Low-dose clozapine but not haloperidol attenuates ketamine-induced deficits under an incremental repeated acquisition procedure

by

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Abstract

Ketamine, like other *N*-methyl-*D*-aspartate (NMDA) receptor antagonists, causes both locomotor and cognitive dysfunction, decrements that may be mediated by distinct neurotransmitter systems. The present study was designed to characterize the contributions of dopamine and serotonin to the behavioral effects of ketamine. BALB/c mice responding under an incremental repeated acquisition procedure were administered ketamine alone and in combination with haloperidol pretreatment or clozapine pretreatment. Ketamine (1-30 mg/kg) dose-dependently decreased response rate, reinforcer rate, maximum chain length, and progress quotient (a weighted measure of overall performance). The ***Performance*** chain was more sensitive to ketamine's effect than were the ***Learning*** chains. Pretreatment with clozapine (0.1-4.0 mg/kg) dose-dependently attenuated disruption of IRA responding by systemic ketamine (30 mg/kg). No dose of haloperidol pretreatment (0.01-0.1 mg/kg) alleviated ketamine-induced IRA deficits (30 mg/kg). The effectiveness of clozapine relative to haloperidol suggests a more central role of specific serotonergic receptors over dopaminergic receptors in mediating the behavioral effects of ketamine.

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Chapter 1: Introduction

Different definitions of learning exist, and with each are attached theoretical implications about behavior. In his *Tactics of Scientific Research*, Sidman stated that learning involves the acquisition of behavior during "transition states," or those contexts under which behavior moves towards a steady state of responding (Sidman, 1961; Thompson & Moerschbaecher, 1978). Included is the notion that learning arises from the reinforced practice of behavior of interest (Kimble, 1961) and a reduction in errors whilst practicing (Thompson & Moerschbaecher, 1978). This is consistent with Staddon's assertion that reinforcement decreases the relative response strength of alternate responses (Staddon, 1977). In light of the classical operant affirmation that behavior is choice (and vice versa), learning is behavior in transition. Transition may involve changes in behavior occasioned by a shift in the schedule of reinforcement or in response requirement (emitting a well-practiced versus novel behavior).

Boren (1963) developed the repeated acquisition of behavior chains (RA) procedure to study choice in transition. RA requires subjects to emit sequences of responses that varied from session to session, requiring within-session learning. Modifications to Boren's (1963) procedure, like the now-standard multiple schedule design, captured two distinct response patterns between-sessions: a steady state of acquisition and performance. The unique baseline that results from RA lends itself to assessment of acute and chronic drug and toxicant effects (Thompson, 1973).

Developed alongside RA, the incremental repeated acquisition procedure (IRA) is a specialized procedure for studying choice in transition. Although it relies upon conditional discriminations, IRA allows for a more dynamic analysis of learning than RA. Subjects learn response sequences in increments, first emitting a single link in the chain (first or last link, dependent upon the training protocol). Following correct responses, the chain increments in length until a subject emits the full chain length, thus increasing within-session difficulty. These features allow for increased sensitivity to manipulations that affect acquisition and performance, specifically drug and neurotoxicant effects, genetic manipulations, and basic behavioral processes (Cohn & Paule, 1995; Moerschbaecher & Thompson, 1998; Bailey, Johnson, & Newland, 2010). IRA procedures currently used in our lab rely upon alternating performance and learning chains between-session (Bailey et al., 2010; Bailey, Hutsell, & Newland, 2013). While this design grants time to increment through a chain, it does not allow transition from one chain type to another (like most

RA procedures). In addition, the session duration (60min) may adversely affect interpretation of drug effects.

To advance the understanding of general aspects of learning, it is important to study neurobiological correlates of choice in transition. The glutamatergic system is important to many facets of learning and NMDA receptor (NMDAR) function is integral to glutamatergic activity. Importantly, NMDARs are necessary for long-term potentiation (LTP), a process whereby synapses in the brain strengthen through repeated stimulation. During LTP, glutamatergic-based processes involve other neurotransmitters, including dopamine. LTP is likely necessary for memory formation, signal recognition, and discriminatory functions that are integral to learning. The drug ketamine is a fast-acting noncompetitive NMDAR antagonist with a short elimination half-life in rodents. Rodent models of schizophrenia, chronic pain, and depression often employ acute and/or chronic ketamine administration. Because ketamine antagonizes NMDARs, its use may describe more about the role of NMDAR function in learning. However, a body of literature suggests that ketamine is also a potent modulator of the neurotransmitter dopamine (Kapur & Seeman, 2002; Seeman et al., 2005), with additional, but lesser, effects upon adrenergic, cholinergic, and serotonergic systems. If so, then typical and atypical antipsychotic drugs, often used to treat schizophrenia, should be able to reverse behavioral impairments caused by ketamine. Yet, these two drug classes should affect the effects of NMDAR antagonists in divergent ways. This is most likely because typical antipsychotics are vastly more potent at dopamine D_2 receptors than atypical antipsychotics. Further, some atypical antipsychotics preferentially modulate serotonin (5-HT) receptors, moreso than dopaminergic and cholingeric systems. These differences can be exemplified in studies on interval timing using peak-interval procedures that show haloperidol (typical) can shift peak times rightward (decrease in clock speed), and that clozapine (atypical) can proportionally shift peak times leftward (increase in clock speed) (MacDonald & Meck, 2005). Thus, co-administration of ketamine with a typical or atypical antipsychotic may give insight into neurobiological and environmental interactions that contribute to the effects of NMDAR antagonism.

The proposed experiment is designed to expand the utility of IRA when studying drug effects by implementing a multiple schedule design with an IRA procedure and then, using this design, examine ketamine alone or in conjunction with antipsychotic drugs to help elucidate the function of NMDARs and

DA in learning. The proposed experiment seeks to answer the following questions: 1) Can mice learn response sequences under a multiple schedule IRA procedure? 2) What are the effects of acute ketamine administration on well-learned and novel response chains? 3) Does haloperidol or clozapine attenuate the behavioral effects of ketamine? These results may increase understanding of the following: 1) The utility of a multiple schedule IRA procedure, 2) The function of component and session duration in a multiple schedule, 3) interaction between NMDARs and learning, 4) The efficacy of antipsychotic drugs to modulate deficits resulting from ketamine administration.

Repeated Acquisition

Pioneered by Boren (1963) and Boren & Devine (1968) to study the acquisition of conditional discriminations (and individual subject acquisition), RA requires subjects to emit a single response sequence within-session. Briefly, Boren & Devine (1968) shaped lever pressing in the presence of a visual stimulus (a light situated above a lever) in three rhesus monkeys. Following shaping, subjects were required to respond to a row of 12 levers, grouped in sets of three. Each lever had a light fixed above it and the lights for a single set of levers would illuminate at once (starting from left to right). Subjects were required to respond five times, in no specific order or combination, on the three levers in the illuminated set. Upon five responses, the lights switched off and the lights above the next set of levers illuminated. Again, five non-specific responses switched the stimuli above the levers, with this continuing until subjects responded on all four sets of levers. Correct four-link sequences were reinforced under a FR 2 schedule of reinforcement. Boren and Devine (1968) reinforced complete response sequences with access to a food dispenser. After stable responding, the criteria for a correct response sequence changed, such that only a response to one lever in a set was correct, advancing the chain. The same four- lever-press sequence, or performance chain, remained in place until subjects met a predetermined criterion. Finally, the sequence used in each session alternated between novel chains of lever-presses, such that the subject had to learn a new sequence each session.

Boren and Divine (1968) tested two conditions, the first of which manipulated the duration of the timeout following an incorrect response and the second of which included the addition of chain link-specific discriminative stimuli. In the former condition, the authors found that no delay following and incorrect response produced more incorrect responses whereas delays between 1s and 4min significantly

decreased incorrect responding. In the latter condition, the authors compared the impact of discriminative versus no discriminative chain link stimuli. The discriminative stimuli sessions used here paired distinct lights with the correct levers of the sequence, while no discriminative stimuli sessions had all lights above the levers illuminated. Boren and Devine (1968) found that the discriminative stimuli did not aid in emitting the response sequences (this was not a robust finding, and subsequent research has shown the importance of chain link-specific stimuli).

The Boren and Devine (1968) procedure is advantageous because it allows for the direct comparison of acute drug effects on learned and unlearned response sequences (using the same basic unit of behavior in both contexts, i.e. a nose-poke or lever-press) (Moerschbaecher, 1976). Additionally, a within-subjects design allows for comparison of an individual's behavior over time. That is, each animal provides baseline data that serves as its own control, allowing for repeated measures analysis while reducing the impact of extraneous variables. Finally, RA allows for dynamic changes in response sequences while keeping stable the unit of behavior under scrutiny. Therefore, it is possible to examine the role that specific variables play in rate and accuracy during RA (e.g. distance between levers, frequency of performance and learning sessions).

Adopting the Boren and Devine (1968) model of acquisition, Thompson and Moerschbaecher (Thompson, 1973, 1979; Thompson & Moerschbaecher, 1978, 1980) sought to streamline the RA procedure for use in evaluating acute and chronic drug effects. Thompson (1973) created a simplified RA procedure that reduced the number of manipulanda while minimizing the effect of the location in favor of discriminative visual stimuli correlated with each lever. Similar to Boren and Devine (1968), Thompson (1973) trained pigeons to emit a four-response performance sequence and, following training, learning chains alternated between-session under an FR 5 with 300 trials per session. Thompson (1973) administered four drugs under the RA procedure: phenobarbital, chlordiazepoxide, chlorpromazine, and *d*-amphetamine. Results showed that phenobarbital and chlordiazepoxide decreased accuracy as a function of dose, although a lower dose of chlordiazepoxide (10mg/kg) than phenobarbital (20-40mg/kg) achieved this reduction in accuracy. Chlorpromazine did not significantly affect accuracy, although it did increase session time. *D*-Amphetamine's effects varied amongst the birds, with a single bird showing decreases in accuracy at 0.5 and 1.0 mg/kg and, two birds showing no impairment at these doses. The

RA procedure allowed for accurate analysis of individual subjects relative to acute drug administration. Meanwhile, Thompson & Moerschbaecher (1978) applied their RA procedure to characterize the effects of a group of stimulants: *d*-amphetamine, cocaine, and fenfluramine. Results showed a dose-dependent decrease in accuracy for *d*-amphetamine and cocaine (0.3 – 10 mg/kg). Interestingly, Thompson and Moerschbaecher (1978)'s study revealed that 1.0 and 3.0 mg/kg *d*-amphetamine and cocaine did not reduce pausing between responses, reflected in the negligible effect on total trial time. Accordingly, they were able to show that RA can be sensitive to acute drug effects, even when dependent measures diverge in a dose-dependent manner.

Two of the more important modifications to Boren and Devine's (1968) RA procedure were implemented by Thompson (1979) and Thompson and Moerschbaecher (1980). First, Thompson (1979) revisited the last experiment of Boren and Devine (1968) and reassessed the value of using chain-link specific discriminative stimuli to facilitate correct response sequences. Thompson's (1979) findings showed subjects did indeed benefit from the presence of chain-link specific stimuli. Subsequent studies of RA have greatly benefited from the addition of discriminative stimuli that signal the current link in the chain, reducing incorrect responses and response latencies. Second, Thompson and Moerschbaecher (1980) altered the schedule of reinforcement, moving to a multiple schedule rather than a simple schedule. This modification allowed them to study the interaction between drug effects (*d*-amphetamine, cocaine, and phencyclidine (PCP)) and fading of discriminative stimuli by having three components,: performance, learning, and faded learning. The new multiple schedule design was able to reveal that, in monkeys, lower doses of *d*-amphetamine increased errors and decreased responding in the learning component compared to the faded-learning component. Cocaine and PCP increased errors at all doses in the learning condition relative to the faded learning and performance conditions. Additionally, cocaine and PCP decreased response rate in a dose-dependent manner. This procedure was able to show that the addition of stimulus fading may attenuate disruption by acute drug administration (Thompson & Moerschbaecher, 1980). Accurate description of chained behavior benefitted greatly from the manipulations of early researchers, e.g. timeout length, discriminative stimuli, multiple schedules of reinforcement (Boren & Devine, 1968; Thompson, 1979; Thompson & Moerschbaecher, 1978, 1980).

These modifications have also led researchers to a model of acute drug administration that is sensitive to acquisition accuracy and response rate.

RA procedures have not been limited to studying the effects of acute drug administration. The study of acute and chronic exposure to environmental neurotoxicants has also used RA procedures (Cohn, Cox, & Cory-Slechta, 1993). Rats were exposed to chronic lead (Pb) via drinking water (0, 50, 250ppm Pb acetate), then assessed using a three-response sequence RA procedure. Chronic Pb exposure caused a decrease in accuracy in the learning but not the performance component, highlighting the importance of a task that is sensitive to well learned versus novel response sequences (Cohn et al., 1993). Results also showed differential effects of lead on learning chains. That is, learning chains, though novel, may be more or less similar to the performance chain. Those chains closer in form to the performance chain tend to show a resistance to disruption by lead. Finding from Bailey et al. (2010) and unpublished data (Newland lab, 2011-2013) using IRA shows that learning chains most similar to the performance chain tend to sustain higher response and reinforcer rates as well as higher maximum chain lengths, supporting claims made by Cohn et al. (1993). RA procedures allow individuals to serve as their own baseline, and thus differences in responding can be investigated as a proportion of baseline responding. As such, examination of individual data by Cohn et al. (1993) revealed “learners” and “non-learners” within each exposure group, though more non-learners were in higher proportion in Pb-exposed groups.

In addition to animal work, human research into acute drug effects has used RA procedures. Bickel, Hughes, and Higgins (1990) used a RA procedure to assess the effects of acute and chronic benzodiazepine exposure (diazepam, alprazolam, and triazolam) in humans. Results indicated that diazepam, alprazolam, and triazolam increased the number of errors in the learning component relative to the performance component in a dose-dependent fashion. Kamien, Bickel, Higgins and Hughes (1994) also used a multiple schedule RA procedure to study the effects of acute Δ^9-tetrahydrocannabinal (THC) administration in humans. The researchers found that THC caused a small, but noticeable deficit in the learning component, and led to increased Profile of Mood State ratings of confusion, depression, and general mood disturbance (Kamien et al., 1994).

Incremental Repeated Acquisition

The range and scope of the RA procedure made it a cornerstone in understanding acquisition and the function of discriminative stimuli in chained responding. Modifications to Boren's (1963) procedure allowed for the study of acute and chronic drug and toxicant studies in humans and a range of non-human animals. RA is not without shortcomings, and alternative and advantageous chaining procedures exist. In particular, RA procedures do not allow for direct determination of errors within the sequence of responses. Furthermore, the design lacks functionality when the aim of a study is to assess the speed at which an individual can emit complete well-learned or novel chains. The IRA procedure appears to allow for more control over the response sequence, the speed of acquisition, and includes dynamic levels of difficulty better suited to detecting drug effects.

Pieper (1976) first remodeled the RA procedure into an incremental-based procedure, during which the length of desired response sequence increased within-session. Briefly, a 4-link response sequence started with a one-link chain and, upon meeting specific criteria, the chain length incremented to a two-link chain, a three-link chain, etc., within-session. The benefit of Pieper's (1976) design was that it allowed for determination of the location of errors within a given sequence. The incrementing procedure also introduced a new element of difficulty into the session. Whereas in RA the chain length was set, here the chain length increased, and the difficulty, progressed throughout the session.

