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DISCRIMINATIVE STIMULUS EFFECTS OF GABAPENTIN

By

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THESIS

Submitted to
Northern Michigan University
In partial fulfillment of the requirements
For the degree of

MASTERS OF SCIENCE

Office of Graduate Education and Research

May 2018

SIGNATURE APPROVAL FORM

Discriminative Stimulus Effects of Gabapentin

This thesis by Michael Ryan Zuidema Jr. is recommended for approval by the student's Thesis Committee and Department Head in the Department of Psychological Sciences and by the Assistant Provost of Graduate Education and Research.

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ABSTRACT

DISCRIMINATIVE STIMULUS EFFECTS OF GABAPENTIN

By

Michael Ryan Zuidema Jr.

The present study sought to evaluate the discriminative stimulus effects of the anticonvulsant gabapentin in rats trained to discriminate 30.0 mg/kg gabapentin from vehicle in a two-lever drug discrimination task. All of the ten rats tested were able to establish gabapentin as an interoceptive cue. Gabapentin produced full generalization (≥ 80% gabapentin-lever responding) for itself at 30.0, 60.0, and 120.0 mg/kg doses. Pentobarbital produced full substitution, while pregabalin, carbamazepine, fentanyl, and buspirone produced partial substitution (≥ 60% gabapentin-lever responding) for gabapentin. Ethanol and raclopride did not substitute for gabapentin. The psychostimulant amphetamine did not produce substitution; however, the 0.25 mg/kg dose of amphetamine fully substituted in five of ten rats. Based on these findings, some depressant (i.e., pentobarbital and fentanyl), anxiolytic (i.e., buspirone), and anticonvulsant compounds (i.e., pregabalin and carbamazepine) produce full or partial substitution to 30.0 mg/kg gabapentin. Additionally, the dopamine releaser amphetamine also produced full substitution in half of the rats tested. Many of the compounds that produced substitution in this study are controlled substances capable of producing rewarding subjective effects. The substitution demonstrated in this study coincides with the past reports of poly-drug misuse, indicating the ability of gabapentin to modulate neurotransmitter pathways involved in positive drug effects. Thus, these modulatory effects should be considered by clinicians and researchers when working with gabapentin.

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TABLE OF CONTENTS

INTRODUCTION

Gabapentin, a GABA analog, is an anticonvulsant primarily used for the treatment of epileptic seizures, but also other disorders, such as neuropathic pain. It is used as an off-label (i.e., non-FDA approved uses) treatment for anxiety, insomnia, bipolar disorder, and restless leg syndrome (Sobel, 2012, p. 124), and has been used in the treatment of opioid withdrawal, cocaine dependency, and alcohol, benzodiazepine, and pentazocine detoxification (Victorri-Vigneau, Guerlais & Jolliet, 2007). Although gabapentin was initially synthesized to mimic the neurotransmitter GABA, it does not bind with GABA receptors. Instead, the action of gabapentin may involve interaction with voltage-gated Ca^{2+} channels, ultimately reducing neurotransmitter release from affected neurons (Davies et al., 2007) and postsynaptic excitatory response. There is also evidence to support the drug's modulation of GABA metabolism (Schifano, 2014). Further exploration of the pharmacological actions of gabapentin could be especially beneficial in lieu of the relatively liberal prescription of gabapentin.

Many of the reports indicating psychoactive subjective effects from gabapentin are relatively recent and few studies have been conducted to specifically examine these effects in a laboratory setting. The most common and effective procedure for examining the pharmacological actions mediating a compound's subjective effects is drug discrimination. Drug discrimination is an operant conditioning procedure that establishes a drug's effects as a discriminative cue, signaling when a behavioral response will achieve a reward. From weeks of training in this procedure, laboratory animals (typically rats) learn to respond only when the specific cue is present; what we find from this procedure is that responding will also occur for different compounds, as long as the other compounds closely match the same subjective effects. The interpretive value from testing different compounds comes from knowing the pharmacological

actions of these different compounds. In this way, we can deduce the pharmacological actions likely involved in eliciting a training drug's subjective effects.

No studies have examined the discriminative cue properties of gabapentin as a training drug in a drug discrimination procedure. Therefore, the proposed study will train rats to discriminate gabapentin from its vehicle (the solvent used for gabapentin, acting like a placebo) and then test a series of compounds from different drug classes and with known pharmacological actions. In particular, these compounds will include those with known abuse potential. The aim of this study is to illuminate the mechanisms mediating the subjective effects of gabapentin and the degree to which these effects are similar to those of known drugs of abuse.

Gabapentin

Gabapentin (Neurontin®, 1-(aminomethyl)cyclohexanacetic acid), a GABA (*gamma*-aminobutyric acid) analog, is an anticonvulsant medication primarily used for the treatment of epileptic seizures and neuropathic pain. It is used as an off-label (i.e., non-FDA approved uses) for the treatment of anxiety, insomnia, bipolar disorder, and restless leg syndrome (Sobel, 2012, p. 124), and has been used in the treatment of opioid withdrawal, cocaine dependency, and alcohol, benzodiazepine, and pentazocine detoxification (Victorri-Vigneau, Guerlais & Jolliet, 2007).

Gabapentin is manufactured under the brand name Neurontin by Parke-Davis, a subsidiary of Pfizer. Another subsidiary of Pfizer, Greenstone manufactures a generic version of the drug. Gabapentin became FDA (U.S. Food and Drug Administration) approved in December 1993 for the adjunctive treatment of partial seizures (Mack, 2003). In 2000, the drug's approval was extended to children for the treatment of partial seizures, and in 2004 for treating postherpetic neuralgia (complication of shingles in which pain lasts long after the condition disappears) (Pfizer, 2011; Mack, 2003). In 2011, a prodrug form of gabapentin designed to increase oral bioavailability (gabapentin enacarbil) was approved for the treatment of restless leg syndrome (Landmark & Johannessen, 2008).

