavior-PV+ correlations between ketamine-exposed animals and controls, (4) the failure to replicate the PV+ neuron reduction in PL and altered behavior-PV+ correlations, (5) the failure to replicate the choice reversal learning effect, and (6) the neurological data from the current experiment (Pickens et al., in prep). The findings and possible implications are discussed further below.

## Ketamine impaired go/no-go reversal learning

Our data show that anesthetic ketamine exposure increased the number of learning errors, errors made before the rat reached criterion for the first time, in go/no-go reversal learning compared to saline exposed rats. More specifically, an exploratory analysis conducted post hoc found that the increase was specifically due to an increased number in perseverative errors. The ketamine-induced impairments are in line with previous literature looking at NMDAR antagonist exposure and choice reversal learning. Operant lever-press choice reversal learning tasks can be impaired by the long-term effects of anesthetic ketamine (Pickens et al., 2017) and subanesthetic PCP (Abdul-Monim, Reynolds, & Neill, 2006; Abdul-Monim, Neill, & Reynolds, 2007; Idris et al., 2009; McLean, Idris, Woolley, & Neill, 2009; McLean, Woolley, Thomas, & Neill, 2009; McLean et al., 2010; McLean et al., 2011) and there are mixed results for subanesthetic ketamine exposure (Floresco et al., 2009; but see Nikiforuk & Popik, 2012). When reversal learning errors are subdivided into perseverative and non-perseverative error classes, anesthetic (Pickens et al., 2017) and subanesthetic (Floresco et al., 2009) ketamine exposure can result in increased perseverative responding. While these results show similarities to previous literature, they are in complete contrast to results we have found in previous studies where anesthetic ketamine exposure reduced the number of learning errors in go/no-go reversal learning (Pickens et al., in prep). While we are unsure what the cause of this difference was, there were two methodological differences that have the potential to explain these differing results: the number of injections each day and the washout period between the final injection and the beginning of behavioral training.

Our secondary analysis using trials to criterion as the dependent variable showed a marginal effect of ketamine, with the Ketamine groups almost needing more trials to criterion than the Saline groups. Unlike the analysis of errors to criterion, there was no interaction or trend towards an interaction of Exposure Group with Criterion Phase. This suggests that the Ketamine groups may have been making errors differently than the Saline groups. Because our criterion is 26 correct responses in a row, there are patterns of behavior that can produce more trials to criterion with the rat making fewer errors than a different rat that made more errors in fewer trials. Our exploratory analysis of error class found that the Ketamine groups made more perseverative errors (errors when performance was 50% or lower during a 12-trial block) than the Saline groups but the two groups did not differ on new learning errors (errors when performance was greater than 50% during a 12-trial block). This difference likely explains the lack of effect in the trials to criterion analysis because perseverative responding will have less of an effect on trials to criterion than new learning errors. New learning errors represent errors made after the rat has begun to respond based on the current contingencies and is performing above 50%. Therefore, new learning errors are likely to be nested within a string of correct response, which will all count towards the trials to criterion measure. Conversely, perseverative errors are trial blocks when the majority responses are errors. Each perseverative error made is likely preceded by only 1-2 correct responses and is therefore associated with fewer additional trials to criterion than a new learning error. As such, the discrepancy between the errors to criterion and trials to criterion analyses may represent that trials to criterion is a less sensitive measure of reversal learning performance when perseverative errors rates are high.