The two principle methods for training IRA are the forward and backward chaining. Forward chaining begins by training the last link in the complete chain (furthest from reinforcement), with subsequent links added after the first link. Thus, responses at each link in the chain receive reinforcement. Backward chaining requires that animals first learn link 1 in the complete chain (the link closest to reinforcement). Additional links are necessarily in front of the first link, and thus the first link response remains the only response followed by reinforcement. It is debatable as to which training method produces faster acquisition, and although there are proponents of forward chaining (Weiss, 1978; Smith, 1999), the majority of IRA studies use backward chaining. A recent study showed that there are modest differences between the two, but that backward chaining is slightly more resistant to disruption (Bailey et al., 2010). Additionally, multiple schedule designs, a standard in RA, are not common to IRA

procedures. This is most likely due to the time required to increment from short to longer chains in a single session. It is common to alternate performance and acquisition chains between-session.

IRA procedures have been used to study a wide variety non-human animals, including monkeys, pigeons, rodents, and pigs (Pieper, 1976; Paule & McMillan, 1984; Ferguson et al., 2009). Mayorga et al. (2000) used an IRA procedure with rats as part of an operant test battery (OTB). The OTB is series of food-reinforced tasks designed to measure an array of dependent variables correlated with time estimation, short-term memory, motivation, discrimination, and learning. In the OTB, IRA is a standard to measure learning. Here, the researchers used IRA to assess drug effects of *d*-amphetamine and methylphenidate on learning. Rats were required to emit a chain incrementing from 1- to 6-links. Mayorga et al. (2000) featured chain-link stimuli, indicating position within the chain and used backward chaining to train the sequence of lever presses. Importantly, Mayorga et al. (2000) did not impose a consecutive correct response requirement: after 20 correct responses incremented the chain by one (similar to Frederick et al., 1995). Incorrect responses resulted in a blackout, but did not reset the counter for the current chain length. The IRA revealed non-specific effects of both *d*-amphetamine and methylphenidate – both dose-dependently decreased response rate and accuracy.

Wenger, Schmidt, and Davisson (2004) used an IRA procedure to assess learning in Ts65Dn mice (a mouse model of Down syndrome; Baxter et al., 2000) relative to control mice. Wenger et al. (2004), similar to the OTB, implemented backward chaining and used a 6-link chain. They found that at short chain lengths (1-link and 2-link), there was little difference between the two strains of mice. However, Ts65Dn mice exhibited marked difficulty when they reached 3- and 4-link chains compared to the controls. Because of the incrementing nature of the IRA procedure and the progression from acquisition of simple to complex chains within-session, Wenger et al. (2004) were able to describe a difficulty-induced learning deficit in the Ts65Dn mice. In addition to the normal incrementing difficulty of the IRA procedure, Wright & Paule (2007) noted that different response chains are not equal in difficulty and analyses of acute drug effects need account for differences in chain type. Thus, accuracy, even at short chains, is subject to bias (perhaps because of order of sequences, response and/or reinforcer delivery location (Wright & Paule, 2007).

Most recently, IRA has been used in humans to assess learning in children. Paule et al. (1999) investigated a possible correlation between performance on the OTB and intelligence. Children emitted response sequences on four levers with lights located above. The lights signaled the remaining number of responses in the current sequence. Additionally, participants received feedback as to whether their most recent response was correct or incorrect via white stimulus lights located to the left and right of the levers. Similar to Mayorga et al. (2000) and Wenger et al. (2004), the procedure used a six-link incrementing chain (generated randomly before each session). Results from Paule et al. (1999) showed that participants' full scale IQ was highly correlated (R=0.532, p<0.0001) with accuracy during the IRA component of the OTB. Most recently, Baldwin et al. (2012) used IRA with backward chaining to train response sequences in children with varying IQs, ages, and sexes. Like Paule et al. (1999), children received feedback following responses, with incorrect responses illuminating the "incorrect-answer light" for 2s. Baldwin et al. (2012) found that children with higher IQs reached longer chains and did so with higher accuracy than children with lower IQs. In addition, the authors found an age-dependent increase in accuracy and percent-task completed. Thus, IRA procedures have efficacy when measuring learning under acute and chronic drug and toxicant exposure in a variety of animals. There is also a great deal of reliability in the translation to human behavior, as noted by Paule et al. (1999) and Baldwin et al. (2012).

Glutamate

The neurotransmitter glutamate is the predominant excitatory amino acid in the mammalian brain and implicated as a key component in learning, memory, and synaptic plasticity, as well as a major component of drug addiction (Kalivas, 2009). Two broad classes of glutamate receptors identified thus far are metabotropic and ionotropic. The excitatory ionotropic receptors contain three receptor types: NMDA, AMPA, and kainate receptors. Briefly, the NMDAR is a voltage-dependent and ligand-gated ion channel, composed of an amalgamation of subunits (Laurie & Seeburg, 1994; Sun et al., 1998). Interestingly, NMDARs have numerous binding sites, though the most salient binding sites are for the neurotransmitters glutamate and glycine. Additionally, there are binding sites for Mg^{2+} and phencyclidine (PCP) (Dingledine et al., 1999; Williams et al., 1989; Javitt, 2007). Located within the ion channel, binding at the Mg^{2+} and PCP sites requires depolarization of the cell membrane. The PCP binding site is a common target for several noncompetitive NMDAR antagonists, including ketamine and MK-801 (Javitt,

2007). Binding of both glutamate and glycine *and* depolarization of the cell membrane causes the removal of the Mg^{2+} bound in the ion channel, a crucial component of Ca^{2+} and glutamate homeostasis. The dynamics of NMDAR structure and binding are integral to synaptic transmission, long-term potentiation (LTP), dendritic spine density and function, and a host of other roles. Glutamatergic neurons function as interneurons, and as such, they diffusely synapse with a variety of other neurons. For example, these synapses form in abundance with dopaminergic neurons in the basal ganglia. Together, the interdependent actions of glutamate and dopamine likely modulate reinforcement, learning, and memory.

Dopamine

Dopamine (DA) is a predominant catecholamine neurotransmitter with expansive innervations in mammalian brains, implicated in the regulation of learning and reinforcement properties. DA has two distinct groups of G protein-coupled receptors: D_1-like (D_1 and D_5) and D_2-like (D_2 - D_4). D_1-like activation results in stimulation of adenylate cyclase (AC) and subsequent stimulation of cAMP, while D_2-like activation reduces adenylate cyclase activity (Seigel et al., 2006). D_1 and D_2-like receptors are most commonly located post-synaptically, although a smaller percentage of these receptors are located pre-synaptically. Presynaptic D_2 receptors function as autoreceptors (provides a negative feedback loop capable of regulating firing rate, synthesis, and release of DA) (Garris et al., 2003; Nicola et al., 1996; Behr et al., 2000; Paspalas & Goldman-Rakic, 2005; Iverson et al., 2009). DAergic neurons form three distinct pathways in the rodent brain: nigrostriatal (substantia nigra to the striatum), mesolimbic (VTA to NAcc), and mesocortical (VTA to the cortex) (Hu et al., 2004). Because of this extensive circuitry, DA functions to regulate movement, learning, reinforcement, sleep, memory, and attention processes. Outside of the CNS, DA functions to regulate hormone release, cardiovascular functions, and renal functions, among others.

D_1 and D_2-like receptors are located throughout the brain, with the highest concentration of D_1 receptors found in the nigrostriatal, mesolimbic, and mesocortical pathways (Beaulieu & Gainetdinov, 2011). DAergic activity is involved with many processes, and D_1, D_2, and D_4 receptors function to facilitate movement and play a role in reinforcement-mediated learning. Reinforcement-induced DA firing is discriminatory and in many circumstances context-dependent. That is, unpredicted presentation of

reinforcers leads to phasic DA firing important for associative learning (stimulus-reinforcer relations). Repeated administration of a static reinforcer leads to reduction in DAergic activation. In addition, presentation of a lesser reinforcer following administration of more preferable reinforcer leads to a depression in DA firing (Schultz, 1997, 2013). Taken together, these observations suggest that a threshold exists, and reinforcer delivery must reach or surpass this threshold to engage DA firing (e.g. repeated delivery of the same reinforcer results in a net decrease in DA firing).

Serotonin (5-HT)

Evolutionarily, 5-HT is one of the oldest neurotransmitters with an extensive and diverse receptor-family. 5-HT belongs to the GPCR family (with the exception of 5-HT_3, which is a ligand-gated ion channel), similar to DA. There are at least14 5-HT receptors and their functions are widespread throughout the body (Hoyer et al., 2002; Hannon & Hoyer, 2008). Most importantly, 5-HT_1 and 5-HT_2 receptor function contribute to learning and memory, with greater localization in limbic structures, like the hippocampus. These receptor groups are also the most common targets for pharmacological manipulation (i.e. anxiolytics, antipsychotics, gastrointestinal medicines). 5-HT_1 receptors couple to G-proteins to inhibit cAMP phosphorylation. This group includes the receptors subtypes 1A, 1B, and 1D (1C is now 5-HT_{2C}), which may function pre-synaptically as autoreceptors or heteroreceptors or post-synaptically by modulating fast excitatory postsynaptic potentials. Moreover, subtype 2C has been shown to regulate mesolimbic DAergic function, exerting an inhibitory influence over DA signaling (Nichols & Nicols, 2008). That the function of several prominent 5-HT receptor subtypes are enmeshed with GLU and DA function may point to the mechanisms by which atypical antipsychotic medications, including clozapine and ziprasidone, exert their influence.

Neurotransmitter signaling

Glutamatergic and dopaminergic functions combine in learning and reinforcement, and long-term potentiation (LTP) may represent a modality that accounts for a portion of their interactions. Post-synaptically, activity-dependent activation of NMDARs leads to a torrent of downstream signaling. First recognized in 1966, Bliss & Lømo (1973) found that prolonged high-frequency stimulation (tetanic stimulation) increased the efficacy of synaptic transmission. This led to a greater understanding of the Hebbian nature of synaptic transmission and plasticity. Generally, LTP is NMDA-dependent, although the

entire process requires many components.. Support for a LTP-mediated model of memory and learning garners support from robust findings that both LTP and memory require synaptic restructuring, as well as the hippocampal pathways that utilize LTP (Lynch, 2004). DA receptor activity may play a more predominant role in LTP than previously thought, as some medium spiny neurons of the basal ganglia require D_1 activation at glutamatergic synapses for LTP (Surmeier et al., 2011). Thus, LTP within the basal ganglia (particularly the striatum) may be dependent upon glutamatergic activation and inhibition by D_1 and D_2 receptor activation, respectively.

Stimulation of DAergic neurons also appears to have regulatory effects upon NMDA and AMPA receptors. Again, DA receptor subtype is important, as D_1 and D_2 receptors appear to control different processes. In particular, D_1 activity-induced PKA cascade facilitates AMPA and NMDA receptor function. The converse appears true of D_2 activity, causing diminished AMPA receptor conductance and trafficking (Surmeier et al., 2007). In addition to modulating AMPA and NMDARs, DAergic neurons may be able to release glutamate. Tecuapetla et al. (2010) were able to show, using a variety of techniques, that mesolimibic DAergic neurons released glutamate as a signaling mechanism to NAcc target sites. This may represent a novel reinforcement-signaling pathway that corresponds to transient DA firing following delivery of a reinforcer. Discussed in the appendix are more complex and vetted examples of DA and GLU interactions. A line of evidence also shows that GLU activity regulates DA firing. DAergic neurons in the SN express glutamatergic NMDA and AMPA receptors, as Christoffersen & Meltzer (1995) showed that *in vivo* application of glutamate in rats increased nigrostriatal DA firing. Importantly, Blythe et al. (2007) observed that *in vitro* activation of dendritic NMDA and AMPA receptors generated transient high frequency firing in SN DAergic neurons. High-frequency stimulation of DAergic neurons in the SN thus appears integral to LTP and synaptic plasticity. Thus, activation of GLU receptors may similarly be a mechanism that drives DA-modulated reinforcement learning.

The neurobiological effects and behavioral manifestations of drugs that modulate NMDA (ketamine) and DA receptors (haloperidol, clozapine) are likely to be complex. The following is a brief review of the pharmacology of ketamine, haloperidol, and clozapine. Included are seminal findings of the behavior effects of these drugs administered alone, and in combination with one another.

Ketamine

Pharmacology. Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, binds to the PCP site on NMDARs, blocking the Ca^{2+} channel. The immediate consequence of NMDAR blockage is a decrease in glutamatergic activity, thought to produce ketamine's effects. Convincing evidence for this assertion, originating from models of schizophrenia, describe a lack of control by NMDAR over GABAergic neurons following prolonged NMDAR antagonism. Glutamate hypofunction as well as cortical hypofunction are hallmarks of NMDAR antagonism (via ketamine, PCP, and MK-801), often presenting in humans diagnosed with schizophrenia (Anand et al, 2000; Brody et al., 2003). Yet, a decrease in glutamate is just one result of NMDAR antagonism. Hypotheses as to other mechanisms of action point to hyperfunction in the prefrontal cortex, specifically to increases in extracellular glutamate, glutamine, and GABA (Moghaddam et al., 1997; Maeng et al., 2008), as well as increases in ACh efflux (Nelson et al., 2002). Though compelling, these findings are somewhat at odds with literature indicating glutamate *hypofunction*. It is likely that ketamine's effects are due, in part, to glutamate hypofunction and downstream hyperfunction, as well as the activation and deactivation of multiple systems (Trujillo et al., 2011). Effects of ketamine also include those effects that stem from the active metabolite norketamine. It exhibits approx. 20-30% of the potency of ketamine, with an elimination half-life greater than its parent compound (approx. 5h and 2-3h, respectively). Norketamine may contribute to the observed behavioral effects, particularly the lasting analgesic effects seen in chronic pain experiments (Blonk et al., 2010). That ketamine exerts robust GLUergic action plausibly implies the recruitment of interconnected systems downstream, specifically DA.

DAergic modulation is a fundamental consequence of NMDAR antagonism, demonstrated in numerous *in vivo, in vitro,* and *in situ* studies. Specifically, French et al. (1990, 1993) showed that MK-801 and PCP (both NMDAR antagonists) increased burst firing in the VTA DAergic neurons of rats. Similarly, Hondo et al. (1994) showed that PCP increased the PFC DAergic activity of rats. In a mutant mouse model of schizophrenia (mice lacking the GluRζ subunit), Miyamoto et al. (2001) found that mutant mice showed increases in DA metabolism in the frontal cortex and striatum (postmortem). In support of this evidence, Chatterjee et al. (2011) found that acute and chronic doses of ketamine in male Swiss albino mice increased levels of DA and its metabolite DOPAC in the striatum, while increasing the DA metabolite

HVA in the cortex, striatum, and hippocampus. Further analysis showed that ketamine increased gene expression for DA D_1 and D_2 receptors, as well as DAT and tyrosine hydroxylase (TH) (Chatterjee et al., 2011). These studies coincide with reports of hyper-dopaminergic function in schizophrenia and correspond with human research showing exacerbated DAergic responses to *d*-amphetamine in patients with schizophrenia, interpreted as the net result of NMDAR hypofunction and DA hyperfunction (Kegeles et al, 2000). In 2000, Vollenweider and colleagues used PET to identify increases in extracellular DA in the striatum of healthy male participants following administration of the (S) enantiomer of ketamine (using a subanesthetic dose).