Pharmacology. Gabapentin is synthesized by adding a cyclohexyl group to the backbone of GABA (Petroianu & Schmitt, 2002). The molecular weight is 171 and the pKA is 3.7. The chemical is not fully metabolized in humans, and bioavailability of gabapentin is not proportional to the dose administered. This means that as the dose increases (900, 1200, 2400, 3600, and 4800 mg/day given in three doses), bioavailability of the drug decreases (60%, 47%, 34%, 33%, and 27%, respectively) with food playing only a small role on absorption (Pfizer,

2011). Gabapentin distribution occurs through blood circulation via plasma protein binding, and the drug is eliminated from the system as an unchanged drug through renal excretion. The half-life of gabapentin is 2 to 3 hours in rats (Radulovic et al., 1995; Vollmer & Koelle, 1986), 5 to 7 hours in humans, and is unaffected by dose or dose schedule (Pfizer, 2011).

Although gabapentin was initially synthesized to mimic the neurotransmitter GABA, it does not bind to GABA receptors. Gabapentin is not converted into GABA, a GABA agonist (Pfizer, 2011), nor does it inhibit GABA reuptake. Gabapentin lacks an appreciable affinity for monoamine receptors, cholinergic receptors, excitatory amino acid receptors, and calcium channels (Petroianu & Schmitt, 2002).

Researchers first investigated gabapentin's interaction with the L-amino acid transport system to identify the drug's primary mechanism of action (Sills, 2006). Although this system is a major Na^+-independent carrier for large *alpha*-amino acids in mammalian cells, gabapentin, a *gamma*-amino acid, is also transported via this network (Su, Lunney, Campbell & Oxender, 1995). Gabapentin is absorbed through the small intestine and transported through the blood-brain barrier and distributed to the nervous system via this transport system (Su et al., 1995). Yet, recent research suggests that although the L-amino acid transport system directly correlates with both drug absorption in the gastrointestinal tract and distribution across the blood-brain barrier, this interaction does not contribute to the drug's clinical efficacy (Belliotti et al., 2005; Schwarz et al., 2005).

Instead, the action of gabapentin may involve interaction with voltage-gated Ca^{2+} channels (Davies et al., 2007; Sills, 2006). Gee et. al. (1996) more accurately identified this binding site to be the α2δ accessory subunit of the voltage-gated calcium channel complex. Gabapentin binds with two of the four Ca^{2+} channel α2δ isoforms, α2δ-1 and α2δ-2 (Taylor,

2004). Knock-in of the R217A mutation of the α2δ-1 subunit results in decreased gabapentin binding, limiting analgesic, anxiolytic, and anticonvulsant effects (Taylor, 2004). This evidence strongly supports the action of these drugs occurring at the α2δ-1 subunit. In a study by Field, Hughes & Singh (2000), further evidence was reported in support of the mediation of gabapentin on α2δ accessory subunits. 3-methyl gabapentin (binds to α2δ accessory subunit with high affinity, K_D = 38 nM) was found to dose-dependently block the effects of static and dynamic allodynia in two rat models of pain (Field, Hughes & Singh, 2000; Suman-Chauhan, Webdale, Hill & Woodruff, 1993), further implicating the α2δ accessory subunit in gabapentin's mechanism in pain models. A more recent study by Brown and Randall (2005) concluded that gabapentin acts to selectively block calcium channels containing the α2δ-1 subunit, rather than inhibiting the α2δ-1 receptor. This α2δ-1 subunit blockage is currently thought to be the primary pharmacological mechanism behind the action of gabapentin.

Evidence also exists to suggest the drug's modulation of GABA metabolism (Leach et al., 1997; Schifano, 2014) synthesis (Taylor, Vartanian, Andruszkiewicz & Silverman, 1992), and non-vesicular release of GABA (Gotz, Feuerstein, Lais & Meyer, 1993; Honmou, Kocsis & Richerson, 1995). Due to the drug's structural similarities with baclofen, a $GABA_B$ receptor agonist, Ng et. al. (2001) suggests that gabapentin may act similarly as a postsynaptic $GABA_B$ agonist to select receptor subtypes in the hippocampus. However, because the co-administration of $GABA_B$ antagonists have not been shown to reverse the antihyperalgesic effects of gabapentin, other mechanisms are thought to more likely mediate the drug's effects (Patel et. al., 2001). While gabapentin has been shown to increase overall GABA levels in neocortical *ex vivo* analysis (Errante, Williamson, Spencer & Petroff, 2002) and *in vivo* nuclear magnetic resonance spectroscopy of the occipital cortex (Errante et al., 2002; Petroff, Rothman, Behar, Lamoureux &

Mattson, 1996), these findings have not been replicable in other studies measuring GABA concentrations in rodent brains (Leach et al., 1997; Errante & Petroff, 2003).

Some studies suggest that gabapentin is able to produce a delayed allosteric enhancement of voltage gated K^+ channels in rat dorsal root ganglions, possibly through protein kinase A activation (McClelland, Evans, Barkworth, Martin & Scott, 2004). Although the drug has been shown to modulate ATP-sensitive K^+ channels in human neocortical and rat hippocampal slices (Freiman et al., 2001), this effect has not been able to be replicated in other models such as the rat dorsal root ganglion (Sarantopoulos, 2003).