In the current study, the rats received two injections daily consisting of the treatment dose (Veh, 1 mg/kg Hal, or 10 mg/kg Hal) and then the exposure dose (Sal or Ket) 20 minutes later. In the previous studies, the rats only received one injection daily (Sal or Ket). While it is difficult to compare across studies, the saline control groups in our previous studies (Study 1: *M*=34.65 untransformed reversal learning errors, *SEM*=3.33, 95% CI [27.55, 41.74]; Study 2: *M*=41.5 untransformed reversal learning errors, *SEM*=6.20, 95% CI [27.19, 55.81]) performed somewhat similarly to the group that received the vehicle co-administered with saline (*M*=29.27 untransformed reversal learning errors, *SEM*=3.73, 95% CI [29.27, 37.49]), despite receiving two injections. This suggests that stress alone from 2 injections likely did not cause the difference between the studies. However, similar behavior in our control groups across studies does not rule out the possibility that stress-induced changes in neuronal activation from the first injection may have interacted with the ketamine injection and altered its long-term effects. Studies have shown that a saline/vehicle injection can cause an increase in extracellular dopamine in the nucleus accumbens shell (NAs) (Barrot et al., 1999) and increased expression of c-fos, an early immediate gene indicative of neuronal activation, in the prefrontal cortex (Panhelainen & Korpi, 2012), basolateral amygdala (Panhelainen & Korpi, 2012; Ryabinin, Wang, & Finn, 1999), and NAs (Ryabinin et al., 1999; Barrot et al., 1999; but see Panhelainen & Korpi, 2012). However, the extracellular dopamine release in the NAs was back to baseline levels 20 minutes following the injection. In addition when a second injection was given 2 hours later, it produced no increase in c-fos in the NAs, suggesting rats quickly habituate to the injection stress (Barrot et al., 1999). Along with increased neural activation, vehicle injections can also increase corticosterone (Drude et al., 2011; Simone, 2018; but see Deutsch-Feldman et al., 2015), a stress hormone. As with neural activity, rats seem to habituate to the stress after repeated injections (Hohlbaum et al., 2018). The quick habituation observed in rats makes it unlikely that a spike in neural activity from injection stress may have altered how ketamine affects the brain, but we cannot rule out this possibility.

The other methodological change from the current experiment was the washout period. In the previous studies, the rats started behavioral training 19-20 days following the final injection (Pickens et al., in prep). In the current study, the rats began behavioral training 12 days following the final injection. The washout period was altered for internal logistical reasons. We had no reason to suspect that changing the washout period would alter the pattern of results in the study.

In retrospect, if the washout period is functionally relevant, the change in washout period between the final injection and start of behavioral training in our different studies may represent a critical reorganization/recovery period where performance may differ depending on if testing occurs before or after the reorganization/recovery process is complete. A previous study in our lab has shown that ketamine exposure decreased PV+ neurons in the PL and altered the pattern of behavior-PV correlations in saline- vs ketamine-exposed rats (e.g. maintenance errors in the task correlated with PV+ neurons in controls, but not ketamine-exposed rats (Pickens et al., in prep)). In addition to the altered PV-behavior correlations in the dorsal medial striatum (DMS) (correlates in controls but not the ketamine group), we have observed PV-behavior correlations in the ketamine-exposed animals in the PL, infralimbic cortex, and NAs that are not present in controls. These altered PV-behavior correlations may be indicative of functional reorganization of brain circuitry after the ketamine exposure. This is similar to compensatory changes seen after METH exposure that results in partial dopamine loss in the striatum (Daberkow et al., 2008; Pastuzyn & Keefe, 2013). The METH exposure altered which brain regions showed correlations between reversal learning performance and *Arc* mRNA expression, an early immediate gene that is thought to be indicative of synaptic plasticity and learning (Korb & Finkbeiner, 2011; Shepherd & Bear, 2011). Even though METH exposure altered these correlations, overall reversal learning performance was unaffected, suggesting METH exposure changed the neural circuitry used to successfully perform the task. A similar reorganization could be occurring following our anesthetic ketamine exposure regimen, but this reorganization may be insufficient to normalize learning/performance after three ketamine injections.

If reorganization is occurring, the different washout periods could represent different functional stages in the reorganization/recovery process. While the time course of damage after anesthetic ketamine exposure is unknown, exposure to other neurotoxins can produce different recovery timelines between different brain regions (Bueno, Olmos, Heimer, & Olmos, 2003; Friedman, Castañeda, & Hodge, 1998; Kazi & Oommen, 2012; Smolen, Smolen, Han, & Collins, 1987) or over-compensation following toxicity (Pian, Criado, Milner, & Ehlers, 2010). For instance, following exposure to a toxin that affects acetylcholine expression, differential recovery timelines are observed such that expression in the frontal cortex and cerebellum recover within 7 days, whereas normalization in the striatum and hippocampus is not achieved until Day 30 (Kazi & Oommen, 2012). Differences in recovery speed between brain regions may not mean permanent reorganization is occurring, but it could be that brain areas not usually involved in go/no-go reversal learning may be recruited while the recovery process occurs, producing the opposite behavioral effect. Alternatively, while the brain is recovering, there may be a behavioral impairment until the reorganization process is complete as there are no brain areas able to support the behavior at the time of testing.