In addition to the downstream effects of ketamine on DA levels, ketamine itself appears to bind with DA receptors. Kapur & Seeman (2002) and Seeman et al. (2005) showed that ketamine has direct binding affinity for DA D_2 receptors in the high state[1]. The binding affinity of ketamine for D_2^{HIGH} receptors relative to NMDARs is drastically greater, 55nM and 3100nM respectively. PCP showed even greater binding affinity at D_2^{HIGH} receptors relative to NMDARs (2.7nM and 313 nM, respectively) (Kapur & Seeman, 2005). These results and the studies mentioned above elucidate hyper-dopaminergic function resulting from ketamine administration that co-occurs with glutamate hypofunction. It is important to note that the neurobiological effects of ketamine may also depend upon the dosing regimen, as acute and chronic studies of ketamine yield discrepancies. For example, Li et al. (2010) found that acute ketamine increased spine density in cortical neurons, and by contrast, Ramsey et al. (2011) found that sub-chronic MK-801 decreased spine density in the striatum.

The distribution and elimination of ketamine from the body varies between non-human animals and humans, and depends on route of administration. The distribution throughout the body and brain is rapid and early phase elimination is rapid. The drug is highly lipid-soluble (as much as 5-10 times more so than thiopentone), and as such, it easily crosses the blood-brain barrier (Cohen & Trevor, 1974; Ghoneim & Korttila, 1977). Ketamine binds as much as 47% to plasma proteins in humans, relatively more than other animals (dog, 33%; baboon, 21.8%; monkey, 29.5%) (Dayton et al., 1983). In male Sprague-Dawley rats, Cohen & Trevor (1974) found, *in vitro*, near maximal uptake of ketamine in cerebral cortex slices

[1] Dopamine D_2 receptors exist in two basic states, high and low affinity. In the high-affinity state, they are coupled with second messenger systems, responsible for functional effects. In the low-affinity state, they are functionally inert (Graff-Guerrero et al., 2009).

after 5 min (solution of 10 ug/ml ketamine HCL). In the same study, testing brain levels of ketamine after a bolus *i.v.* injection found that levels spiked 1 min after injection to near 100 ug/g and declined thereafter, approaching 1ug/g 60 min after injection (Cohen & Trevor, 1974). Similarly, following *i.v.* injection of 30 mg/kg ketamine, Marietta et al. (1976) found maximum brain and plasma levels of approx. 90 ug/g and 10 ug/ml, respectively, 1 min post-injection. Ketamine levels in the liver, muscle, and gut appeared to peak at 10 min, while skin samples peaked at approximately 20 min. Similar studies in rodents by Marietta et al. (1977) and Livingston & Waterman (1978) have found comparable brain and serum concentrations. Maxwell et al. (2006) studied pharmacokinetic differences in four strains of mice (C3H, FVB, C57, and DBA). Mice were injected *i.p.* with 100 mg/kg ketamine, and sacrificed at 5, 10, 20, 30, and 60 min post-injection. Serum concentrations at 5 min were between 37-40 ug/ml and near 1.0 ug/ml after 60 minutes. In an additional experiment, Maxwell et al. (2005) also showed that serum concentrations of ketamine, taken 15 min post-injection, were 3.33 times greater than brain concentrations (ug/ml and ng/mg, respectively) in FVB mice. Collapsed across strains, the elimination half-life of ketamine was 13min, C3H mice exhibiting the shortest half-life of 11.3min. Ketamine elimination may be 9-15 times faster in the rodent than human. One drawback is that Maxwell et al. (2005) did not sacrifice at 1 min post-injection, and the initial rapid spike and peak concentration may be missing. The distribution and elimination time courses appear stable across a variety of non-human animals, including rabbits, cats, dogs, and elephant seals (Pedraz et al., 1985; Hanna et al., 1998; Pypendop et al., 2005; Woods et al., 1999).

The above literature uses a two-compartment open model to describe the pharmacokinetics of ketamine (often expanded to four compartments when including metabolites). The two-compartment model often describes two phases, α and β, (also referred to as initial and later phases) that relate distribution and elimination of ketamine, respectively. Thus, the distribution half-life of ketamine in the α phase is rapid, while the half-life of ketamine in the β phase is much slower.

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq.1)}$$

In Eq. 1, C is the concentration of ketamine at a given time *t*, *A* and *B* are zero time intercepts, and α and β are first order rate constants for the distribution and elimination phases of ketamine, respectively.

The elimination of ketamine from plasma and brain points to a bi-exponential curve for the concentration following *i.v.* and *i.p.* administration of ketamine. As the literature above describes,

ketamine levels in plasma and brain decrease rapidly in the initial phase of elimination, and decline slowly thereafter. The results of Maxwell et al. (2005) do not appear bi-exponential, likely a result of the starting sampling time-point (5 min post-injection).

Behavioral effects. To date, the focus of ketamine research centers on its use in animal models of schizophrenia (sensorimotor gating, prepulse inhibition of startle responses) and cataloging NMDAR function in processes of memory (many studies focus on spatial and working memory) and basic learning (conditioned place preference, fear conditioning). Contemporary insight into NMDAR structure and binding has led to new therapeutic uses for ketamine. In humans, subanesthetic doses of ketamine aid in the treatment of anxiety, treatment-resistant depression, and chronic pain management. The following review emphasizes ketamine research (also included are several studies using PCP and MK-801) utilizing methodologies better suited to the study of behavior-in-transition.

In a seminal experiment investigating behavioral effects of ketamine, Wenger and Dews (1976) administered a spectrum of psychoactive drugs, including amphetamine, PCP, and ketamine, to male C57 mice. Subjects level-pressed under a two-component multiple FR30 FI300s schedule, each schedule component correlated with a discriminative stimulus (green light or clicking relay, respectively). Wenger and Dews (1976) noted schedule-specific effects of PCP and ketamine. Low doses of PCP (0.3, 1.0 mg/kg) produced negligible differences between schedules. Moderate to high doses (3-30 mg/kg) showed a biphasic response pattern under the FI 300s, with a sharp rate increase at 3.0 mg/kg followed by a decline at 10-30 mg/kg to near zero levels relative to baseline. Response rate decreased monotonically at moderate to high doses (3-30 mg/kg) under the FR 30. Effects of ketamine on response rate were similar to PCP. At low doses (1.0-10 mg/kg), there was no difference in either schedule relative to baseline. Moderate to high doses (30-180 mg/kg) produced schedule-dependent differences, with a biphasic curve under the FI 300s; increase in rate until 180 mg/kg. Under the FR 30, response rate declined in a monotonic fashion, similar to PCP. Notably, for PCP and ketamine, low control rates correlated with greater drug-induced rate increases, the inverse being true as well. Importantly, Wenger & Dews' (1976) experiment elucidated the schedule and baseline rate-dependent effects of ketamine. Additionally, the FI 300s schedule revealed differences between the potency of PCP and ketamine.

There exists little research combining rodent behavior under incremental or simple repeated acquisition procedures and NMDAR antagonists, but there is primate research to draw upon. Thompson and colleagues (1980, 1984, 1987) studied ketamine and PCP effects using RA procedures with patas monkeys. Ketamine and PCP alike dose-dependently increased errors and decreased response rate. In all three studies, ketamine and PCP markedly disrupted acquisition to a greater extent than performance (Moerschbaecher & Thompson, 1980; Thompson et al., 1987). The success of RA primate research with NMDA antagonists led directly to multifaceted primate research with similar compounds. Frederick, et al. (1995) used a version of the OTB (previously described) to assess acute effects of PCP in male rhesus monkeys. Under a progressive ratio (PR) schedule, lever-presses to one extended lever received reinforcement. The PR value increased within-session by adding the starting PR value following each reinforcer delivery (i.e. 2, 4, 6, 8, etc.). PCP reduced breakpoints and increased pausing at doses of 0.13 mg/kg and higher, although the lowest dose of 0.003 mg/kg increased the frequency of short duration IRTs (Frederick et al., 1995). In the IRA portion of the OTB, four levers extended into the operant chamber, and responses to a single correct lever (IRA1) resulted in reinforcer delivery. Following 20 total correct responses, the chain incremented in length, required two a two-lever sequence of responses (IRA2) for reinforcer delivery. After 20 correct two-lever responses, the chain incremented again (max six-link chain). Visual stimuli signaled position within the chain, and incorrect responses resulted in a 2s ITI blackout period. Results showed that PCP dose-dependently increased acquisition errors relative to the performance condition. At the highest dose (0.30 mg/kg), no subject was able to advance beyond a 1-link chain. Interestingly, low to moderate doses of PCP (0.01-0.13 mg/kg) produced some decrements in IRA that did not appear on the short-term memory task (Frederick et al., 1995). Taffe et al. (2002) expounded upon these results, using a version of the OTB with ketamine administration in rhesus monkeys. The PR schedule produced results similar to Frederick et al. (1995), with 1.0 and 1.78 mg/kg reducing breakpoint. A delayed match-to-sample task replaced IRA in the battery. Again, doses of 1.0 and 1.78 mg/kg decreased accuracy in a delay-dependent manner of which the most significant reductions occurring at the longest delays.

In work with rodents, measures of impulsivity have been used to provide insight into the mechanism of action of NMDAR antagonists. Impulsivity is a construct often dimensioned into impulsive

action and impulsive choice (Evenden, 1999). Impulsive action is generally thought to comprise behavior that manifests as an inability to withhold a response, while impulsive choice represents the selection of a smaller, more immediate reinforcer over a more favorable, but delayed reinforcer. Often, measurement of impulsivity utilizes indirect methodology. For example, the five-choice serial response time task (5-CSRTT), modeled as an attentional task, has an element of impulsivity. Premature responses or an inability to inhibit responses may be an indicator of impulsive action. Findings from 5-CSRTT experiments reveal that PCP, MK-801, and ketamine dose-dependently increase premature and perseverative responses (Greco et al., 2005; Oliver et al., 2009; for a review, see Amitai & Markou, 2010).

In a more direct measurement of impulsivity, Cottone et al. (2013) used a modified adjusting delay procedure (similar to Blasio et al., 2011) with late-adolescent male Wistar rats and a liquid reinforcer. Following training, sessions consisted of blocks of trials. Within a block, two levers extended; one correlated with a short delay (1s) and a sucrose reinforcer, the other correlated with a long delay (6s) and a super-saccharin reinforcer (preferred). Here, the first two trials of each block were forced-choice and the second two free-choice. Free-choice responses to the smaller sooner lever decreased the larger later delay by 1s, while responses to the larger later increased the larger later by 1s (delays bound between 0-36s). Subjects received *i.p.* injections of ketamine or memantine (also a noncompetitive NMDA antagonist) 30 min prior to adjusting-delay sessions[2]. Ketamine decreased the mean adjusting delay (MAD) in a dose-dependent manner, with the highest doses (10 and 20 mg/kg) significantly decreasing the MAD. The highest doses significantly increased the "impulsivity score," a direct function of the obtained, maximum, and minimum MAD (Cottone et al., 2012). Based upon the results, a binary split of subjects produced low and high-impulsive groups. Ketamine preferentially affected the low-impulsive group, increasing choice for the immediate reinforcer at 10 and 20mg/kg, not seen in the high-impulsive group.

Established tests of ketamine-induced memory deficits often employ a T-maze or Morris water maze with varying delays, similar to Imre et al. (2006). Results from the test showed that 12 but not 8 mg/kg decreased accuracy relative to vehicle (saline) injections. The findings of Imre et al. (2006) support a multitude of literature showing NMDAR antagonist-induced working and spatial memory deficits in T-

[2] Subjects also received microinfusions of the competitive NMDA antagonists D-AP-5 and CGS19755, although not discussed here.

mazes (Verma & Moghaddam, 1996; Chrobak et al., 2008) and water mazes (Tsien et al., 1996; Sabbagh et al., 2012). Galizio et al. (2003) adapted the Morris water maze to use a multiple schedule RA procedure. In the performance component, the elevated platform never changed position but its location varied in the acquisition component. In male Hotlzman rats, PCP dose-dependently increased latency and path ratio without affecting speed. Most importantly, the RA procedure revealed increased latency and path ratio during acquisition relative to performance at 3.0 mg/kg.

Featherstone et al. (2012) used a progressive ratio schedule (PR) and extinction to test the effects of subchronic (14 daily injections) ketamine on male C3H mice. The PR value was adjusted such that after three reinforcers were earned, the ratio was increased by three (i.e. 1,1,1,4,4,4,7,7,7,…etc.). Extinction, or discontinuation of reinforcer delivery, lasted two sessions. The authors found no differences in saline or ketamine-treated mice under the PR schedule, but distinct differences emerged under extinction. Ketamine-treated mice responded significantly more than did control mice on the second, but not the first, day of extinction, the implication of which is a deficit in encoding and behavioral flexibility (Featherstone et al., 2012). Additionally, the fact that control and ketamine-treated mice looked qualitatively similar on the first day suggests that both groups acquired the extinction similarly, yet the ketamine-treated mice displayed a deficit in cognitive flexibility, i.e. the speed at which they adjusted to the contingencies during extinction.

Haloperidol

Pharmacology. Considered a typical antipsychotic drug, haloperidol belongs to the butyrophenone drug class and commonly used in the treatment of schizophrenia and stereotypies in development disorders (Cohen et al., 1980). Characteristic of most typical antipsychotics, haloperidol is a highly selective and potent DA D_2 antagonist. In human cloned cells, haloperidol has a dissociation constant of 0.55, more than 100 times less (thus more potent) than that of some atypical antipsychotics, including quetiapine and clozapine (Seeman, 2002). The mechanism by which haloperidol exerts its behavioral effect appears to be blockade of postsynaptic D_2 receptors throughout the basal ganglia. Recently, new insight into D_2 autoreceptor regulation of DA release and reuptake has led to the theory that haloperidol disrupts presynaptic D_2 autoreceptors (Wu et al., 2002; Garris et al., 2003; Frank & Reilly, 2006). It has been posited that a portion of haloperidol's effects result from this blockade, which leads to a

disinhibition of DA synthesis and release into the synapse. Subsequently, antagonism of autoreceptors quickly leads to pharmacodynamic tolerance, causing a downregulation in extracellular DA, allowing haloperidol to bind with postsynaptic DA receptors (Garris et al., 2003).

Haloperidol also increases the density of D_2 receptor sites in primary visual and motor cortical regions, as well as the somatosensory and temporal association regions (Lidow & Goldman Rakic, 1994). These increases in D_2 receptor density are coupled with reductions in the density of D_1 receptors in the PFC and association cortex. More recently, haloperidol has been shown to occupy $D_{2/3}$ receptors of the midbrain and left and right temporal poles to a greater degree than olanzapine or clozapine. In addition, haloperidol increases NMDA-evoked depolarization, the frequency of NMDA-evoked EPSPs, and NMDA-evoked inward current, but reduces the amplitude of NMDA-evoked EPSPs and AMPA-evoked inward current (Arvanov et al., 1997).

Haloperidol is highly lipophilic and is widely metabolized in human patients, with maximal plasma concentrations after 20-33 min and an elimination half-life of 14-26 h. This time-course is similar to that seen in rhesus monkeys, with plasma elimination half-lives between 7.56-15.97 h (Stafford et al., 1981). A consistent finding is that, much like ketamine, haloperidol preferentially accumulates in brain at concentrations 10-30 times higher than in plasma (given a therapeutic dose in humans). Kornhuber et al. (1999) found the elimination half-life from human brain tissue to be approx. 6.8 days. Zetler & Bauman (1985) found maximal serum concentrations of haloperidol in CF-1 mice following *s.c.* 0.6 mg/kg after 2 min. Brain levels rose steadily to maximal levels at 15 min, and accumulation in brain was approx. 40 time greater than the serum concentration. Consistent with findings in humans, the elimination half-life of haloperidol from rodent brain tissue has been reported to be approx. 6.6 days, and haloperidol was detectable via HPLC 21 days after administration of 1 mg/kg *i.p.* (Cohen et al., 1992). In Fischer-344 rats, Kapetanovic et al. (1982) found the greatest concentration of haloperidol in the frontal cortex, following by the striatum, mesolimbic DA pathway, and finally the cerebellum following *i.v*. 0.50 mg/kg haloperidol. The decline in haloperidol plasma concentrations following *i.v.* or *i.p.* administration is biphasic in nature for both humans and rodents (Kudo & Ishizaki, 1999; Kapetanovic et al., 1982)

Typical antipsychotics like haloperidol still receive widespread usage in the treatment of acute and chronic schizophrenia as well acute mania in adults with type I bipolar disorder (Hegerl, 2012).