Because gabapentin contains anticonvulsant properties, neuronal voltage-gated Na^+ channels have been investigated to evaluate the drug's ability to block repetitive firing. Although moderate inhibitory effects can be elicited with drug exposure, research suggests that the effect of gabapentin is mediated by yet another unidentified mechanism rather than through blockades of voltage-gated Na^+ channels (Sills, 2006). For example, gabapentin fails to inhibit batrachotoxin, a Na^+ channel-specific agent shown to induce depolarization in vesicular preparation (Creveling, McNeal, Daly & Brown, 1983), rat neocortical membranes (Dooley, Donovan, Meder & Whetzel, 2002), Na^+ currents in Chinese hamster ovary cells (Xie et al., 2001), or Ca^{2+} influx in rat cortical synaptosomes (Meder & Dooley, 2000).

Based on the findings discussed above, a single common mechanism is thought to be most prominent in gabapentin's mechanism of action. Gabapentin most likely predominantly works to inhibit voltage gated calcium channels via the $\alpha 2\delta$ subunit, ultimately reducing neurotransmitter release and postsynaptic excitability (Sills, 2006). Because of the presynaptic nature of gabapentin's proposed mechanism, it is not unreasonable to theorize that this accounts for other effects of the drug, including $GABA_B$ receptor activation, presynaptic NMDA receptor

modulation, and the overall decrease of neurotransmitter release (Sills, 2006). Further investigation is required to determine whether calcium channel interaction alone is able to explain the broad clinical spectrum of gabapentin.

Effects. Gabapentin is commonly thought to exhibit few cognitive and neuropsychological side effects at therapeutic doses (Loring, Marino & Meador, 2007). Many studies suggest even at relatively high doses, gabapentin demonstrates a favorable CNS-related profile (Chadwick et al., 1998). For example, single-dose administration of gabapentin in humans was associated with improved concentration and EEG slowing (Saletu et al., 1986). In long-term administration, gabapentin scored better on 8 of 31 neuropsychological measures compared to carbamazepine, only scoring lower than placebo on four measures (Meador et al., 1999). However, although well tolerated cognitively, gabapentin does seem to pose certain risks to neuropsychological function (Loring, Marino & Meador, 2007).

As prescribing rates of gabapentin have increased, so too have reports of drug misuse and overdose fatalities. Recent data indicate that gabapentin is abused across a wide range of doses including therapeutic (900-3600 mg/day) and supratherapeutic doses (3-20 times greater than clinically advisable doses) (Smith, Havens & Walsh, 2016; Schifano, 2014). Individuals who possess a prescription for gabapentin and abuse the drug often take doses much higher than the amount prescribed, while, in contrast, those who abuse the drug without a prescription are more likely to take doses within clinical guidelines (Wilens, Zulauf, Ryland, Carrellas & Catalina-Wellington, 2014; Smith, Lofwall & Havens, 2015). Use of these high amounts suggest the development of tolerance, one of many indicators of substance dependence.

Clinicians have begun noting patients' histories that may account for unexpected effects from gabapentin. According to Smith, Higgins, Baldacchino, Kidd & Bannister (2012), for

example, “effects vary with the user, dosage, past experience, psychiatric history, and expectations.” Reports reveal that gabapentin may elicit an array of subjective effects reminiscent of opioids, benzodiazepines, and psychedelics (Smith, Havens & Walsh, 2016). Case studies report that gabapentin alone (1500-12,000 mg doses) and in combination with other drugs (such as buprenorphine, naloxone, methadone, baclofen, quetiapine, and alcohol) can elicit a type of euphoria similar opioid-induced euphoria (Reeves & Burke, 2014; Reeves & Ladner, 2014; Fischer, Ban, Rogers, Fischer & Trudeau, 1994; Baird, Fox & Colvin, 2014; Smith et al., 2012; Schifano et al., 2011). In another case study, individuals experienced a cocaine-like high after snorting powder from gabapentin capsule medication (Reccoppa, Malcolm & Ware, 2004). More commonly reported psychoactive effects of gabapentin include sedation, relaxation, and calmness, sometimes in combination with other drugs such as quetiapine, alcohol, cannabis, or buprenorphine (Pittenger & Desan, 2007; Markowitz, Finkenbine, Myrick, King & Carson, 1997; Reeves & Burke, 2014; Reeves & Ladner, 2014; Schifano et al., 2011). Other effects shown consist of an improved sociability, a marijuana-like high (Smith et al., 2012), cocaine-like high (Reccoppa, Malcolm & Ware, 2004), 3,4-methylenediocy-mehtamphetamine (MDMA)-like high, ‘amphetamine rush’, dissociative effects (Schifano et al., 2011), increased focus and energy, improved sleep (Satish, Kandasamy, Jayarajan & Benegal, 2015), and becoming more talkative (Schifano et al., 2011).

Effects of gabapentin for the treatment of epilepsy. Epilepsy is a disease of the central nervous system in which nerve cell activity becomes disrupted (Meyer, Dua, Ma, Saxena & Birbeck, 2010). This disruption of nerve activity can cause seizures (often characterized by involuntary jerking movements of the arms and legs), abnormal behavior, temporary confusion, and loss of consciousness or awareness (Mayo Clinic Staff Print, 2015). Genetic influence is

likely to play a role in many cases of idiopathic epilepsy (Pandolfo, 2011), and the majority of genes associated with epilepsy represent subunits of receptor channels that mediate sodium, potassium, calcium, and GABA neurotransmission (Rees, 2010).