To our knowledge, no other group has examined the effects of anesthetic ketamine exposure on go/no-go or choice reversal learning so it is difficult to pinpoint the reason why the opposite effect occurred in our study. Future research should replicate the impairment following a 12-day washout period with 2X injections per day and the improvement found in our previous experiments following a 19-20-day washout period with one injection per day (Pickens et al., in prep). If these effects are replicable, research should parse out the cause by manipulating the washout period or creating drug cocktails, such that only one injection is given per day, to minimize injection stress.

In order to not add to the growing replication crisis (Shrout & Rodgers, 2018; John, Loewenstein, & Prelec, 2012; Yong, 2012; Open Science, 2015), we will conduct follow-up experiments to attempt to replicate our effects and determine the cause of our opposite finding here, namely investigate the effects of the washout period and/or injection stress. If we fail to determine the cause of these discrepant findings or fail to replicate our original effects, we plan to publish the current study with our previous work and any follow-up experiments that fail to replicate any of our previous findings. In addition, we will publish (Pickens et al., in prep) our recent attempts (and failure) to replicate the effects we observed in choice reversal learning (Pickens et al., 2017). We aim to publish our ketamine data together to be transparent about the unreliable nature of ketamine's effects. In a related, but separate, experiment in our lab, we gave rats 3 injections of ketamine and processed their brains for PV+ neurons to replicate the finding that ketamine reduces the number of PV+ neurons in PL. We failed to replicate this finding, which we believe to be because ketamine has unreliable effects on the brain, specifically on PL. As others have noted, the effects of ketamine on PV+ neurons in the medial prefrontal cortex, which includes PL, are variable (Benneyworth, Roseman, Basu, & Coyle, 2011). Therefore, we will publish all of our findings together in order to add to the contradictory evidence of ketamine's effect on PL and reversal learning. We do not want to hinder scientific progress by not publishing our data and have others pursue research on an unreliable observation or be unethical by only publishing a subset of the ketamine data we have collected. Should another research group begin researching anesthetic ketamine, publishing our data together would serve to temper any results in either direction and hopefully encourage more rigorous experimentation when using anesthetic ketamine.

### Haloperidol did not alter ketamine's effects on go/no-go reversal learning

We found no evidence to suggest haloperidol affected go/no-go reversal learning performance alone or in combination with ketamine exposure. While no one has examined co-administration of haloperidol and ketamine on reversal learning, our null finding is in agreement with the schizophrenia literature examining effects of acute haloperidol at the time of test on counteracting effects of previous NMDAR antagonist exposure. In these studies, rats are typically administered subanesthetic PCP and later tested on the behavioral task following acute administration of haloperidol, in an attempt to reverse deficits caused by NMDAR antagonist exposure. Several groups have repeatedly found no effect of haloperidol on reversing PCP-induced deficits on reversal learning (Abdul-Monim et al., 2006) or attentional set-shifting (McLean, Beck, Woolley, & Neill, 2008; Goetghebeur & Dias, 2009; Rodefer, Nguyen, Karlsson, & Arnt, 2007). While haloperidol does not reverse these deficits, the atypical antipsychotics clozapine, ziprasidone, sertindole and olanzapine reverse PCP-induced reversal learning (Abdul-Monim et al., 2006) and sertindole (Goetghebeur & Dias, 2009; Rodefer et al., 2007), clozapine (McLean et al., 2008; but see Rodefer et al., 2007), and risperidone (McLean et al., 2008; but see Rodefer et al., 2007) reverse attentional set-shifting deficits from PCP exposure. Our data add to the literature by showing that co-administration of haloperidol with ketamine does not affect ketamine-induced changes in go/no-go reversal learning. In addition, our results suggest that ketamine is not producing behavioral changes in go/no-go reversal learning via a D2R/$\sigma_1$ or $\sigma_2$ receptor mechanism since the haloperidol dose had no effect on how ketamine rats performed. However, while our data suggest haloperidol does not affect anesthetic ketamine-induced impairments in go/no-go reversal learning, haloperidol may be protective when ketamine produces an improvement in go/no-go reversal learning. More research is needed to determine if haloperidol can protect against the long-term effects of ketamine exposure and under what circumstances haloperidol is and is not protective.