However, extrapyramidal side effects (EPS) often accompany haloperidol treatment in patients. To assess the preclinical effects of a number of antipsychotic drugs, Varvel et al. (2002) used a multiple FR30 FI60-s schedule of reinforcement. Sessions alternated between the FR and FI components twice, with a discrete auditory stimulus correlated with the FI component. Haloperidol dose-dependently decreased response rate and increased response duration in both components. Haloperidol-induced reductions in response rate did not differ from those produced by relative doses of the atypical antipsychotics quetiapine, olanzapine or clozapine. However, increased response durations were only seen in haloperidol and risperidone, not in any atypical antipsychotics (Varvel et al., 2002). In the 5-CSRTT, relative to vehicle, 0.1 mg/kg haloperidol did not decrease accuracy, increase anticipatory responses, or perseverative responses in male Lister hooded rats (Carli et al., 2011). Haloperidol may increase sensitivity to extinction under FR requirements. This may be representative of the interaction between FR schedules and DA depletion, as findings are not consistent for all schedules. For example, Paterson et al. (2010) found that haloperidol increased responding under a DRL 72-s schedule. Yet, Salamone (1986) trained rats to lever-press under a FR20 schedule of reinforcement and then assessed effects of haloperidol on responding during extinction. Compared to controls, subjects that received 0.1 mg/kg haloperidol showed reductions in initial-session responding (a lack of extinction-busting). Subsequently, Salamone (1986) found that 0.4 mg/kg haloperidol reduced motor function measured by an open field test. Interestingly, the same dose did not decrease food-reinforced responses (FI 30-s). In a time-constrained ratio task, haloperidol dose-dependently decreased the break-point in female Wistar rats. However, only 0.1 mg/kg decreased peak response rate relative to vehicle (Mobini et al., 2000). Comparable results were obtained using the same time-constrained PR schedule by Zhang et al. (2005), with 0.1 mg/kg haloperidol causing the most disruption. In addition to decreasing break-points in PR schedules, haloperidol modulates effort-discounting via DAergic modulation. Reviewed by Salamone (2012), dopamine antagonists, including but not limited to haloperidol, can decrease responding for a preferred food in favor of non-contingently available but less preferred food. Other investigations utilizing T-maze tasks report similar results. For example, in a T-maze task in which the arms differed in the density of reinforcement, haloperidol did not decrease choice for the high-density arm. However, when a barrier was inserted into the high-density arm, haloperidol increased responses to the low-density arm,

relative to control (Salamone et al., 1994; Cousins et al., 1996). Bardgett et al. (2009) obtained similar results when using an adjusting-amount version of the T-maze task, with haloperidol dose-dependently increasing selection of the low-density arm. .

Clozapine

Pharmacology. Clozapine is an atypical antipsychotic belonging to the dibenzodiazepine family, often used to treat schizophrenia. Studied extensively, clozapine exhibits a unique binding profile, preferentially binding with and acting as an antagonist at a variety of 5-HT receptors, while also weakly binding with and blocking DA D_1 and D_2-like receptors. In particular, clozapine selectively binds with 5-HT_{2A} and 5-HT_{2C} receptors, and with lower affinity to 5-$HT_{1A,1D}$ receptors, where it may act as a partial agonist (Bardin et al., 2006). Additionally, clozapine has affinity for α_1 and α_2-adrenergic, cholinergic (predominantly muscarinic), and histamine receptors (Richelson et al., 2000; Philibin et al., 2005); a description of clozapine's actions at these receptors may be found in the appendix. Observed in a broad number of studies, clozapine also reduces LTP via fronto-cortical hypofunction of NMDAR, although this appears to manifest via chronic, not acute, administration (Gemperle & Olpe, 2004; Jardemark et al., 2000). Under in vitro conditions, acute clozapine administration may enhance the release of $_D$-serine and $_L$-glutamate in rat neurons and astrocytes, perhaps facilitating clozapine's acute ameliorate effect on LTP-mediated processes (Tanahashi et al., 2012).

Part of clozapine's unique binding profile is that it is a weak D_2 antagonist, with low occupancy in human patients relative to other atypical antipsychotics, e.g. olanzapine (Tuppurainen et al., 2009). Evidence shows support for clozapine binding to D_1, D_2, and D_4 receptors to varying extents, with the highest affinity for D_4 receptors (Salmi & Ahlenius, 1996; Van Tol et al., 1991; Gelernter et al., 1992). To that effect, recent attempts at pharmacological interventions for the treatment of schizophrenia have focused on the interaction between D_4 receptor modulation and 5-HT. The in vivo work of Gobbi & Janiri (1999) with Wistart rats showed that clozapine blocked the inhibitory action of dopamine applied to mPFC neurons. In addition, clozapine blocked the inhibitory action of DOI (5-HT agonist) and PBG (5-HT_3 agonist) applied to mPFC neurons. The findings that clozapine affects DA function corresponds with acute studies that observed dose-dependent (10-40 mg/kg) decreases in D_2 binding in the NAcc and striatum. Decreased binding to cortical 5-HT_2 receptors accompanied that decrease. However, chronic

treatment with clozapine did not affect the number of D_2 receptors in those brain regions, but did produce a 55% reduction in cortical 5-HT_2 receptors (Wilmont & Szczepanik, 1989). Indeed, it appears that acute administration of clozapine produces an increase in mPFC DA efflux, while chronic administration decreases this effect, down-regulating TH in the VTA and up-regulating the vesicular monoamine transporter (VMAT) in the mPFC (Jaskiw et al., 2006). These studies and other suggests that an interaction between DA and 5-HT is important to clozapine's therapeutic effect. That atypical antipsychotics appear to fair better in the treatment of schizophrenia may be a result of brain regions modified during treatment, as D_1-like and 5-HT receptor sites (targets of atypical antipsychotics) are more abundant in the limibic system than D_2 sites (target of typical antipsychotics).

The distribution half-live of clozapine in serum and brain is similar to that of haloperidol, but the elimination half-life is much shorter. In humans, the serum elimination half-life of clozapine ranges from 10.3-15.8 h, but may be as high as 17.5 h if given orally (Magliozzi & Hollister, 1985) [3]. Shown in an array of studies, genetic factors and the co-administration of drugs often observed in schizophrenic patients (e.g. anti-depressants) makes pharmacokinetic data pertaining to clozapine difficult to interpret. However, the observed distribution and elimination half-lives of clozapine are significantly shorter in rodents than in humans. In Sprague-Dawley rats, the distrubition half-life of clozapine in serum was 32.88 min and the elimination half-life was 98.34 min (Baldessarini et al., 1993). The distribution and elimination half-lives in brain tissue were 30.42 min and 90.6 min, respectively. Peak serum concentrations were observed 10 min following *i.p.* injection, while peak brain concentrations (corpus striatum) were observed at 30 min post-injection. Relative to serum, brain concentrations of clozapine are 24.3 times higher in rodents (Baldessarini et al., 1993). Also using Sprague-Dawley rats, Ma & Lau (1998) administered *i.v.* 2.5 mg/kg clozapine, finding serum distribution and elimination half-lives of 2.2 and 41.9 min, respectively (no brain data collected). The difference between these results and those of Baldessarini are due, in part, to advances in the techniques used to obtain pharmacokinetic data.

Similar to typical antipsychotics, atypical antipsychotics decrease motor function in rodent models. In an open field test of motor function, clozapine reduced exploratory behavior and increased anxiety in Wistar-Kyoto rats (WKY), but not the spontaneously hypertensive rat (SHR) (McFie et al.,

[3] The standard deviation given for oral administration was 8.7 h, showing the extreme variability in human serum concentrations.

2012). This is likely to due to increased D_4 receptor expression in the PFC of WKY rats, but may also reflect clozapine's effect on α_1-adrenergic receptors. These results coincide with previous reports in Holtzman rats, showing a dose-dependent decrease in responding during an FI 60s schedule of reinforcement and open field motor activity (Kaempf & Porter, 1987). Clozapine also reduced responding during a force-sensitive FR 20 schedule (Ford et al., 1979). As dose and force increased, response rate and duration of response decreased, and these results were similar to results found with chloropromazine. Taken together, these studies reveal that while atypical antipsychotics may reduce the incidence of EPS, they still present sedative and rate-reducing effects in rodents. Similar to most DA antagonists, in classic conditioned avoidance paradigms, clozapine dose-dependently reduces escape and avoidance from shock in rodents (Aguilar et al., 2000).

Haloperidol versus Clozapine

Haloperidol and clozapine differ in behavioral effects under the same procedures. In a multiple FR 30 FI 600s schedule, both mice and pigeons show a rate-dependent effect during only the FI component at lower doses of haloperidol ref?. Clozapine did not elicit stark rate-dependent effects in pigeons, although at the highest doses in mice (9.0 and 30.0 mg/kg) responding decreased under the FR 30 before it did under the FI 600s. Measuring the effects of haloperidol and clozapine, Fowler et al. (1994) used a water fountain task. In the task, male Sprague-Dawley rats pressed and held a force lever down to receive water. Presentation of water was contingent upon the force lever being depressed. In a dose-dependent manner, both haloperidol and clozapine reduced time on task. However, haloperidol increased the peak force output and the overall force while the lever was depressed. This is congruent with Fowler et al. (1986), in which haloperidol increased force output while decreasing response rate. In contrast, clozapine decreased the force while the level was depressed, but did not augment the peak force. Haloperidol may increase the steady output of force because it evokes EPS, including tremor, while clozapine is known to exert anti-tremor properties (Fowler et al., 1994). Both drugs have shown sedation at moderate to high doses in rodents, with increased clozapine sedation relative to haloperidol (Salamone et al., 1996).

At moderate doses (0.3 haloperidol and 3.0 clozapine), both of these drugs decrease locomotor function and impair spatial learning assessed by the Morris water maze (Hou et al., 2006). Using

quantitative assessments to parse out drug effects, Zhang et al. (2005) compared differences between haloperidol and clozapine under PR schedules. Acting in relatively opposite fashions, haloperidol dose-dependently decreased the highest completed ratio and reduced reinforcer efficacy at low doses (specific activation, derived from Killeen's (1994) MPR). In contrast, clozapine dose-dependently increased the highest completed ratio and increased reinforcer efficacy. Interestingly, both drugs increased the minimum response time, a parameter indicative of the capacity to respond (Zhang et al., 2005; Mobini et al., 2000). Reanalyzing data from Olarte-Sanchez et al. (2012a), Bradshaw & Killeen (2012) found similar results pertaining to haloperidol. Inferred from these results, it is possible that reductions in responding due to typical antipsychotics are not due to changes in reinforcer efficacy, but to motor impairment.

More recently, in a delayed non-matching-to-position procedure, haloperidol and clozapine affected male Lister Hooded rats differentially Gemperle et al., 2003). Haloperidol and clozapine did not negatively affect choice accuracy, except at the longest delays combined with the highest doses. However, clozapine did increase the number of omitted trials while haloperidol increased the number of incomplete trials (reduced nose-poking between sample and test). It should be noted that the doses of clozapine (0.1 and 0.3 mg/kg) used by Gemperle et al. (2003) represent the low end of the dosing range for clozapine, as many studies of PPI, operate behavior, and force-dynamics use considerably higher dose ranges (1-30 mg/kg). Using a full spectrum of doses in a delayed radial maze task with Sprague-Dawley rats, Wolff & Leander (2003) found that neither haloperidol nor clozapine, at substantially high doses (ranging from 0.01-3.0

Several investigators have begun to characterize differences in the efficacy of haloperidol and clozapine to attenuate NMDAR antagonism-induced neurobehavioral deficits using different procedures. The effectiveness of antipsychotic drugs to attenuate pharmacologically induced deficits in prepulse inhibition (PPI[4]) varies. In the task, a weak auditory (usually) stimulus precedes a strong auditory stimulus that is loud enough to induce a startle response. The preceding stimulus inhibits the strength of the startle response, a compensatory response that appears impaired in many patients with schizophrenia. NMDA antagonists, like PCP and MK-801, decrease overall PPI in rodents at normal doses (5.0 and 0.5 mg/kg, respectively). That is, relative to saline (vehicle), NMDAR antagonists diminish the inhibitory power that

[4] PPI is a non-human animal task considered a standard analogue to the sensorimotor gating issues observed in many schizophrenic patients.

the prepulse exerts over the startle response to strong auditory stimulus (Keith et al., 1991). Pretreatment with 0.02 and 0.1 mg/kg haloperidol in rats was unable to recover deficits in PPI of acoustic or tactile startle deficits caused by PCP or MK-801 (Keith et al., 1991). Studies with clozapine found a similar inability to attenuate PCP-induced deficits in PPI of an acoustic startle (Wiley, 1994; Celia et al., 2007). In contrast, several studies in mice (Andreasen et al., 2006) and rats (Bakshi et al., 1994; Swerdlow et al., 1998) have found clozapine effective in reducing PPI deficits. Results of these studies may differ based on the strength of the startle response used, the modality, or training procedures. In studies that have found clozapine effective, non-DAergic mechanisms are often thought to underlie behavioral changes not seen when using typical antipsychotics, including haloperidol.

Using a conditional discrimination task, Dunn & Killcross (2007) tested the effects of several drugs, including haloperidol and clozapine, on PCP-induced deficits. During the task, two levers extended and responding on both levers was reinforced under a VI 30s schedule; clicks and tones signaled the correct lever, which alternated throughout the 30 min session (10, 5 min trials). During an extinction probe, PCP (1.5, 2.5, and 5.0 mg/kg) markedly disrupted performance (equal responding on both correct and incorrect levers). During a second extinction probe and a dose of 1.5 mg/kg PCP, pretreatment with clozapine (5 mg/kg) increased the number of responses on the correct lever while haloperidol (0.3 mg/kg) did not change behavior from the PCP baseline (Dunn & Killcross, 2007). In male ICR mice subchronically treated with PCP (10 mg/kg), acute *i.p.* injections of haloperidol (0.1 mg/kg) or clozapine (5.0 mg/kg) did not rescue PCP-induced deficits a novel object recognition task (Hashimoto et al., 2005) but subchronic administration of clozapine not only abated the PCP-induced deficits, but increased performance beyond control levels. Subchronic haloperidol narrowly and non-significantly increased performance, but not to the extent of clozapine. Thus, both haloperidol and clozapine cause decrements in locomotor function, but clozapine appears superior at reducing NMDAR-induced deficits in sensorimotor gating and discrimination tasks.

As animal models that use ketamine increase, it is important to characterize the drug's effectsand possible avenues for treatment of side effects. To date, most studies using pharmacologically induced NMDAR antagonism in animal models rely on neurochemical analyses (Meshul et al., 1992; Daly & Moghaddam, 1993; Lopez-Gil et al., 2007), basic behavioral paradigms (Andreasen et al., 2006; Zhang et

al., 2005), or maze tasks (Hou et al., 2006). Few utilize complex operant procedures to measure neurobiological correlates of NMDAR antagonism and compare efficacy of drug treatments.