Sodium channels are one of the primary targets amongst traditional antiepileptic medications. For example, anticonvulsant medications such as lamotrigine, topiramate, and zonisamide all modulate Na^+ channels (Leach, Marden & Miller, 1986; Loring, Marino & Meador, 2007). Although, as noted previously, evidence would suggest gabapentin's action to be mediated by mechanisms other than interaction with Na^+ channels, gabapentin does act on other similar mechanisms seen in typical anticonvulsants. In addition to Na^+ channel interaction, lamotrigine works by glutamate reduction (Leach, Marden & Miller, 1986), topiramate by GABA potentiation and glutamate antagonism, zonisamide by blocking presynaptic Ca^{2+} channels, and tiagabine by inhibiting GABA reuptake (Loring, Marino & Meador, 2007).

Effects of gabapentin for the treatment of neuropathy. Peripheral neuropathy is a disease or damage to the peripheral nervous system (National Institute of Neurological Disorders and Stroke, n.d.). Affected areas can become hypersensitive to stimuli, resulting in the perception of pain from stimuli that do not normally evoke pain. In severe cases, symptoms may worsen to burning pain, paralysis, muscle atrophy, and organ dysfunction. Nerve damage to organs responsible for vital functions may result in failure or functional impairment in the form of sweating, digestive complications, sexual dysfunction, and difficulty breathing (National Institute of Neurological Disorders and Stroke, n.d.).

Medications used to treat peripheral neuropathy generally act on the central nervous system and include antidepressants, anticonvulsants and narcotics. Tricyclic antidepressants (e.g. amitriptyline) and serotonin-norepinephrine reuptake inhibitors (SNRI) (e.g. duloxetine

hydrochloride) require chronic treatment and are thought to exert their effects through noradrenergic descending pathways and the recruitment of noradrenaline via sympathetic fibers (Kremer, Salvat, Muller, Yalcin & Barrot, 2016). These compounds seem to target both α2 and β2 adrenoreceptors and require μ and δ opioid receptor interaction to produce therapeutic action (Kremer et al., 2016). Although tricyclic antidepressants provide effective pain relief, adverse effects are often seen such as arrhythmia, sedation, dry mouth, constipation and urinary retention (Rowbotham et. al., 1998).

In animal models, gabapentin has been shown to decrease allodynia induced by lesions (Chen, Eisenach, McCaslin, & Pan, 2000; Field, Gonzalez, Tallarida, & Singh, 2002; Miranda et al., 2015). Although the mechanism of action remains unclear, gabapentin may exert its antiallodynic effects by binding to the α2δ-1 subunit of voltage-gated Ca^{2+} channels, decreasing excitatory neurotransmitter release. Additionally, gabapentin has been shown to activate NO-cyclic GMP-ATP sensitive K^{+} channel pathways (Ortiz, Medina-Tato, Sarmiento-Heredia, Palma-Martinez, & Granados-Soto, 2006; Godinez-Chaparro, Quinonez-Bastidas, Rojas-Hernandez, Austrich-Olivares, Mata-Bermudez, 2017), non-competitvely inhibit NMDA receptors (Hara & Sata, 2007), and activate descending noradrenergic pain inhibitory system via α2 adrenoreceptors, which explains the drug's antinociceptive effects (Kremer et al., 2016).

Gabapentin is a single nonopioid medication that provides safety and pain relief to postherpetic neuralgia patients, making it a strong candidate for treatment. In a multicenter, randomized, double-blind, placebo-controlled, parallel design, 8-week trial conducted by Rowbotham et. al. (1998), 229 subjects were used to determine the efficacy and safety of gabapentin in reducing postherpetic neuralgia pain. Over a 4-week period, gabapentin was titrated up to a maximum dose of 3600 mg/d in the experimental group, followed by 4 weeks at

the maximum tolerated dose (Rowbotham et. al., 1998). Efficacy was measured based on an 11-point Likert scale rating average daily pain (0, no pain; 10, worst pain) from the baseline week until the final week of therapy (Rowbotham et. al., 1998). Patients receiving gabapentin had significantly lower daily pain scores (6.3 to 4.2) compared with change in subjects that received placebo ($p < 0.001$) (Rowbotham et. al., 1998), demonstrating that gabapentin is an effective treatment of pain associated with postherpetic neuralgia.

More recently, a double-blind, randomized, placebo-controlled 8-week study was conducted by Serpell & Neuropathic Pain Study Group (2002) in order to determine the efficacy and safety of gabapentin in neuropathic pain. Patients exhibited a range of symptoms, including allodynia, burning pain, shooting pain or hyperalgesia (Serpell & Neuropathic Pain Study Group, 2002). In the experimental group, gabapentin was titrated up to 900 mg/d over 3 days, and ultimately up to 2400 mg/d by the end of week 5 (Serpell & Neuropathic Pain Study Group, 2002). Efficacy was measured using an average daily pain score. In patients receiving gabapentin treatment, average daily pain scores significantly decreased by 21% ($p < 0.05$) (Serpell & Neuropathic Pain Study Group, 2002), demonstrating that gabapentin is effective is reducing pain-like symptoms in patients with neuropathic pain symptoms.