One possible reason haloperidol failed to protect against ketamine exposure may be because the haloperidol dose was too low. The high dose (10 mg/kg) was chosen to affect $\sigma_2$

receptors and because it blocks about 75% of MK-801 induced neurotoxicity in the RSC (Farber et al., 1993). However, Morimoto and colleagues (2002) reported the haloperidol dose needed to block 50% of neurotoxicity in the RSC was 19 mg/kg. There are methodological differences between the studies that may account for the large discrepancy between effective doses. In Farber and colleagues' study (1993), the rats received the haloperidol via an intraperitoneal injection before the MK-801. Conversely, Morimoto and colleagues (2002) administered the haloperidol orally 3 hours before the MK-801. It is likely that the slower absorption from oral administration and 3-hour period between haloperidol administration and MK-801 exposure resulted in the haloperidol dose needing to be higher to be effective. This suggests our 10 mg/kg dose administered via intraperitoneal injection 20 minutes prior to ketamine exposure should be more similar to Farber and colleagues' study (1993) and at least effective at blocking neurotoxicity in the RSC, although we have not yet confirmed whether haloperidol protected the RSC. We chose to use the 10 mg/kg dose delivered via intraperitoneal injection over the higher dose delivered via oral administration to reduce variability in drug absorption and bioavailability across subjects. However, both previous studies focused their attention on the RSC, which is vulnerable to NMDAR antagonist exposure. In order for haloperidol to be protective in areas other than the RSC, the dose either may need to be higher than 10 mg/kg or haloperidol may be ineffective at blocking NMDAR antagonist exposure in regions other than the RSC. Gross observation of the rats at the time of injection suggested there were dose-dependent acute behavioral effects at both the low and high dose of haloperidol. Anecdotally, rats that received the high 10 mg/kg dose of haloperidol showed more pronounced and prolonged catalepsy than rats that received the low 1 mg/kg dose, suggesting both groups received active amounts of haloperidol.

It is also possible that haloperidol may have protected against neural changes in some but not all brain areas responsible for ketamine-induced long-term alterations in behavior, which is why we still observed a behavioral effect of ketamine. At low doses, haloperidol blocks METH-induced neurotoxicity in the DMS (Bowyer et al., 1994), an area where we have observed altered correlations between PV+ neurons and maintenance reversal learning errors in the go/no-go task (correlated in controls but not the ketamine group) (Pickens et al., in prep). As discussed earlier, these altered correlations between groups may be due to changes in the brain circuitry recruited to successfully perform the go/no-go reversal learning task. If this is the case, while haloperidol does not prevent behavioral alterations following ketamine exposure, it may have protective effects against the more subtle effects of anesthetic ketamine exposure in the brain. We are currently processing the brains for PV+ neurons to see if haloperidol was protective against ketamine-induced alterations in PV-behavior correlations.

Even though haloperidol did not protect against ketamine-induced behavioral alterations, other drugs that show efficacy in blocking NMDAR antagonist-induced neurotoxicity and reversing PCP or ketamine behavioral deficits may be effective. NMDAR antagonist neurotoxicity can be prevented in the RSC by drugs that work through the GABAergic, cholinergic, serotoninergic, dopaminergic, or noradrenergic systems (Farber et al., 1995; Farber et al., 1996; Farber et al., 1998; Farber et al., 2002; Kim et al., 1999; Olney et al., 1991; F. R. Sharp et al., 1992; Morimoto et al., 2002). Clozapine and other drugs that work through alpha-7 nicotinic receptors (α7R), are also promising candidates for future research preventing anesthetic ketamine's effects on go/no-go reversal learning. Clozapine, which directly affects the serotonergic system and indirectly affects α7Rs (Arnt & Skarsfeldt, 1998; Simosky, Stevens, Adler, & Freedman, 2003), blocks NMDAR antagonist neurotoxicity (Farber et al., 1993; Fujimura, Hashimoto, & Yamagami, 2000; Morimoto et al., 2002; Okamura et al., 2003) and reverses PCP-induced reversal learning deficits (Abdul-Monim, 2006). More selective drugs for α7Rs also block PCP-induced deficits when co-administered on Y-maze performance (Thomsen, Christensen, Hansen, Redrobe, & Mikkelsen, 2009) and reverse deficits on Y-maze (Thomsen et al., 2009) and reversal learning performance (McLean et al., 2011). Their efficacy at reversing NMDAR antagonist neurotoxicity and behavioral deficits suggests they may also be protective against behavioral changes when co-administered with ketamine. As such, there are several promising candidates to block anesthetic ketamine-induced behavior changes, even though haloperidol was ineffective.