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Chapter 2: Experiment

Abstract

Ketamine, like other *N*-methyl-*D*-aspartate (NMDA) receptor antagonists, causes both locomotor and cognitive dysfunction, decrements that may be mediated by distinct neurotransmitter systems. The present study was designed to characterize the contributions of dopamine and serotonin to the behavioral effects of ketamine. BALB/c mice responding under an incremental repeated acquisition procedure were administered ketamine alone and in combination with haloperidol pretreatment or clozapine pretreatment. Ketamine (1-30 mg/kg) dose-dependently decreased response rate, reinforcer rate, maximum chain length, and progress quotient (a weighted measure of overall performance). The ***Performance*** chain was more sensitive to ketamine's effect than were the ***Learning*** chains. Pretreatment with clozapine (0.1-4.0 mg/kg) dose-dependently attenuated disruption of IRA responding by systemic ketamine (30 mg/kg). No dose of haloperidol pretreatment (0.01-0.1 mg/kg) alleviated ketamine-induced IRA deficits (30 mg/kg). The effectiveness of clozapine relative to haloperidol suggests a more central role of specific serotonergic receptors over dopaminergic receptors in mediating the behavioral effects of ketamine.

Introduction

Glutamatergic *N*-methyl-*D*-aspartate receptors (NMDARs) are vital to fundamental processes such as learning and memory (Riedel, Platt, & Micheau, 2003). Increasingly, evidence points to NMDAR dysfunction as a neurobiological correlate in neurodegenerative and psychiatric illnesses, including schizophrenia and autism (Jentch & Roth, 1999; Olney, Newcomer, & Farber, 1999; Carlsson M. , 1998; Goff & Coyle, 2001). More recently, experimental and clinical studies suggest that NMDAR antagonists like ketamine may have efficacy in the treatment of major depressive disorder and chronic pain (Krishmam & Nestler, 2008; Hewitt, 2000). However, several neurotransmitters, including dopamine (DA) and serotonin (5-HT), are also involved in the behavioral effects of NMDAR antagonism. As the role of NMDAR antagonists in preclinical animal models and experimental clinical practice increases, there is a growing need to accurately dissociate contributions of different neurotransmitter receptor sub-types that mediate the effects of NMDAR antagonists.

Acute NMDAR antagonism interferes with reflexive behavior (Swerdlow, Bakshi, Waikar, Taaid, & Geyer, 1998; De Bruin, Ellenbroek, Cools, Coenen, & van Luijtelaar, 1999), locomotor function (Irifune, Shimizu, & Nomoto, 1991; Imre, Fokkema, Den Boer, & Ter Horst, 2006) and executive or cognitive control (Riedel, Platt, & Micheau, 2003; Verma & Moghaddam, 1996; Amitai & Markou, 2010; Cottone, et al., 2013). NMDAR antagonism may interfere with the ability of endogenous NMDARs to exert tonic inhibitory control over striatal DA efflux (Miller & Abercrombie, 1996). Operant studies of interval timing have elucidated the specificity of ketamine's effects; a lack of disruption of timing following ketamine administration is indicative of disruption within ventral striatum and not the dorsal striatum, as interval timing is thought to depend on dorsal striatal DAergic activity (Cheng, MacDonald, & Meck, 2006; Cheng, Ali, & Meck, 2007). NMDAR antagonists also disrupt the interaction between DA, $_{Y}$-amino butyric acid (GABA), and glutamate neurons of the prefrontal cortex (PFC) and limbic system (Verma & Moghaddam, 1996; Littlewood, et al., 2006; Korotkova, Fuchs, Ponomarenko, von Engelhardt, & Monyer, 2010; Carlen, et al., 2012; Caixeta, Cornelio, Scheffer-Teixeria, Ribeiro, & Tort, 2013). Ketamine, a prototypical NMDAR antagonist, also appears to act directly at dopamine (DA) and serotonin (5-HT) receptors in the high affinity state and its affinity for DA D_2 receptors may be greater than its affinity for NMDARs (Kapur & Seeman, 2002; Seeman, Ko, & Tallerico, 2005). Indeed, considerable evidence exists showing the

contribution of DA and 5-HT systems to the locomotor and cognitive effects of ketamine (Byrd, Standish, & Howell, 1987; Moghaddam, Adams, Verma, & Daly, 1997; French & Ceci, 1990; Chatterjee, Ganguly, Srivastava, & Palit, 2011; Meltzer & Huang, 2008).

In animal models of NMDAR dysfunction, neurochemical and behavioral effects of ketamine and other NMDAR antagonists like PCP and dizocilpine (MK-801) can be blocked by pretreatment with typical (haloperidol) or atypical (clozapine) antipsychotics, but pretreatment efficacy and reliability appears to be procedure-dependent. Haloperidol is a potent and relatively specific D_2 antagonists (Garris, et al., 2003), while clozapine is a 5-$HT_{2A, 2C}$ receptor antagonist with affinity for DA D_2 receptors, but to a lesser extent that haloperidol (Seeman, 2002; Bardin, Kleven, Depoortere, & Newman-Trancredi, 2006).

The present study was designed to answer broad questions regarding the contribution of DA and 5-HT receptors to ketamine's behavioral effects. Specifically, were ketamine-induced deficits in IRA responding a function of an overall *hypo*glutamatergic state, a *hyper*dopaminergic state, down-stream modulation of 5-HTergic activity, or perhaps a combination of all of the above? Given that ketamine may function as a D_2 receptor agonist or partial agonist, haloperidol, a potent D_2 antagonist, may block the contribution of D_2 agonism to ketamine-induced deficits seen in the IRA procedure. Conversely, if clozapine, which has high affinity for 5-$HT_{2A, 2C}$ receptors and lower affinity for D_2 receptors, blocks ketamine-induced deficits in responding, it may lend support to a 5-HT-mediated mechanism of disruption. To that end, an incremental repeated acquisition (IRA) procedure assessed responding of BALB/c mice following injections of ketamine alone (1-30 mg/kg), ketamine (30mg/kg) with haloperidol pretreatment (0.01-0.1 mg/kg), and ketamine (30mg/kg) with clozapine pretreatment (0.1-4.0 mg/kg).

Methods

Subjects. Eleven adult, male BALC/cAnNHsd mice obtained from Harlan laboratories (Indianapolis, IN) were kept in a temperature- and humidity-controlled vivarium, maintained on a 12-hour light-dark cycle (lights on at 6:00am). Adult mice, nine months of age, were individually housed in an AAALAC-accredited facility in clear polycarbonate cages (two mice per cage separated by a clear, plastic divider and wire cage-top). All procedures were approved by the Auburn University Institutional Animal Care and Use Committee (IACUC) guidelines.

Apparatus. Subjects' daily experimental sessions were conducted in 11 standard Med Associates operant conditioning chambers (Med Associates Inc., product #ENV-007). Each chamber measured 12″L×9.5″W×11.5″H and contained two stainless steel front and back walls and two *Plexiglas*© side walls. Mounted on the front wall were two nose-poke holes (L and R), separated by a pellet dispenser, and a third nose-poke hole was located in the center of the back wall (B). A pellet dispenser delivered 20mg white sucrose pellets. Chambers also had two Sonalert™ tones (2900 and 4500 Hz, nominally) calibrated to amplitude of 70 dBc for presentation of auditory stimuli. Located near the ceiling of the chamber on the back wall was a single 2.8-W house light. Operant chambers were enclosed in sound-attenuating cabinets with a fan to circulate air for ventilation.

General procedure. Mice had prior nose-poke experience, including approximately 70 IRA sessions, with subjects experiencing the performance chain and the 11 learning chains used in the current experiment. Thus, after confirming a stable baseline, subjects progressed through the following phases: steady-state baseline, ketamine dose-effect curves (total of two), exploratory haloperidol and clozapine doses, ketamine with haloperidol pretreatment, and finally ketamine with clozapine pretreatment (Table 1). Following each round of drug administration (ketamine alone, exploratory haloperidol and clozapine, haloperidol pretreatment, clozapine pretreatment), subjects received at least five consecutive non-drug sessions to reestablish baseline. Four days separated drug sessions in the haloperidol pretreatment condition to allow for complete drug elimination. Lasting 30 min, experimental sessions took place every day at approximately 2:00 p.m. Subjects had no access to water during experimental sessions, but received water and their daily ration of food 10 min post-session.

Incremental repeated acquisition. Based on the repeated acquisition (RA) procedure (Boren, 1963; Boren & Devine, 1968; Thompson, 1973), the IRA procedure requires subjects to acquire response changes within a session (Pieper, 1976; Paule & McMillan, Incremental repeated acquisition in the rat: Acute effects of drugs, 1984). The current procedure employed a number of methods established for use with IRA, most notably: 1) backwards chaining, 2) discrete stimulus conditions for each link, and 3) the establishment of a *Performance* chain versus a set of *Learning* chains (Thompson & Moerschbaecher, 1978; Moerschbaecher, Boren, & Schrot, 1978; Weiss, 1978; Moerschbaecher & Thompson, 1980; Smith, 1999; Wright & Paule, 2007). In addition, there were stringent mastery-based criteria for advancing

chain length. Also, as part of the multiple schedule of reinforcement, discrete discriminatory stimuli signaled each component.

Subjects acquired a chain of nose-pokes (up to six links in length) by means of backward chaining (for details, see Bailey, Johnson, & Newland, 2010). Briefly, backward chaining required the mice to emit the final link of a sequence first (link 1). Subsequent links were added in front of the link 1, consequently increasing the chain to the desired length (Table 2). Thus, link 1 was always the final response in the chain and the only response directly followed by reinforcement - one 20mg sucrose pellet paired with a high-tone for 0.2-s. The final target sequence always consisted of six nose-pokes across three different locations (L=left, R=right, B=back), excluding repeating locations. No sequence included two or more successive responses at the same response location (i.e. R-B-L-L-R-B was not a target chain). For example, if the behavior chain was R-L-B-L-B-R, the first response chain learned was simply R, followed by B-R, L-B-R, and so on until subjects learned to emit the entire sequence.

Each link in a chain also had a set of unique stimulus conditions to facilitate within-session acquisition of the chain. Regardless of chain composition, a given link always had with the same stimulus conditions. For example, a pure low tone accompanied the first link of a chain and a pulsating low tone accompanied the second link (see Table 2 for the full list). When an animal progressed through a chain, the stimuli changed with each link, but terminated following reinforcement or an incorrect response. Incorrect responses at any point during the sequence resulted in a 2-s inter-trial interval (ITI), during which the nose-poke holes remained unlit. Responses during the ITI increased its duration by 1s such that ITI responses were not adventitiously reinforced by termination of the ITI and the onset of the next trial. Following an error, subjects restarted the current chain. For example, an error in the third link of 4-link chain reset the chain to the fourth link of the 4-link chain. Unlike standard IRA procedures, mastery-based criteria were imposed to increment the length of the response chain within-session. The criterion to move from a 1-link to a 2-link sequence was six consecutive correct responses. The criterion to increase link length from 2 to 3, 3 to 4, 4 to 5, and 5 to 6 was 3 consecutive correctly produced chains.

A two-ply multiple schedule comprising a P*erformance* and *Learning* component was imposed with one alteration in a session. Within and across sessions, P*erformance* used the well-learned sequence mentioned above (R-L-B-L-B-R). The chain during *Learning* varied across sessions, randomly

selected from 11 sequences that all differed from the performance chain. Reinforcement was under a FR1 schedule of reinforcement, i.e., reinforcement followed every correctly emitted chain. Components had durations of 14 min each. During P*erformance*, the chamber remained dark, with the exception of the illuminated nose-poke holes. During *Learning*, the house-light flashed at a 0.5s interval. A 1 min inter-component interval (ICI) divided the two components, during which the chamber, including nose-poke holes, remained dark. Nose-pokes during the ICI had no programmed consequences.

Drugs. Ketamine (racemic), haloperidol, and clozapine were procured from Sigma Aldrich (St. Louis, MO). Ketamine was prepared by dissolving drug into a 0.9% saline solution (NaCl), while haloperidol and clozapine were mixed with a 1.0 M solution of HCl and diluted with NaCl to achieve a pH of 6-7. All drugs were prepared to achieve an injection volume of 0.1 ml (0.1ml/kg) and filtered through a five micron filter needle to produce a sterile injectate. The same 0.9% saline solution functioned as a vehicle control injection. All injections of ketamine, haloperidol, clozapine, and vehicle were administered intraperitoneally [*i.p*.].

Ketamine Alone. Injections occurred five min pre-session due to the short elimination half-life of ketamine. Vehicle injections followed in the same manner as drug, five min pre-session. Interspersed between drug days were vehicle control and control days. Ketamine doses of 1.0, 3.0, 5.6, 10.0, and 30.0 mg/kg were given in ascending order. Subjects received the full range of doses twice, once with P*erformance* first and then with *Learning* first. Following the reversal in component order, a new baseline was established and after five consecutive sessions with no systematic change in the productivity, the next dose-effect curve started. In all subsequent drug conditions (haloperidol and clozapine alone, haloperidol pretreatment, and clozapine pretreatment), the component order was *Performance* first because, during baseline, this provided greater separation in behavior measures by component (see Fig. 1 below).

Haloperidol and clozapine alone. Subjects received a single injection of 0.1 mg/kg haloperidol and 2.5 mg/kg clozapine 30 or 50 min pre-session, respectively or a vehicle control injection. These represented the high end of the planned dose-response functions for each antipsychotic pretreatment, although they represent moderate doses as compared with many behavioral studies of their acute effects. The goal was to determine their behavioral effects when administered alone

Haloperidol pretreatment. To assess the role of D_2 receptors in ketamine-induced deficits, injections of haloperidol preceded injections of 30 mg/kg ketamine prior to the start of experimental sessions. The dose of ketamine was chosen because it produced the greatest disruption in the acute dose-effect determinations. Mice received pretreatment doses of 0.01, 0.03, and 0.1 mg/kg 30 min pre-session, 25 min prior to ketamine. Subjects received doses in ascending order only once during sessions in which *Performance* came first. These represent low to moderate doses of haloperidol in mice. Subjects also received a vehicle control injection 30 min pre-session followed by ketamine five min prior to the session. Only three doses of haloperidol were used in an attempt to minimize the cumulative dosing effects of haloperidol, which can lead to DA upregulation (Liskowsky & Potter, 1987).

Clozapine pretreatment. To test the hypothesis that ketamine-induced deficits may be mediated by $5\text{-HT}_{2A,\ 2C}$ receptors clozapine injections preceded injections of 30 mg/kg ketamine prior to experimental sessions. Mice received eight pretreatment doses of clozapine ranging from 0.1-4.0 mg/kg in ascending order. Unlike haloperidol, these injections occurred 50 min pre-session, 45 min prior to ketamine. Again, subjects received doses in ascending order only once during sessions in which *Performance* came first. Because acute clozapine has the potential to cause sedation, the doses here mostly represent the low to moderate dosing spectrum for clozapine with rodents. Similar to haloperidol, subjects received a vehicle control injection 50 min pre-session followed by ketamine five min prior to the session.