Drug misuse. Gabapentin is currently labeled an uncontrolled substance lacking abuse potential, and thus, is widely prescribed as an off-label medication. However, there have been many documented cases of gabapentin misuse, abuse, dependence, and withdrawal. Accordingly, evidence suggests that the drug may exert reinforcing effects that are dissociable from its anticonvulsant effects (Bossert & Franklin, 2003). A recent meta-analysis by Smith, Havens, & Walsh (2016) reported gabapentin misuse to be prevalent in 1% of the general population. Of this one percent, 40-65% misuse gabapentin in conjunction with other prescription medications,

and 15-22% misuse gabapentin specifically with opioids (Smith, Havens, & Walsh, 2016). Motives behind gabapentin abuse were identified as: recreational use, control of mood/anxiety, pain relief, reduced cravings from other drugs, substitution for other drugs, potentiating effects of drug abuse treatment, addiction to gabapentin, and intentional self-harm (Smith, Havens, & Walsh, 2016).

As previously stated, users report subjective effects reminiscent of opioids, benzodiazepines, and psychedelics. These subjective effects were reported over a range of doses, including clinically therapeutic doses (Smith, Havens, & Walsh, 2016). In addition to these drug effects, evidence suggests that gabapentin may be abused in conjunction with other drugs, possibly providing potentiating or modulatory drug effects. Accordingly, gabapentin is reported to be most commonly abused with prescription opioids (Smith et al., 2012; Smith, Lofwall, & Havens, 2015), benzodiazepines (Smith, Lofwall, & Havens, 2015; Peterson, 2009), and alcohol (Schifano et al., 2011); although, reports exist of conjunctive use with cannabis, selective serotonin reuptake inhibitors, lysergic acid diethylamide (LSD), gamma-hydroxybutyric acid (GHB), and amphetamine (Schifano et al., 2011).

Drug Discrimination

Drug discrimination (DD) is an operant conditioning procedure that establishes a drug's effects as a discriminative cue. The subjective effects of the drug act to signal when a laboratory animal's response (e.g., lever-pressing behavior) will achieve a reward (e.g., food, water, etc.). In both human and non-human DD models, the subject must perform a response or behavior that distinguishes drug and nondrug conditions (Buccafusco, 2009). When applied in non-human models, subjects are trained to discriminate between a drug and vehicle (often 0.9% sodium chloride solution that is also the solvent for the drug) by pressing the drug-appropriate lever in an

operant chamber to receive reinforcement. The established distinction between drug and nondrug conditions allows an experimenter to deduce that the drug has been perceived and investigate the stimulus effects of the drug being studied (Buccafusco, 2009). Drug discrimination provides a powerful tool to studying in vivo drug properties, and can be used accordingly to study non-training drugs for similar actions. Thus, drug discrimination provides a model for screening novel drugs that are able to be established as a discriminative cue. The specificity of a discriminative stimulus for certain CNS receptors can be demonstrated by stimulus generalization to receptor-selective compounds.

One study sought to assess the discriminative properties of tiagabine, a drug which exerts its anticonvulsant and sleep-enhancing effects by inhibiting reuptake at the GABA transporter (GAT-1). Using 30.0 mg/kg tiagabine-trained rats, McDonald et al. (2008) reported no stimulus generalization to gabapentin, which has also been thought to inhibit GABA GAT-1 (Loscher, Honack, & Taylor, 1991; Sills, 2006; Goldlust, Su, Welty, Taylor, & Oxender, 1995; Leach et al., 1997). Moreover, tiagabine also produced no generalization for zolpidem and zopiclone ($GABA_A$ agonists). Full substitution was defined as ≥ 80% condition-appropriate responding, and partial substitution was defined as a statistically significant ($p < 0.05$) increase in generalization compared to vehicle (while not approaching full substitution, i.e., < 80% condition-appropriate responding).

While no studies have utilized gabapentin as the training drug in a drug discrimination procedure, studies have evaluated the stimulus effects of gabapentin as a substitute for other training drugs. McDonald et al. (2008) trained rats to discriminate the anticonvulsant drug tiagabine from vehicle in a two-lever drug discrimination task and evaluated drugs that may bind to GABA receptors. Gabapentin (30.0 – 300.0 mg/kg, po) did not fully substitute for tiagabine,

engendering up to 40% tiagabine-appropriate responding. The only drug that fully substituted for tiagabine was the $GABA_A$ receptor agonist gaboxadol, although this result could only be shown in three rats due to rate suppression. In addition, the discriminative stimulus effects of tiagabine were partially blocked by the $GABA_A$ receptor antagonist bicuculline. Based on these findings, the discriminative stimulus effects of tiagabine appear to be mediated, at least in part, by the activation of $GABA_A$ receptors. Further, these findings suggest that the stimulus effects elicited by gabapentin are likely not mediated by $GABA_A$ receptor agonism, which is consistent with pharmacological results noted earlier.

Other studies have compared the discriminative stimulus effects of cannabinoids to those of gabapentin in order to better understand the drug's mechanism of action. In a study by Lile, Wesley, Michael, Thomas & Lon (2016), eight cannabis users were trained to discriminate 30 mg of the $CB_{1/2}$ receptor partial agonist Δ^9-THC from placebo, and then received gabapentin (600, 1200 mg), Δ^9-THC (5, 15, and 30 mg), and placebo both in combination and alone as test compounds for substitution testing. Both doses of gabapentin alone fully substituted for the discriminative stimulus effects of Δ^9-THC and the combination of gabapentin with Δ^9-THC shifted the dose response for Δ^9-THC to the left (Lile et al., 2016). In addition, it is noted that CB1 receptor agonists act as L-voltage gated Ca^{2+} channel blockers (Ross, Napier & Connor, 2008; Lozovaya, Min, Tsintsadze & Burnashev, 2009), and therefore Δ^9-THC-like discriminative stimulus effects may result from the blocking mechanisms of gabapentin and Δ^9-THC at L-voltage gated Ca^{2+} channels (Stefani, Spadoni & Bernardi, 1998; Fink et al., 2002).