## Conclusion

Together our data show that, in contrast to previous studies in our lab, anesthetic ketamine exposure impaired go/no-go reversal learning. These findings are in line with previous literature investigating effects of NMDAR exposure on choice reversal learning. It is unclear why the ketamine exposure results differed from our previous studies but investigating the conditions under which the discrepancy occurred could reveal important information about injection stress or neural recovery following neurotoxicity. In addition, this work suggests haloperidol does not protect against ketamine-induced behavioral impairments in go/no-go reversal learning when ketamine exposure results in impaired reversal learning performance, although further research is needed to support a complete lack of protective effect. While haloperidol was not protective against ketamine's effects in these circumstances, this null finding can direct research towards other drugs with different mechanisms, like clozapine or selective alpha-7 nicotinic agonist drugs, to determine how anesthetic ketamine exposure affects go/no-go reversal learning.

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# Table

**Table 1**

***Summary of Groups***

| | Exposure | |
|---|---|---|
| Treatment | Saline (Sal) | 100 mg/kg Ketamine (Ket) |
| Vehicle (Veh) | Veh + Sal | Veh + Ket |
| 1 mg/kg Haloperidol (Hal) | 1 mg/kg Hal + Sal | 1 mg/kg Hal + Ket |
| 10 mg/kg Haloperidol (Hal) | 10 mg/kg Hal + Sal | 10 mg/kg Hal + Ket |