Dependent measures. Dependent measures included progress quotient (PQ), response rate (correct and error rate), reinforcer rate, and maximum chain length (MCL). PQ marked IRA performance by taking into account the inherent changes in response difficulty within-session (i.e. increasing chain length).

$$PQ = \frac{\left(\sum_{i=1}^{6} wR_i\right)}{R_t} \qquad \text{(Eq. 2)}$$

Here, R_i was the number of reinforcers earned in a given link, and R_t was the total reinforcers earned within a session and w, chain length, was a weighting factor. Thus, reinforcers earned following longer chains (e.g. 5-link) received more weight than those earned following shorter chains (e.g. 2-link). If a subject earned no reinforcers in a given session (either all incorrect responses or no responses at all), no

PQ score was given to that animal. Response rates (total component responses/available component time) and reinforcer rates (total reinforcers/available component time) were calculated for each component, based upon available component time (total component time – (time in ITI + time following reinforcer delivery + ICI)). Also, the Maximum Chain Length (MCL) reached in an individual session, regardless of how many times that chain was executed successfully, was determined. Data from drug conditions, including PQ, MCL, response rate, and reinforcer rate, were converted to proportion of control.

Data analysis. Repeated measures ANOVA (RMANOVA) compared baseline differences with Component (*Performance* and *Learning)* and Component order (*Performance* first, *Learning* first) as factors (significant at $p<0.05$). Ketamine dose-response functions were fit using the non-linear four-parameter Hill equation.

$$Y = \min + \frac{\max - \min}{1 + \frac{x}{EC50}^{-Hillslope}} \quad \text{(Eq. 3)}$$

Here, Y was the dependent measure of interest, x was the dose of ketamine, min and max were lower and upper limits of the sigmoidal curve, EC_{50} was an estimate of the dose of ketamine that would produce a response halfway between the min and max, and Hillslope described the steepest slope of the curve (Motulsky & Christopoulos, 2004).

Model comparison allowed for assessment of differences between *Performance* and *Learning*; a model in which all four parameters were shared between *Performance* and *Learning* data was compared against one in which *Performance* and *Learning* data were fit separately to obtain two distinct curves with four parameters for each data set. Differences between models with shared or separate parameters were assessed using the Akaike's Information Criterion - corrected (AICc). Finally, the effects of haloperidol and clozapine pretreatment on ketamine-induced IRA deficits were assessed using RMANOVA with Dose as a repeated measure. When post-hoc testing was necessary, Tukey's correct was applied. Data analysis used the following programs: SigmaPlot v.12©, Microsoft Excel©, and R v. 3.0.2©.

Results

Incremental repeated acquisition (IRA). Figure 1 shows PQ, MCL, and response and reinforcer rate (rows) for *Performance* and *Learning* (filled versus open circles) during the last five sessions of baseline of each component order (columns) before the start of the ketamine doses. Averaging these five

session together for each component order, RMANOVA revealed a significant Component Order X Component interactions for PQ [$F(1,10) = 38.09$, $p<0.001$] , MCL [$F(1,10) = 43.63$, $p<0.001$], response rate, [$F(1,10) = 33.22$, $p<0.001$], and reinforcer rate [$F(1,10) = 23.79$, $p<0.001$]. Post-hoc tests (Tukey's test) revealed a series of complex multiple comparisons (all p's<0.05). When *Performance* was first, PQ and MCL were higher during *Performance* than *Learning,* but there was no difference when *Learning* was first. Also, when *Performance* was first, response and reinforcer rate were higher during *Performance*, but when *Learning* was first, these two measures were higher during *Learning*. Upon comparing *Performance* to *Learning* when both components occurred first, PQ, MCL, and response rate were higher during *Performance*, while there was no difference in reinforcer rate between components.

Ketamine. Five acute injections of ketamine (1-30 mg/kg, *i.p.,* 5 min pre-session) comprised dose-response functions for both component orders: *Performance* first and *Learning* first. The behavioral time-course of ketamine's effects was closely tied to the drug's elimination half-life, approximately13 min (Maxwell, et al., 2005). Thus, disruption occurred primarily during the first component only, regardless of whether it was *Performance* or *Learning,* while responding recovered to near control levels during the second component (also regardless of chain type). Thus, analyses of ketamine's effects on IRA responding focused on the first component. Figure 2 shows dose-response functions for PQ, MCL, response rate, and reinforcer rate (converted to proportion of control) during *Performance* and *Learning,* but only when those components occurred first. Lines represent the best fit of the data to the four parameter Hill equation (Eq. 3). Featured next to each curve are scatterplots of individual subjects' dose-response data by component (top: *Performance*; bottom: *Learning*).

AICc was used to assess the likelihood of a difference between *Performance* and *Learning* curves for each dependent measure. That is, a singular curve in which the four parameters were held constant (null; no difference between *Performance* and *Learning*) was compared against two separate curves (alternative; a difference between *Performance* and *Learning*) with four parameters each. Table 3 shows the results of the model comparisons using AICc; for PQ, MCL, and reinforcer rate two curves fit better than one and for response rate one curve fit better than two. Table 4 shows best fit parameter estimates for min, max, Hillslope, and EC_{50}. For PQ, MCL, and reinforcer rate, the EC_{50} was lower and the Hillslope was steeper in for *Performance* relative to *Learning*. There were no differences in these

parameters for response rate, as one curve with shared parameters fit data from the two components best. Taken together, these results suggest that *Performance* showed greater sensitivity to ketamine's effects. However, the obtained evidence ratios were lower than normal standards for indicating definitive differences (Table 3) for PQ, MCL, and reinforcer rate, indicating that differences in the effects of ketamine on *Performance* and *Learning* were modest. There was a high evidence ratio (14.2) for response rate, however, clearly indicating that shared parameters and one curve provided a better fit than separate parameters for two curves.

It should be noted that, for *Performance*, three subjects at 10 mg/kg (802, 805, 809) and two subjects at 30 mg/kg (802, 805) did not earn any reinforcers and therefore did not receive a PQ score. For *Learning* at 30 mg/kg, six subjects (802, 804, 805, 808, 809, and 813) did not receive a PQ score. Overall, subjects 802, 805, and 809 appeared more sensitive to ketamine's motoric effects.

Haloperidol and clozapine alone. Because ketamine's effects dissipated so rapidly, the remainder of the study focused on *Performance*, no longer alternating the first component between *Performance* and *Learning*. This was done, in part, to reduce the number of ketamine injections. To test for haloperidol and clozapine-induced disruption in the IRA procedure, single acute doses, 0.1 and 2.5 mg/kg^{-1} respectively, were administered alone. Both haloperidol and clozapine alone produced a modest reduction in PQ compared to vehicle [Haloperidol: $F(1,8) = 7.45$, $p=0.026$; Clozapine: $F(1,10) = 5.13$, $p=0.047$; Fig. 3], but MCL, response rate and reinforcer rate were unaffected (p's>0.05).

Haloperidol pretreatment. Pretreatment with haloperidol occurred 30 min pre-session, 25 min prior to administration of 30 mg/kg ketamine. Because the interest was in showing a reversal of ketamine's effect, the combination of haloperidol pretreatment and ketamine was compared against the responding produced by the combination of vehicle and 30 mg/kg ketamine (Fig. 2). Thus, protection would be revealed as an upward shift in the dose-effect relation. Visually, it appeared that haloperidol produced a mild reversal of ketamine's effect, but the results did not reach conventional levels of statistical significance. Thus, there was no effect of Dose on PQ [$F(3,22) = 1.90$, $p=0.16$] , MCL [$F(3,30) = 2.32$, $p=0.09$], response rate [$F(3,30) = 2.39$, $p=0.08$], or reinforcer rate [$F(3,30) = 2.09$, $p=0.12$], such that no dose of haloperidol differed from the vehicle saline pretreatment (Fig. 4).

Clozapine pretreatment. As with haloperidol pretreatment, the baseline for examining clozapine-induced rescue was the poor performance produced by 30 mg/kg ketamine. Unlike haloperidol pretreatment, clozapine pretreatment partially blocked the effects of ketamine. There was a significant main effect of Dose on PQ [$F(8, 60) = 4.23$, $p<0.001$], MCL [$F(8,76) = 9.33$, $p<0.001$], response rate [$F(8,76) = 8.49$, $p<0.001$], and reinforcer rate [$F(8,76) = 10.83$, $p<0.001$] (Fig. 5). Tukey's test revealed that the following doses of clozapine reversed ketamine's disruption of PQ, MCL, response rate, and reinforcer rate relative to clozapine vehicle: PQ, 0.3 ($p=0.012$) and 0.56 ($p=0.016$); MCL, 0.17 ($p=0.002$), 0.3 ($p<0.001$), 0.56 ($p<0.001$), 1.0 ($p<0.001$), and 1.5 ($p=0.009$); response rate, 0.3 ($p<0.001$), 0.56 ($p=0.009$), 1.0 ($p=0.023$); reinforcer rate, 0.3 ($p<0.001$), 0.56 ($p=0.008$), 1.0 ($p=0.002$).

At a dose of 1.0 mg/kg, an outlier was detected and removed; the subject's proportion of control response rate and reinforcer rate were more than 2.5 standard deviations above the mean. Though recovery never reached baseline levels, 0.30 mg/kg had the greatest effect on response rate and reinforcer, returning them to 51.8% and 70.7% of baseline, respectively, while also significantly increasing PQ.

Discussion

The goal of the present study was to characterize the contributions of DA and 5-HT to the behavioral effects of ketamine (30mg/kg) by pretreating subjects with clozapine or haloperidol before ketamine administration. The primary mechanisms of action of haloperidol and clozapine are divergent, with haloperidol a specific and potent DA D_2 antagonist, and clozapine a 5-$HT_{2A,2C}$ antagonist with mixed affinity for a variety of other neurotransmitters. A reversal by a D2 or a 5-$HT_{2A,2C}$ antagonist would imply that ketamine's disruption of IRA performance would be caused by its overstimulation of that neurotransmitter system. After showing that ketamine dose-dependently disrupted behavior under the IRA procedure, the two drug-pretreatment conditions were examined at the most disruptive ketamine dose (30 mg/kg). Notably, both of these drugs, when administered alone at higher doses, disrupted IRA in the same direction as ketamine, so this test was conservative. Haloperidol failed to block ketamine-induced deficits while low-dose clozapine partially attenuated ketamine-induced deficits.

Ketamine. The IRA procedure revealed the disruptive effects of the NMDAR antagonist ketamine, while elucidating a link between the drug's pharmacokinetic properties and behavioral effects. Ketamine

disruption was limited to the first component, regardless of component order. Given the 5 min pre-session injection time and component duration of 14 min, ketamine disruption was limited to approximately 19 min, similar to the 13 min half-life of ketamine in rodents reported by Maxwell et al. (2005). Though the IRA procedure produced differences between *Performance* and *Learning*, the short elimination half-life of ketamine complicated the within-session nature of the procedure. Yet, the procedure elucidated the need to use short component or session durations with ketamine, as the behavioral effects appear to mirror the pharmacokinetic properties. The use of a multiple schedule may be more appropriate with drugs that have longer elimination half-lives (e.g. MK-801).

Comparison of *Performance* and *Learning* (occurring in the first component) revealed differential sensitivity to ketamine. Ketamine dose-dependently reduced PQ, MCL, response rate, and reinforcer rate. Importantly, the EC_{50} and Hillslope parameters differed between *Performance* and *Learning* for PQ, MCL, and reinforcer rate, but not for response rate. Thus, the dose necessary to reduce PQ, MCL, and reinforcer rate for *Performance* was lower than for *Learning*, showing greater sensitivity to ketamine's disruptive effects. This is unusual since typically, the learning component is more disrupted by drugs than the performance chain. This suggests that ketamine has a greater impact on well-learned behavior and the acquisition of new behavior, while still affected, is less sensitive. The increased sensitivity to disruption of well-learned behavior seen here has also been observed with procedural disruptions of IRA responding resulting in greater deficits in *Performance* relative to *Learning* (Hutsell, Bailey, & Newland, under review), MK-801-induced deficits of a well-learned maze (Przbyslawski & Sara, 1997), and Pavlovian fear conditioning (Reichelt & Lee, 2013).

While greater disruption *Performance* may seem counterintuitive (as the chain should be well learned), it may be related to the stimulus conditions present in each component. Because the chain never changes *Performance*, the auditory chain stimuli (Table 2) should come to signal not only the current *link*, but also *location* (L, R, or B) of the correct response. Conversely, during *Learning*, chain stimuli initially signal current link, while the relation between a chain stimulus and location is undergoing acquisition and is likely relatively weak. Recall that 11 distinct chains were used during *Learning*, and the same chain was never used in consecutive sessions. Thus, responding during *Performance* may have been more vulnerable to disruption if ketamine interfered with the discrimination of chain stimuli and these

auditory chain stimuli signaled the location of the correct response in a well-learned chain. That is, ketamine may have disrupted a pre-existing auditory discrimination signaling spatial location while having a minimal effect on the acquisition of a discrimination based on an auditory stimulus. Indeed, there is evidence from human (Umbricht, et al., 2000; Javitt, Shelley, Silipo, & Lieberman, 2000); and non-human animal studies (De Bruin, Ellenbroek, Cools, Coenen, & van Luijtelaar, 1999; Siegel, et al., 2003; Maxwell, et al., 2006; Bickel, Lipp, & Umbrecht, 2008) indicating that NMDAR dysfunction produces deficits in auditory processing.

The finding that ketamine dose-dependently impaired IRA responding in mice is consistent with a breadth of rodent literature underscoring cognitive/executive control decrements (set-shifting, memory, and attention impairment) following NMDAR antagonism (Moerschbaecher & Thompson, 1980; Amitai, Semenova, & Markou, 2007; Smith, et al., 2011; Kos, Nikiforuk, Rafa, & Popik, 2011). NMDAR antagonism may cause global impairment characterized by overarching deficits in attention (Amitai, et al., 2007). However, the IRA procedure is a multifaceted, apical, operant procedure that combines aspects of many paradigms. The procedure has high attentional demands, requiring subjects to discriminate between auditory tones signaling chain length (and perhaps spatial location in the performance component), visual stimuli signaling the type of chain in effect (*Performance* or *Learning*), and auditory tones paired with reinforcer delivery following correctly emitted chains. In addition, the spatial configuration of the nose-pokes requires subjects to remain relatively mobile throughout the duration of a session. Thus, it is likely that systemic injections of ketamine disrupted many facets of IRA responding to various degrees, each with distinct neurobiological correlates.

Haloperidol pretreatment. Pretreatment with the D_2 antagonist haloperidol was ineffective at rescuing IRA responding disrupted by ketamine. There was no difference in PQ, MCL, response rate, or reinforcer rate between vehicle and over a 10-fold range of haloperidol. In other studies, haloperidol attenuated hyperlocomotion and decreased vertical jumping (referred to as “popping”) produced by NMDAR antagonists (Deutsch & Hitri, 1993). While it was not part of the present study, we did observe ketamine-induced popping at 30 mg/kg and that 0.1 mg/kg haloperidol attenuated this popping. This suggests that haloperidol’s strict antagonism of subcortical D_2 receptors (e.g. basal ganglia) was enough to attenuate hyperlocomotion but not deficits in IRA responding. Therefore, haloperidol may block

locomotor effects of ketamine caused by increases in DA, while leaving other dysfunctional neurotransmitter systems relatively unaffected. It should be noted that the effects of haloperidol differ greatly depending on the dosing regimen employed (acute versus chronic) and rescue by haloperidol may require chronic pretreatment.