One study attempted to characterize the effects of gabapentin compared to the discriminative stimulus effects of alcohol (1 g/kg) in rats (Besheer, Frisbee, Randall, Jaramillo & Masciello, 2016). Gabapentin (120 mg/kg) produced partial substitution (>40% alcohol-lever

responding) for alcohol. The study also examined the effects of gabapentin on alcohol self-administration in rats. Gabapentin (30 and 120 mg/kg) pre-treatment resulted in increased alcohol self-administration (Besheer et al., 2016).

Several studies have investigated gabapentin's effects on the discriminative stimulus properties of cocaine in rats and humans (Filip et al., 2007; Haney, Hart, Collins & Foltin, 2005; Hart, Ward, Collins, Haney & Foltin, 2004). Filip et al. (2007) trained rats to discriminate cocaine (10 mg/kg) from vehicle. Gabapentin (10 – 30 mg/kg) failed to block the discriminative stimulus effects of cocaine. In this same study, gabapentin did not attenuate cocaine self-administration responding or affect cocaine-induced reinstatement (Filip et al., 2007). Haney et al. (2005) trained cocaine-dependent human volunteers to discriminative the stimulus effects of cocaine from placebo. Following training, participants were assigned a chronic treatment dose of gabapentin (0, 600 or 1200 mg/day) and then later, cocaine was tested for substitution during gabapentin maintenance. Percent cocaine responding was significantly decreased compared to those given gabapentin placebo. The 1,200 mg/day gabapentin also significantly decreased "Good Drug Effect" and "Craving or I want cocaine" subjective ratings following cocaine administration. In another study in humans, gabapentin (2,400 and 3,200 mg/day) did not attenuate cocaine-appropriate responding. Further investigation of varying doses and maintenance periods of both gabapentin and cocaine is warranted, considered both compounds are commonly abused at relatively high doses.

Rationale

In order to evaluate the effects gabapentin, rats were trained to discriminate 30.0 mg/kg gabapentin from vehicle in a two-lever drug discrimination task. The first goal of this investigation was to determine if, in fact, gabapentin could be established as a discriminative cue

in rats. Next, in order to elucidate the pharmacological mechanisms and stimulus properties of gabapentin, compounds from various drug classes (i.e., selective for different receptors; e.g., dopamine, serotonin, GABA, etc.) were tested for stimulus generalization in these animals (ethanol, pregabalin, carbamazepine, pentobarbital, fentanyl, buspirone, amphetamine, and raclopride). A description of these compounds is provided below.

Ethanol produces dose-dependent effects, generally yielding depressant physiological and psychology effects at higher doses and excitatory effects at lower doses (Prus, 2014). Ethanol has been shown to produce its reinforcing effects through $GABA_A$ agonism, ultimately facilitating action in the nucleus accumbens (Harris, Mihic, Brozowski, Hadingham, & Whiting, 1997; Yoshimoto, McBride, Lumeng, & Li, 1992). Additionally, ethanol has been shown to inhibit NMDA receptors (Krystal, Petrakis, Mason, Trevisan, & D'Souza, 2003), inhibit L-type voltage-gated Ca^{2+} channels (Walter & Messing, 1999), increase serotonin in the nucleus accumbens (Yoshimoto et al., 1992), and interact with endocannabinoid systems (Hungund, Szakall, Adam, Basavarajappa, & Vadasz, 2003).

Pregabalin, a gabapentinoid compound structurally, behaviorally, and pharmacologically similar to gabaepentin, demonstrates efficacy in the treatment of seizure and neuropathic pain. Additionally, pregabalin is able to produce benzodiazepine-like anxiolytic effects, which attenuate both psychic and somatic symptoms of anxiety (Kavoussi, 2006). Pregabalin shares a novel mechanism of action with gabapentin, binding selectively to the α2δ subunit of voltage-gated Ca^{2+} channels, ultimately reducing excitatory neurotransmitter release. Coinciding with pregabalin's higher binding affinity (relative to gabapentin) to the α2δ subunit, pregabalin is considered to be more addictive, as demonstrated by the drug's behavioral dependence

symptoms (Bonnet & Scherbaum, 2017). Pregabalin is expected to generalize to the 30.0 mg/kg gabapentin training dose at lower of doses of pregabalin (relative to gabapentin).

Carbamazepine is an anticonvulsant compound which exerts its effect through the blockade of Na+ channels (Rogawski, Loescher, & Rho, 2016; MacDonald, 1995; Czapinski, Blaszczyk, & Czuczwar, 2005), $Na_v1.8$-like sodium channels (Cardenas, Cardenas, de Armendi, & Scroggs, 2006) and by inhibiting serotonin reuptake (Southam, Kirkby, Higgins, & Hagan, 1998; Dailey, Reith, Yan, Li, & Jobe, 1997; Kawata et al., 2001). Although gabapentin does not modulate serotonin reuptake or concentration (Southam et al., 1998), previous studies have shown gabapentin and carbamazepine to share a similar profile, both producing significant antihyperalgesic and anti-allodynic effects within a similar dose range (De Vry, Kuhl, Franken-Kunkel, & Eckel, 2004; Bennet & Xie, 1988; Hunter et al., 1997; Koch, Faurot, McGuirk, Clarke, & Hunter, 1996). Additionally, these drugs have also been evaluated for their ability to attenuate the positive subjective effects of cocaine in rats and humans (Carroll, Lac, Asencio, Halikas, & Kragh, 1990; Hart et al., 2004; Haney, Hart, Collins, & Foltin, 2005; Sharpe, Jaffe, & Katz, 1992; Halikas, Crosby, Pearson, & Graves, 1997).