# Figures

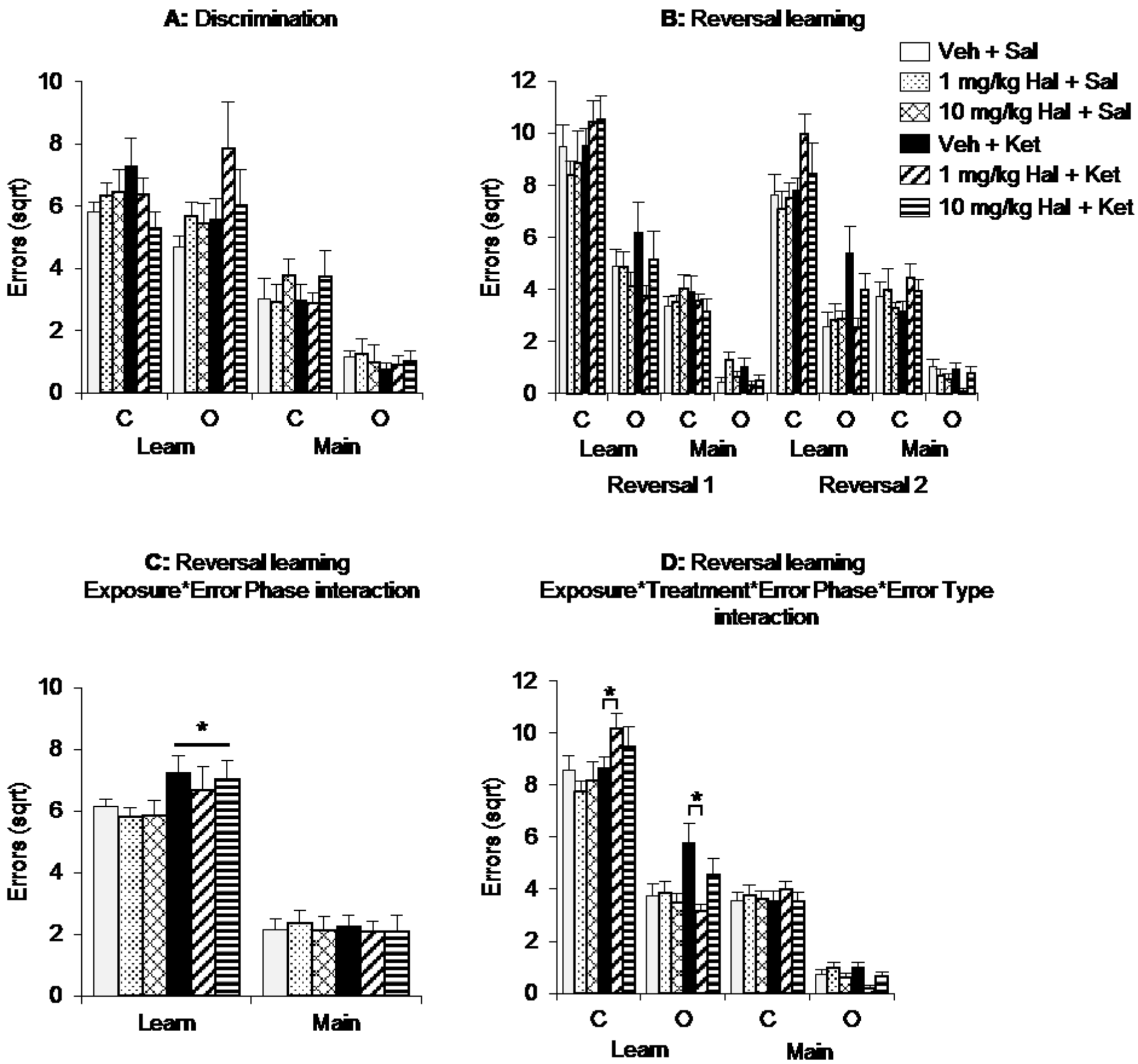



*Figure 1.* A. Errors to criterion in go/no-go discrimination. B. Errors to criterion in go/no-go reversal learning. C. Errors to criterion in go/no-go reversal learning collapsed across Reversal Phase and Error Type. * = Different from saline groups, $p<.05$. D. Number of errors in go/no-go reversal learning collapsed across Reversal Phase. * = $p<.05$.

All data are square root transformed mean±SEM. Learn = learning errors. Main = maintenance errors. C = commission errors. O = omission errors.

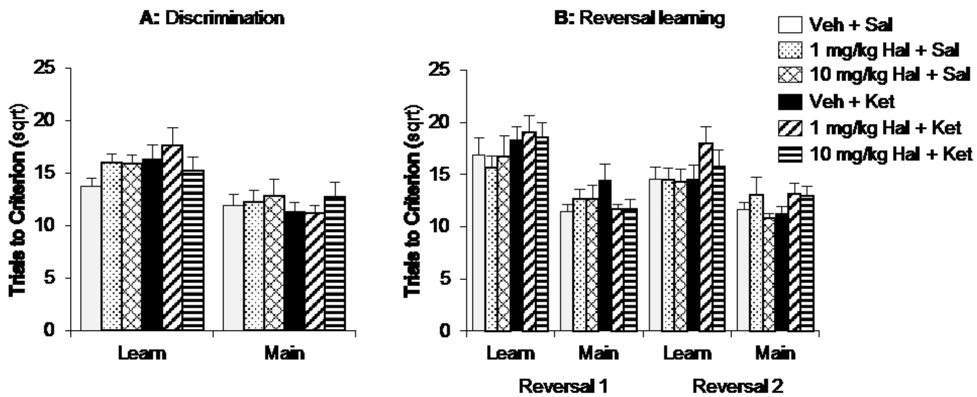



***Figure 2.*** A. Trials to criterion in go/no-go discrimination. B. Trials to criterion in go/no-go reversal learning.

All data are square root transformed mean±SEM. Learn = learning errors. Main = maintenance errors.