Clozapine pretreatment. Clozapine, a 5-$HT_{2A, 2C}$ antagonist, partially and dose-dependently rescued IRA responding; doses between 0.17-1.0 mg/kg constituted the effective dosage range. Doses of 0.3 and 0.56 mg/kg were the only pretreatment doses that increased all four key dependent measures: PQ, MCL, response rate, and reinforcer rate, with the greatest rescue observed at 0.3 mg/kg. Relative to vehicle, 0.3 mg/kg clozapine produced a net recover of approximately 29.4, 35.3, 48.4, and 61.9% of baseline PQ, MCL, response rate, and reinforcer rate, respectively.

These findings correspond to reports showing clozapine's reversal of NMDAR-induced reflexive and cognitive deficits (Hashimoto, Fujita, Shimizu, & Iyo, 2005; Dunn & Killcross, 2007; Linn, Negi, Gerum, & Javitt, 2003; Idris, Repeto, Neill, & Large, 2005; Levin, Caldwell, & Perraut, 2007). Here, the most effective doses reported, 0.3 and 0.56 mg/kg, represent an effective range well below that commonly seen in preclinical animal models of schizophrenia and acute NMDAR antagonism. Often, doses between 2.5-10 mg/kg clozapine have been effective at attenuating deficits induced by ketamine, PCP, and MK-801. However, under the current IRA procedure, doses of 2.5 and 4.0 mg/kg were indistinguishable from pretreatment with vehicle. This finding may be a result of the acute pretreatment dosing regimen, as clozapine has sedative effects that often diminish following chronic administration; the use of the elongated pretreatment period was undertaken to bypass this sedation. While clozapine has been effective in managing some of the behavioral deficits that result from NMDAR antagonists like ketamine, its reliability may be dose- and procedure-dependent. For example, Celia, Hatcher, Reavill, & Jones, 2007 found that clozapine, as well as the drugs risperidone, haloperidol, and lamotrigine failed to attenuate ketamine-induced deficits in prepulse inhibition in rats. It should also be noted that while clozapine has been reported to reverse some behavioral deficits caused by NMDAR antagonists, this study represents the first known report of clozapine blocking ketamine-induced deficits in an apical procedure such as IRA, a procedure with measures correlated to IQ scores in humans (Paule, Chelonis, Buffalo, Blake, & Casey, 1999; Baldwin, Chelonis, Prunty, & Paule, 2012).

As with 0.1 mg/kg haloperidol, it was observed that pretreatment with 0.3-4.0 mg/kg clozapine reduced ketamine-induced popping. Clozapine and other atypical antipsychotics that target 5-HTergic receptors also reduce NMDAR antagonist-induced locomotor dysfunction, including popping. Thus, both clozapine and haloperidol reduced popping but only clozapine attenuated ketamine-induced cognitive dysfunction. Yet, atypical antipsychotics are relatively ineffective at reducing high-dose amphetamine-induced hyperlocomotion, mainly because of their weaker D_2 antagonism relative to drugs like haloperidol (Meltzer, Horiguchi, & Massey, 2011). It is important to note, then, that NMDAR antagonist-induced hyperlocomotion does not require DAergic mechanisms, as catecholamine-depleted animals still display NMDAR antagonist-induced hyperlocomotion (Carlsson & Carlsson, 1989). Indeed, ketamine, which promotes 5-HT release and consequent stimulation of 5-HT_{2A} receptors, produces hyperactivity that is reversed by clozapine's 5-HT_{2A} receptor antagonism (McOmish, Lira, Hanks, & Gingrich, 2012).

In addition to clozapine's action at 5-HTergic receptors, it also has affinity for DA D_1 and D_2 receptors. *In vitro* and *in vivo* research continues to demonstrate that acute clozapine increases the release of cortical but not subcortical glutamate and dopamine, resulting in joint activation of NMDA and D1 receptors (Daly & Moghaddam, 1993; Chen & Yang, 2002; Tanahashi, Yamamura, Nakagawa, Motomura, & Okada, 2012). This concurrent activation may restore cortical NMDAR function at glutamatergic synapses following administration of NMDAR antagonists. Indeed, Wang & Liang, 1998 showed that clozapine but not haloperidol blocked PCP-induced NMDAR antagonism in mPFC slices of rats. Interestingly, reduction in cortical D_1 activation, which might result from over-activation of D_2 receptors via ketamine, has been linked to deficits in spatial working memory and learning. Clozapine has affinity for D_1 receptors where it behaves as an agonist, which may also be a potential target contributing to the drug's therapeutic effects. Thus, clozapine's mixed affinity may be responsible for the reversal of cognitive dysfunction resulting from NMDAR antagonism in the present study. The findings reported here, taken together with *in vitro* and *in vivo* work, support broad mechanisms by which ketamine disrupts IRA responding. The efficacy of clozapine, an atypical antipsychotic with affinity for a broad range of receptors, over haloperidol, a typical antipsychotic with specific affinity for D_2 receptors, points to deficits in cortical signaling which clozapine may be well-suited to alleviate. Since only an attenuation of dysfunction, and not a complete reversal, was observed, it is possible that other systems are involved.

For example, the present findings cannot rule out the contribution of muscarinic ACh receptors to clozapine's efficacy, as the drug has great affinity for muscarinic receptor subtypes (Bolbecker & Shekhar, 2012). In fact, the active metabolite of clozapine, *N*-desmethylclozapine, acts as an allosteric agonist at M_1 muscarinic receptors, resulting in increased hippocampal NMDAR currents, potentially contributing to the drug's therapeutic effects (Sur, et al., 2003; Weiner, et al., 2004).

Conclusion. The present experiment was designed to define better the contributions of DAergic and 5-HTergic receptors to the behavioral effects of acute ketamine (30 mg/kg) using a task that tapped many behavioral functions. Ketamine dose-dependently disrupted IRA responding, with 10.0 and 30.0 mg/kg causing the most disruption for both chain types, although *Performance* was more sensitive than *Learning* for most measures. Haloperidol pretreatment failed to attenuate ketamine-induced deficits. However, clozapine pretreatment attenuated ketamine-induced deficits in IRA responding. Doses ranging from 0.17 -1.0 mg/kg were the most effective at blocking ketamine's detrimental effects, with 0.30 mg/kg the most effective dose at returning PQ to control levels. The IRA procedure provided quantitative measures of complex between- and within-session learning that has translational efficacy to human studies. These finding support the theory that 5-HT_{2A} receptor pathways mediate the behavioral effects of ketamine to a greater extent than do DA D_2 receptor pathways, at least for the behavior under study here. The lack of efficacy of haloperidol infers that the mechanism by which ketamine induces deficits is at least partially due to cortical dysfunction of 5-$HT_{2A,2C}$ receptor systems.

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Tables

Table 1

Timeline of Events

Event	Number of Sessions
(1) Baseline	20
(2) Ketamine alone (1.0, 3.0, 5.6, 10, 30 mg/kg^{-1})	24
(3) Haloperidol and clozapine alone (0.1 and 2.5 mg/kg^{-1}, respectively)	12
(4) Haloperidol pretreatment (0.01, 0.03, 0.1 mg/kg^{-1} with 30 mg/kg^{-1} ketamine)	28
(5) Clozapine pretreatment (0.1 - 4.0 mg/kg^{-1} with 30 mg/kg^{-1} ketamine)	35

Table 2

Backwards chaining and discrete stimulus conditions

Link 6	**Link 5**	**Link 4**	**Link 3**	**Link 2**	**Link 1**	**SR$^+$**
Low, fast pulse	*High, fast pulse*	*High tone, pulse*	*Low tone, pulse*	*Low tone, pure*	*High tone, pure*	*High tone*
					R →	Sucrose (6)
			L →	B →	R →	Sucrose (3)
			L →	B →	R →	Sucrose (3)
		B →	L →	B →	R →	Sucrose (3)
	L →	B →	L →	B →	R →	Sucrose (3)
R →	L →	B →	L →	B →	R →	Sucrose

Table 3

Ketamine dose-response functions: Model comparison

Measure	Model	SS	N	K	AICc	Probability (%)	Evidence Ratio
PQ	Shared	2.69	120	4	-334.66	37.1	1.70
	Separate	2.47	120	8	-333.46	62.9	
MCL	Shared	3.88	131	4	-452.75	43.7	1.28
	Separate	3.62	131	8	-454.11	56.3	
Response Rate	Shared	5.95	131	4	-396.56	93.7	14.87
	Separate	5.80	131	8	-391.15	6.3	
Reinforcer Rate	Shared	6.18	131	4	-391.71	27.8	2.59
	Separate	5.86	131	8	-389.81	72.2	

Table 4

Ketamine dose-response models: parameter estimates by component

Dependent measure	Component	Parameter			
		MIN	*MAX*	EC_{50}	*HILLSLOPE*
Progress quotient	Performance	0.44	0.99	6.1	*-4.38*
	Learning	0.64	0.97	6.9	*-2.84*
Max chain length	Performance	0.28	0.98	7.7	*-5.55*
	Learning	0.33	0.97	11.3	*-3.19*
Response rate	Performance	0.01	0.95	7.1	-3.01
	Learning	0.01	0.95	7.1	-3.01
Reinforcer Rate	Performance	*0.06*	*0.93*	7.4	*-5.09*
	Learning	*0.01*	*1.00*	13.0	*-2.41*

Table captions

Table 1. A timeline of events for the current study. In a within-subjects design, animals progressed from training to doses of ketamine alone, single injections of haloperidol or clozapine alone, haloperidol pretreatment with ketamine, and finally clozapine pretreatment with ketamine. In all, the study lasted approximately 5 months.

Table 2. Backwards chaining was used to incremental chain length in the IRA procedure. Methodologically, learning the first link (or the response closest to reinforcement) occurs first, and subsequent links in the chain are added in front of the first link. Thus, reinforcement follows only correct first link responses. Each link in the chain, regardless of component, had unique stimulus conditions associated with it. Here, these conditions were variations and combinations of pure tones and pulsating tones continuously repeated throughout the session, except during inter-trial intervals (following an incorrect response) and the inter-component interval.

Table 3. To compare performance and learning condition responding, a model with four shared parameters was compared to a model with two curves with four separate parameters each (for each dependent measure). AICc was used to determine the best model. For PQ, MCL, and reinforcer rate, there was a difference between shared and separate parameters in favor of separate parameters, though the evidence ratios were relatively small. For response rate, one curve was suitable for both components.

Table 4. Parameter estimates from the four-parameter sigmoidal function fit to the ketamine dose-response functions. For PQ, MCL, and reinforcer rate, all four parameters varied between performance and learning components, with lower EC_{50}'s and steeper slopes in the performance condition. Response rate was the only measure for which having shared parameters (one curve) between components was a better fit than separate parameters (two curves).

Figure 1

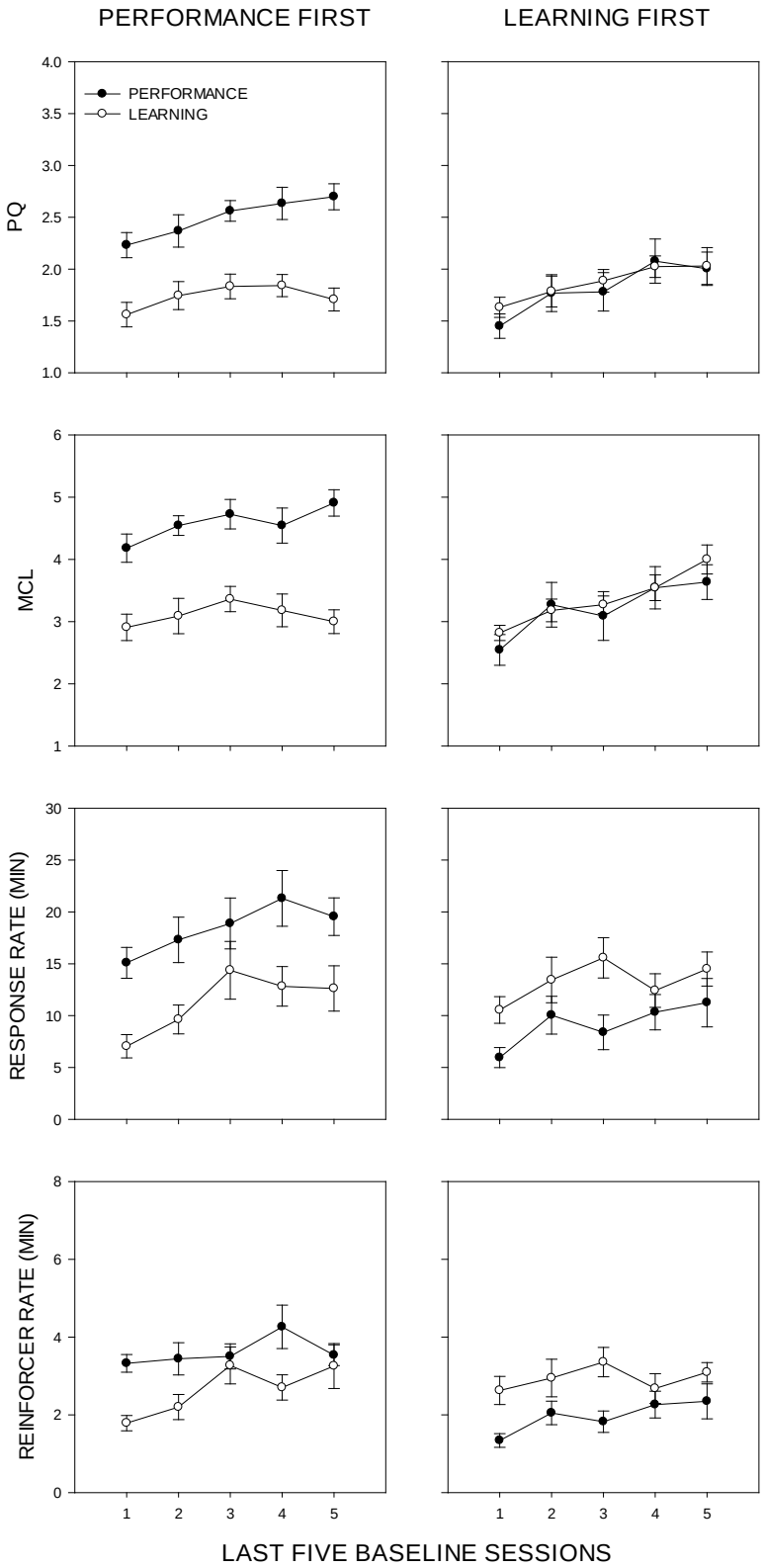
PERFORMANCE FIRST
LEARNING FIRST
PERFORMANCE
LEARNING
PQ
MCL
RESPONSE RATE (MIN)
REINFORCER RATE (MIN)
LAST FIVE BASELINE SESSIONS