Pentobarbital is a highly effective anticonvulsant and sedative (Raines et al., 1979) that exerts its effects through $GABA_A$ agonism (Leeb-Lundberg, Snowman, & Olsen, 1980), diminishing glutamate responses (Macdonald & Barker, 1978), and by inhibiting voltage-gated sodium (Lingamaneni & Hemmings, 2003; Wartenberg, Wartenberg, & Urban, 2001) and calcium channels (Werz & Macdonald, 1982; Barker & Rogawski, 1993; Schoeber, Sokolova, & Gingrich, 2010). Studies have shown that pentobarbital produces no substitution in rats trained to discriminate 1.5 g/kg ethanol from vehicle (De Vry & Slangen, 1986) and is readily discriminated from ethanol in a drug vs. drug discrimination task (Overton, 1977). These

findings imply that pentobarbital produces discernably different subjective effects from ethanol (a test compound used in this study). In rhesus monkeys trained to discriminate amphetamine from vehicle using a signaled shock-avoidance procedure, pentobarbital produced no substitution, further indicating pharmacological specificity (de la Garza & Johanson, 1987).

The synthetic opioid fentanyl exerts its drug effects through the agonism of μ opioid receptors. Zhang, Walker, Sutherland, & Young (2000) showed that fentanyl has high efficacy and specificity for the μ opioid receptor, producing similar qualitative effects at both low and high doses. When established as a discriminative stimulus, fentanyl (0.01 mg/kg and 0.04 mg/kg) has been shown to fully substitute for other potent μ opioid agonists (etonitazene, methadone, and morphine) but not for spiradoline (κ opioid agonist) or amphetamine (dopamine releaser) (Zhang et al., 2000). In another study, phencyclidine (PCP), ketamine, and (+/-)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801) produced partial substitution for fentanyl (0.04 mg/kg) (Koek, Colpaert, & Vignon, 1993). In both of the previous studies, naltrexone antagonized the discriminative effect produced by fentanyl.

Buspirone, a serotonin 5-HT_{1A} receptor agonist, dose-dependently decreases serotonin levels, while increasing dopamine and norepinephrine levels in the brain (Loane & Politis, 2012). Studies have demonstrated efficacy for the use of 5-HT_{1A} agonists in treatment of neuropathic pain in rodent models (Colpaert, 2006). Several studies investigating the discriminative stimulus effects of buspirone in a drug discrimination procedure have found evidence in support of buspirone's serotonin-mediated effects (Hendry, Balster, & Rosecrans, 1983; Ator, 1991; Mansbach & Barrett, 1987). However, there is also evidence that serotonergic receptors do not play an important role in the drug's effects (Davis, Cassella, & Kehne, 1988), but rather antagonism at the dopamine D_2 receptor (Kamien & Woolverton, 1990).

The psychostimulant and rate-stabilizing agent amphetamine produces dose-dependent effects. At low doses, amphetamine typically produces positive subjective effects and characteristic stimulant-like effects (i.e., increased alertness, energy, sense of well-being) (Smith & Davis, 1977), while higher doses are capable of producing euphoria (Prus, 2014). Amphetamine's rate-dependent effects are contingent on predrug administration response rates (Ginsburg, Pinkston, & Lamb, 2011), making the drug useful in the treatment of ADHD. Amphetamine exerts its effects by promoting monoamine (i.e., dopamine, serotonin, norepinephrine) efflux. In vitro, amphetamine has been shown to stimulate monoamine release, as well as inhibit reuptake (Heal et al., 1988). Seidel et al. (2005) more accurately described amphetamine's monoamine-releasing effect to be mediated through the reversal of monoamine transporter proteins and by displacing vesicular monoamines.

The synthetic compound raclopride exerts its effects through antagonism of the dopamine D_2 receptor. Raclopride possesses a high specificity and affinity (K_D = 1.2 nM in the rat striatum) for the dopamine D_2 receptor, and thus, is a useful tool for assessing D_2 receptor activity in studies investigating pharmacological action of compounds (Kohler, Hall, Ogren, & Gawell, 1985). Raclopride has not been shown to produce substitution in drug discrimination paradigms that use a non-dopaminergic modulating compound as a discriminative cue. Based on these findings, raclopride was not expected to substitute for the anticonvulsant gabapentin.

THESIS STATEMENT

Understanding drugs' specific subjective effects and pharmacological actions is imperative to understand the pharmacological effects of compounds used in humans, including the risks that a drug may have abuse potential. Gabapentin is a GABA analogue compound approved for the treatment of epilepsy and neuropathy, but used off-label for treating a number of disorders, such as insomnia, bipolar disorder, and anxiety. Some reports indicate that gabapentin also has abuse potential. There is relatively little known about the behavioral pharmacological effects of gabapentin.

Drug discrimination procedures, which evaluate the subjective effects of drugs and link these effects to pharmacological mechanisms of action, have not yet been conducted as a means to carefully examine the discriminative stimulus effects of gabapentin. Studies using gabapentin as a test compound have reported partial substitution for Δ^9-THC and for alcohol, but no substitution for anticonvulsant drugs mediated by $GABA_A$ receptors. Moreover, gabapentin has not been shown to block the discriminative stimulus effects of cocaine in rats, but it has attenuated the discriminative stimulus effects of cocaine in humans. Yet, gabapentin is used for the treatment of alcohol withdrawal and has been shown to reduce the positive subjective effects produced by cocaine. This study aims to evaluate gabapentin as the training drug in a drug discrimination procedure, which will serve to examine the pharmacological mechanisms that mediate this compounds subjective effects.