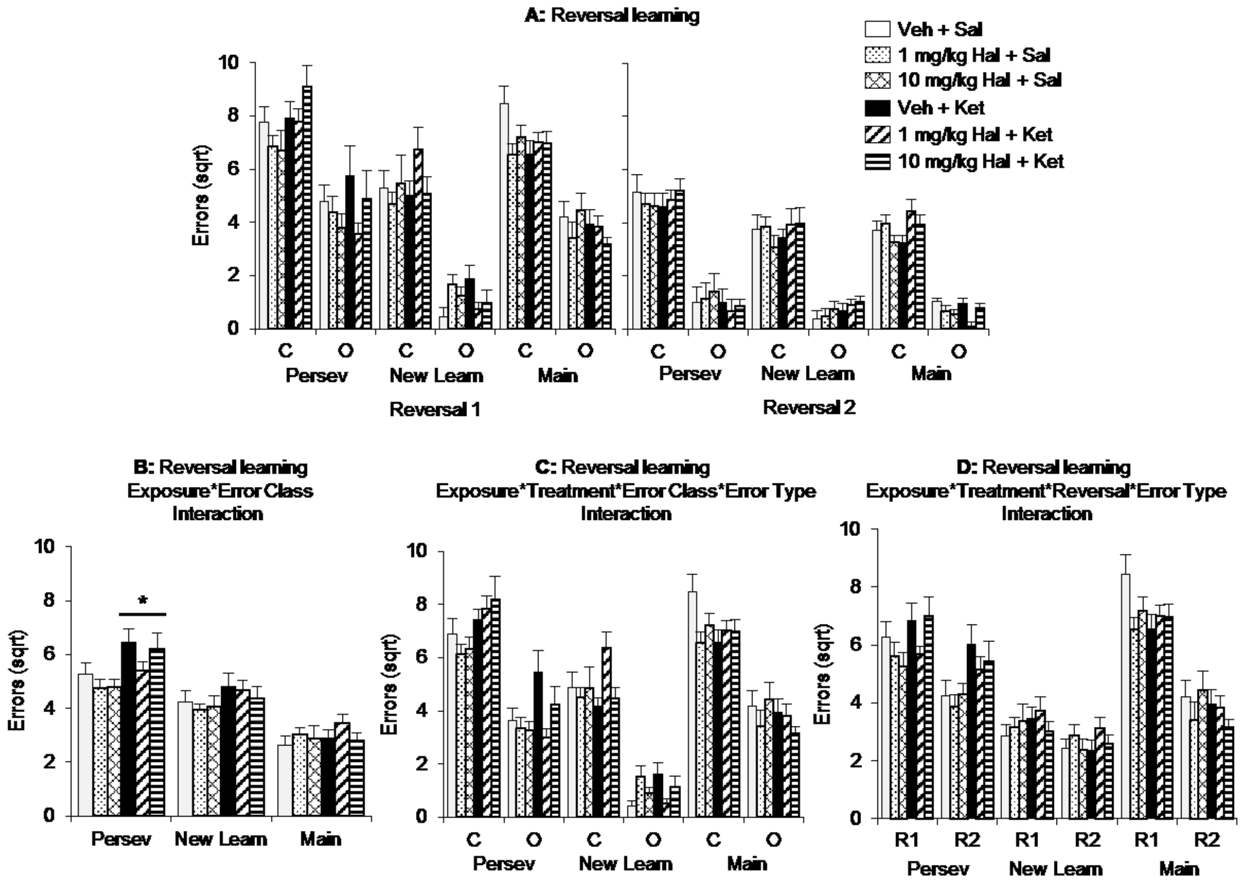


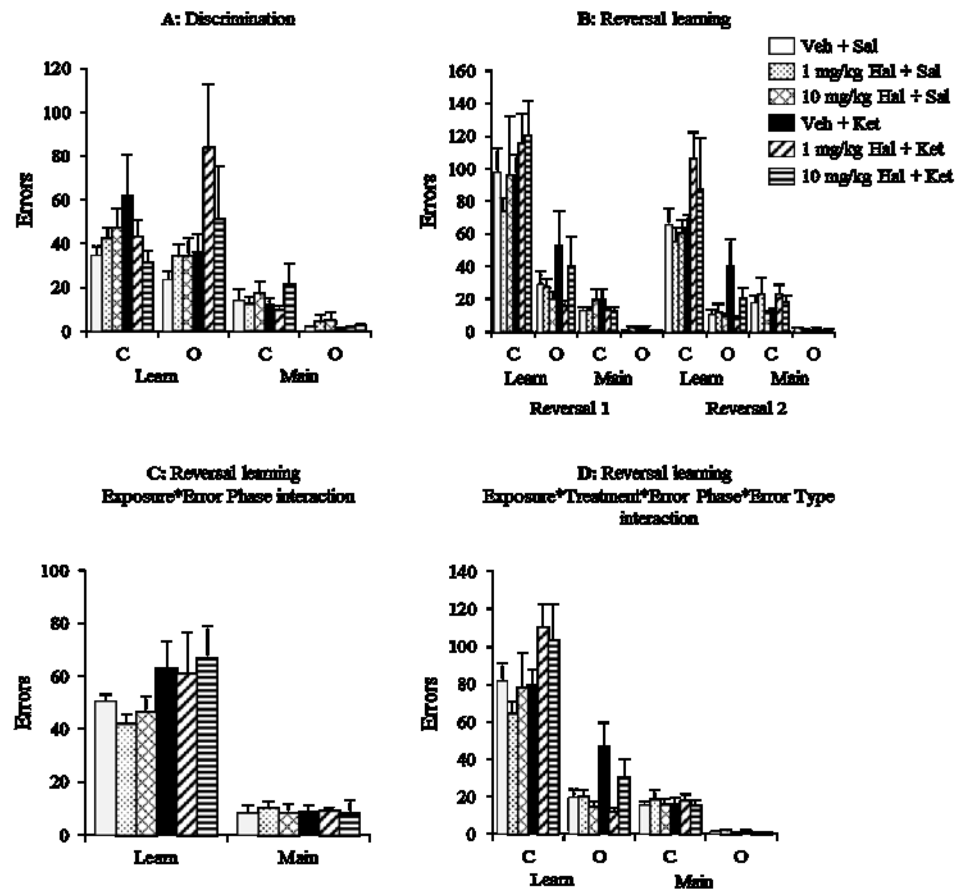

*Figure 3.* A. Errors to criterion subdivided into Error Class in go/no-go reversal learning. B. Errors to criterion subdivided into Error Class in go/no-go reversal learning collapsed across Reversal Phase and Error Type. * = Different from saline groups, $p<.05$. C. Errors to criterion subdivided into Error Class in go/no-go reversal learning collapsed across Reversal Phase and Error Type. D. Errors to criterion subdivided into Error Class in go/no-go reversal learning collapsed across Reversal Phase. E. Errors to criterion subdivided into Error Class in go/no-go reversal learning collapsed across Error Type.

All data are square root transformed mean+SEM. Persev = perseverative errors. New Learn = new learning errors. Main = maintenance errors. C = commission errors. O = omission errors. R1 = reversal 1. R2 = reversal 2.

# Appendix

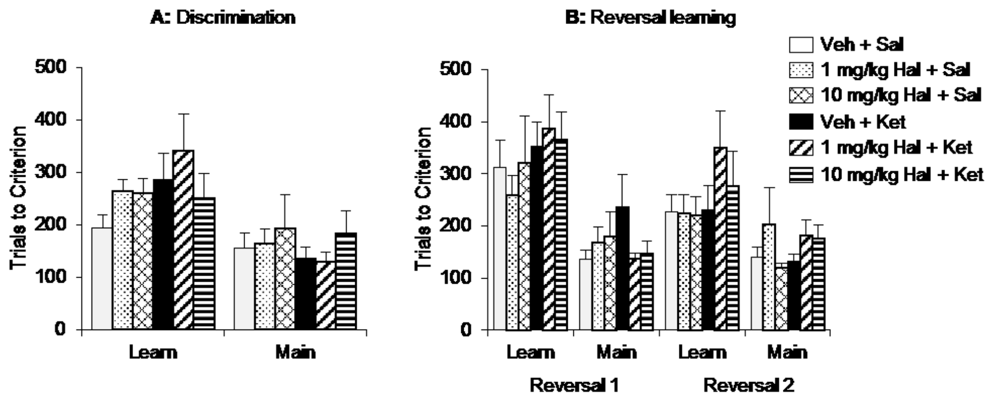
**Appendix A1.** *A. Number of errors (non-transformed) in go/no-go discrimination. B. Number of errors (non-transformed) in go/no-go reversal learning. C. Number of errors (non-transformed) in go/no-go reversal learning collapsed across Reversal Phase and Error Type. D. Number of errors (non-transformed) in go/no-go reversal learning collapsed across Reversal Phase.*

*All data are mean±SEM. Learn = learning errors. Main = maintenance errors. C = commission errors. O = omission errors.*



**Appendix A2.** *A. Trials to criterion (non-transformed) in go/no-go discrimination. B. Trials to criterion (non-transformed) in go/no-go reversal learning.*

*All data are mean±SEM. Learn = learning errors. Main = maintenance errors.*



**Appendix A3.** *A. Errors to criterion (non-transformed) in Reversal 1 subdivided into Error Class in go/no-go reversal learning. B. Errors to criterion (non-transformed) in Reversal 2 subdivided into Error Class in go/no-go reversal learning. C. Errors to criterion (non-transformed) subdivided into Error Class in go/no-go reversal learning collapsed across Reversal Phase and Error Type. D. Errors to criterion (non-transformed) subdivided into Error Class in go/no-go reversal learning collapsed across Reversal Phase. E. Errors to criterion (non-transformed) subdivided into Error Class in go/no-go reversal learning collapsed across Error Type.*

*All data are mean±SEM. Persev = perseverative errors. New Learn = new learning errors. Main = maintenance errors. C = commission errors. O = omission errors. R1 = reversal 1. R2 = reversal 2.*