Figure 2

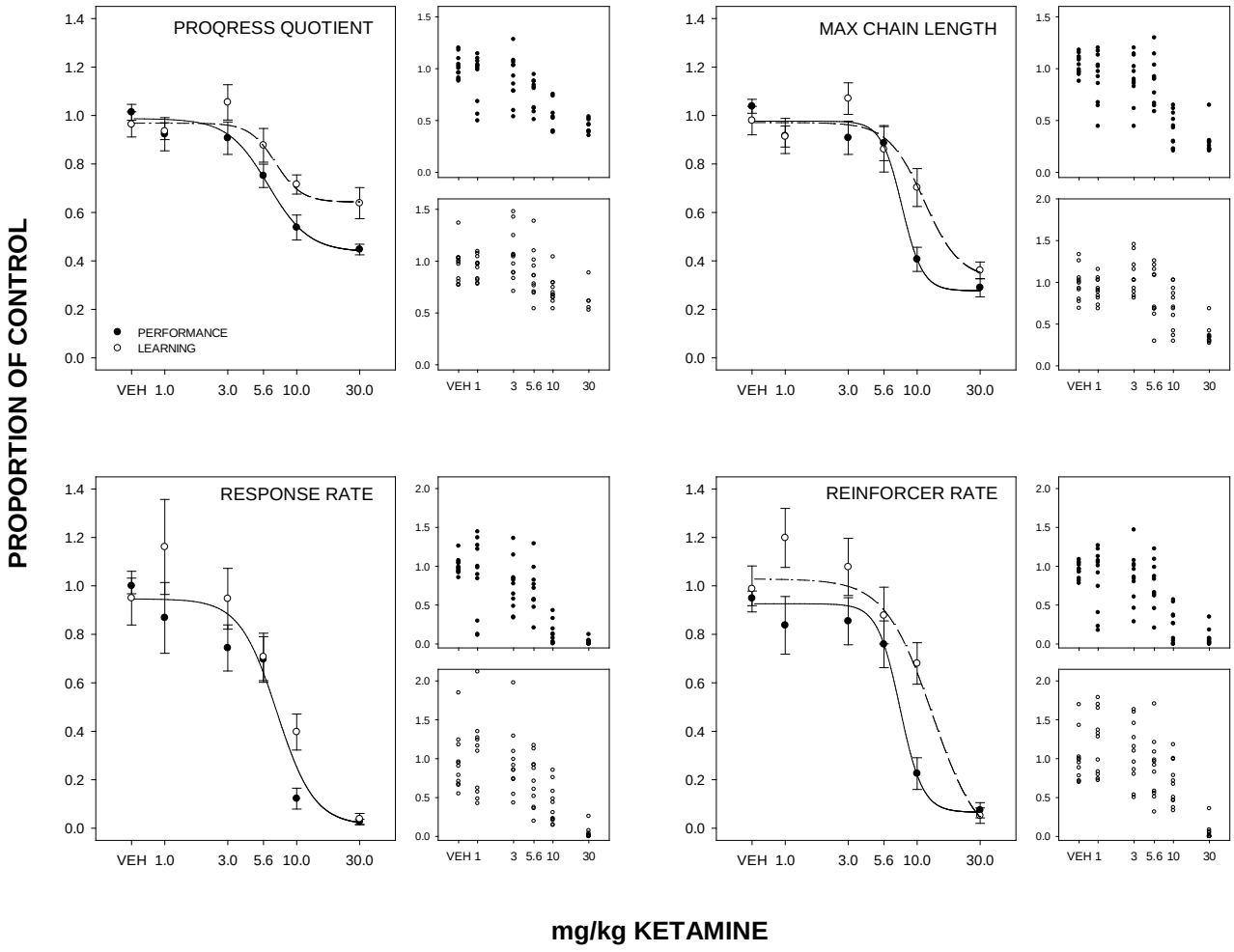
PROQRESS QUOTIENT
MAX CHAIN LENGTH
RESPONSE RATE
REINFORCER RATE
PERFORMANCE
LEARNING
PROPORTION OF CONTROL
mg/kg KETAMINE
VEH 1.0 3.0 5.6 10.0 30.0
VEH 1 3 5.6 10 30

Figure 3

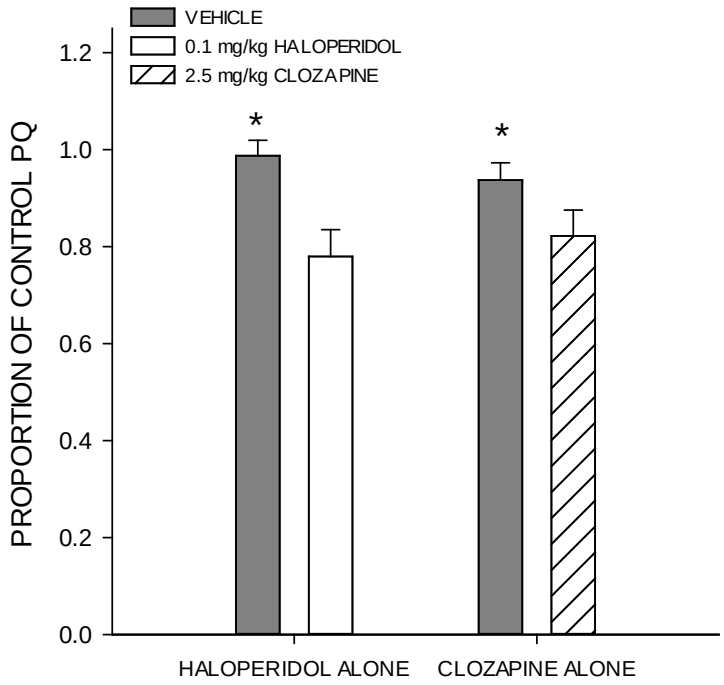
VEHICLE
0.1 mg/kg HALOPERIDOL
2.5 mg/kg CLOZAPINE
PROPORTION OF CONTROL PQ
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
HALOPERIDOL ALONE
CLOZAPINE ALONE

Figure 4

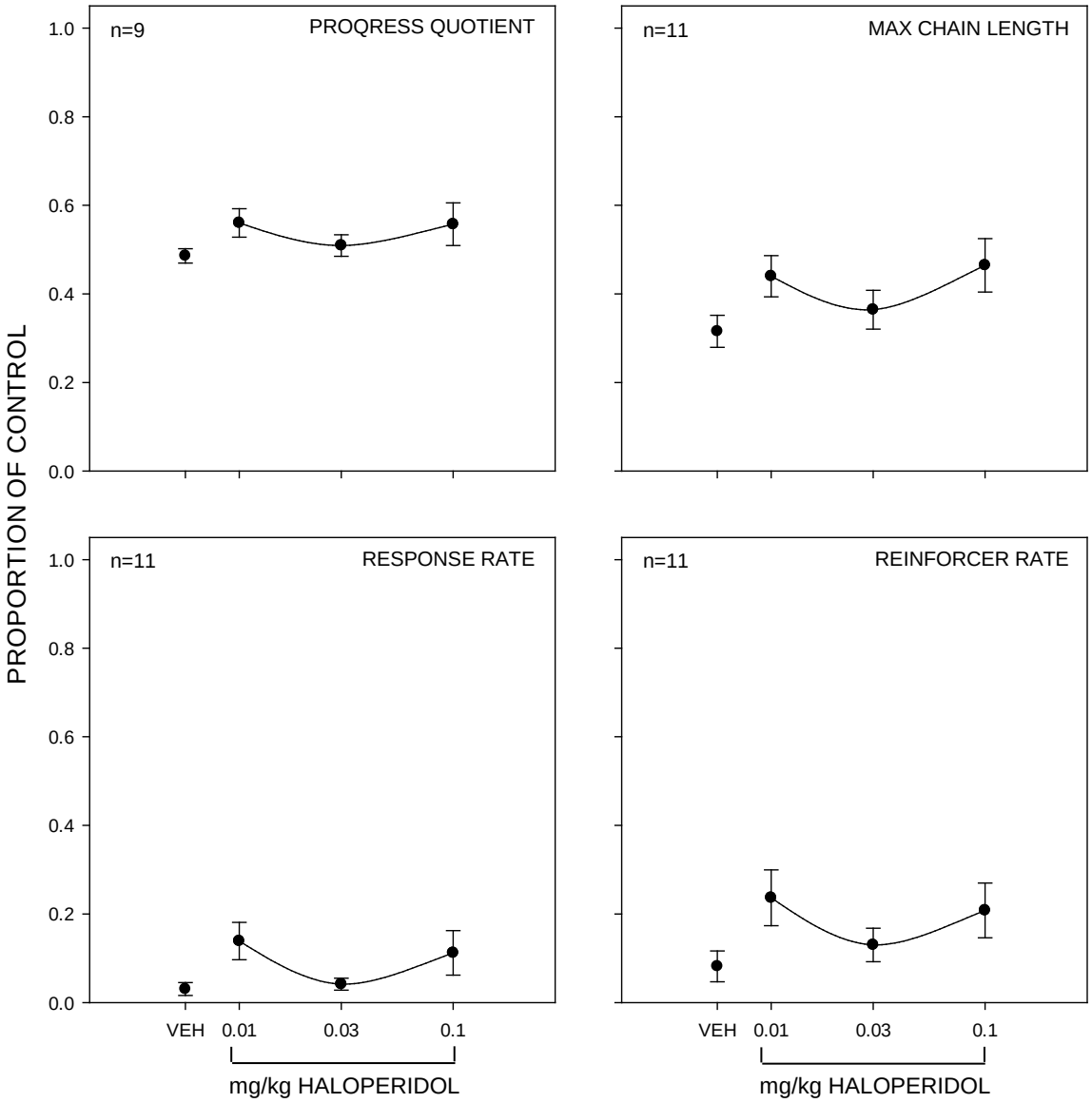
n=9
PROQRESS QUOTIENT
n=11
MAX CHAIN LENGTH
n=11
RESPONSE RATE
n=11
REINFORCER RATE
PROPORTION OF CONTROL
1.0
0.8
0.6
0.4
0.2
0.0
VEH
0.01
0.03
0.1
mg/kg HALOPERIDOL

Figure 5

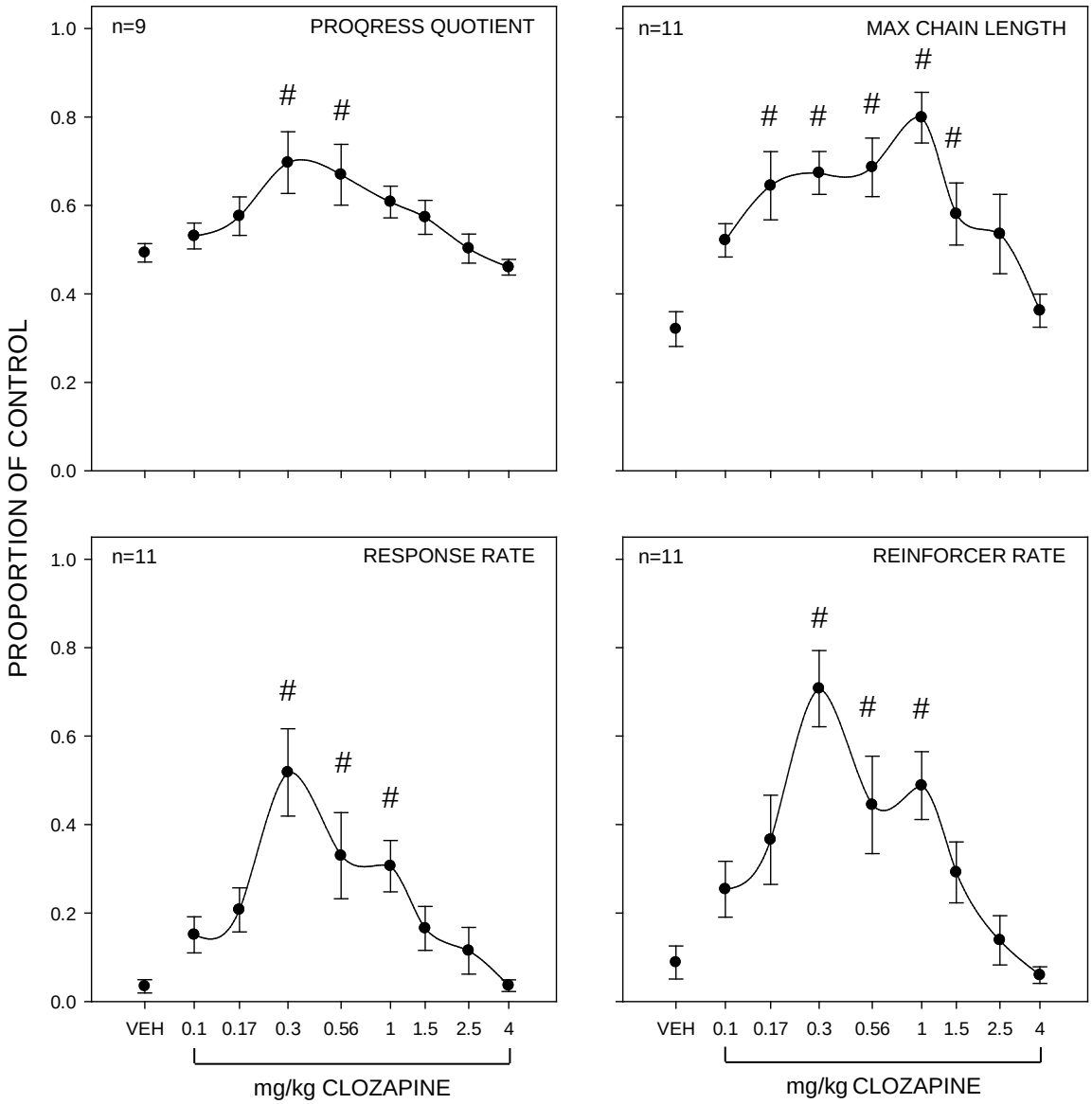
n=9
PROQRESS QUOTIENT
n=11
MAX CHAIN LENGTH
n=11
RESPONSE RATE
n=11
REINFORCER RATE
PROPORTION OF CONTROL
1.0
0.8
0.6
0.4
0.2
0.0
#
VEH
0.1
0.17
0.3
0.56
1
1.5
2.5
4
mg/kg CLOZAPINE

Figure Captions

Figure 1. Line graphs showing PQ, MCL, response rate, and reinforcer rate (rows) for the last five sessions of IRA baseline for each component order (columns). When these sessions were averaged together, RMANOVA revealed that when *Performance* was first, it produced greater separation in behavioral measures, whereas when *Learning* was first, overall productivity during *Performance* declined. When *Performance* and *Learning* occurred in the first component, PQ, MCL, and response rate were higher during *Performance* while there was no difference between reinforcer rates.

Figure 2. Performance (closed circles, solid lines) and learning (open circles, dashed lines) data from the first component only for each dependent measure were converted to proportion of control and plotted as a function of ketamine dose using a $\log_{10}$ x-axis. The smooth lines represent the best-fit of the four-parameter Hill equation (Eq. 3); the scatterplots show individual data from each component.

Figure 3. Single doses of haloperidol (0.1 mg/kg) and clozapine (2.5 mg/kg) were administered. While both doses do not represent high doses of either drug, RMA ANOVA revealed that they both caused a significant decrease in PQ relative to baseline (*: p's<0.05)

Figure 4. PQ, MCL, response rate, and reinforcer are plotted are shown as proportion of baseline plotted as a function of dose of haloperidol. At all doses, pretreatment with haloperidol was ineffective at rescuing ketamine-disrupting responding during the IRA procedure (p's>0.05).

Figure 5. PQ, MCL, response rate, and reinforcer rate are shown plotted as proportion of baseline plot as a function of dose of clozapine. Pretreatment with clozapine dose-dependently rescued responding IRA responding during the performance component (#: p's<0.05). Doses of 0.3-1.0 comprised the effective dosing range, while a doses of 0.3 and 0.56 mg/kg were the only two doses that significantly increased all four measures relative to vehicle. An outlier at 1.0 mg/kg was removed from each graph.

Equations

Equation 1

A two-compartment model that describes a drug's distribution and elimination half-lives.

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

Equation 2

Progress quotient, used to assess overall IRA productivity.

$$PQ = \frac{\left(\sum_{i=1}^{6} wR_i\right)}{R_t}$$

Equation 3

The four-parameter Hill equation (often termed the four-parameter logistic equation) fit to the ketamine dose-response curves.

$$Y = \min + \frac{\max - \min}{1 + \frac{x}{EC_{50}}^{-Hillslope}}$$