MATERIALS AND METHODS

Animals

Ten male Sprague Dawley rats (Charles River Laboratories, Portage, MI) were housed individually under 12-hour light/dark (6 am/6 pm) conditions with regulated temperature and humidity. Rats were trained to discriminate 30.0 mg/kg gabapentin from vehicle. Free feed weights for each rat were collected, then home cage food rations were restricted to achieve 90% of free-feeding weights (M = 370.83 +/- 4.35g). Water was available ad lib. Rats were fed immediately after daily training or testing sessions, occurring at approximately the same time each day.

Apparatus

Five standard rat operant chambers (ENV-008-VP, MED Associates, St. Albans, VT) contained in sound-attenuating cabinets were used in the drug discrimination study (ENV-018MD, MED Associates, St. Albans, VT). Cabinets were equipped with fans for masking noise and ventilation, and all equipment was controlled by MED-PC IV software. The operant chambers (30-cm L × 24-cm W × 29-cm H) were constructed of a Plexiglas top and side door panels, with other walls and components made of stainless steel. A concealed light bulb located near the top of the operant chamber provided illumination during all training and test sessions. Two retractable levers (gabapentin and vehicle levers) were located on either side of a food hopper centered on the stainless steel wall of the chamber. Food reinforcers consisted of 45-mg food pellets (Dustless Precision Pellets, Rodent Grain-Based Diet, Bio-Serv, Flemington, NJ).

Drugs

Generalization testing was conducted with the gabapentin, (Neurontin®), amphetamine (Adderall®), raclopride, pentobarbital (Nembutal®), buspirone (Buspar®), ethanol, fentanyl (Sublimaze®), carbamazepine (Tegretol®), and pregabalin (Lyrica®). All drugs were purchased from Sigma-Aldrich, St. Louis, MO, and all drugs, with the exception of carbamazepine, were dissolved in 0.9% saline solution. Carbamazepine was dissolved in β-cyclodextrin. Vehicle for all drugs consisted of 0.9% saline solution. All drugs were administered intraperitoneally (ip) at a volume of 1 ml/kg body weight, except for ethanol, which was administered intragastrically via oral gavage. All drugs were administered 30 minutes prior to test sessions. Injection times were based on cumulative dosing procedure (Wenger, 1980), and all doses were chosen based on previous published literature to determine a sufficient dose of a compound that produces a cessation of responding (Pfizer, 2011).

Procedures

Training procedures described below are for rats trained to discriminate 30.0 mg/kg gabapentin from vehicle. For all of the following procedures, no more than one session was conducted per day. Training sessions consisted of no more than one trial (i.e., each rat received one injection and underwent training procedures) per day, whereas test sessions consisted of multiple trials (one trial for each level of cumulatively dosed test compound, including vehicle [0 mg/kg test compound]).

Lever-press training. After one magazine training session, in which no levers were available and food pellets (45mg dustless grain pellet) (Bio-Serv, Flemington, NJ) were delivered every 60 seconds (fixed time 60 sec), lever press training sessions began. During lever press training, only the center lever was available in the chamber (other two levers were retracted) and every lever press resulted in the delivery of one food pellet (i.e., a fixed ratio [FR]

1). The center lever was chosen to prevent biased responding, due to either the left or the right lever eventually being paired with the training drug's vehicle. A session ended when either 30 food pellets were delivered or 15 minutes' time had elapsed. As the rats acquired the lever-press response, the FR requirement was gradually increased until FR 30 responding occurred reliably.

Single-lever (errorless) training. During single-lever (errorless) training sessions, rats were administered either the training drug (i.e., gabapentin, 30.0 mg/kg dose) or vehicle (0.9% physiological saline, Sigma-Aldrich, St. Louis, MO) 60 minutes prior to a training session (later shortened to 30 minutes due to cumulative dosing procedure). For each rat, one lever (left or right) was extended for drug-treatment sessions and the other lever was extended for vehicle-treatment sessions. Drug and vehicle lever assignments were counterbalanced between subjects to account for olfactory cues (Extance and Goudie, 1981). Four sessions of each condition were conducted in a single/double alternation design for gabapentin (G) and the discriminate vehicle saline (S) for training sessions (i.e., GSGGSSGS). Animals were required to maintain FR 30 responding and successfully obtain 30 food pellets within each session for seven of eight consecutive training sessions before two-lever discrimination training began.

Two-lever discrimination training. During the two-lever sessions, both levers were extended in the operant chamber. Discrimination training sessions continued to follow a single/double alternation design. During these sessions, a resetting counter was used for FR responding (e.g., if a rat pressds the incorrect lever before 30 responses on the condition-appropriate lever, FR response requirement was reset to 0, and the next food pellet will require thirty consecutive presses on the condition-appropriate lever). In order to complete two-lever training, the rats had to meet the following criteria for five of six consecutive sessions: (1) the first completed FR 30 requirement must have been on the condition-appropriate lever, (2)

cumulative response rates of no less than 5 RPM, (3) at least 80% condition appropriate responding prior to the first fixed ratio, and (4) at least 80% cumulative condition-appropriate responding over the entire session. These sessions continued throughout the study to ensure discriminative accuracy was maintained.

Generalization testing. Prior to a test sessions, rats were required to successfully complete a minimum of two discrimination training sessions (i.e., all four training criteria had to be met during the training sessions immediately preceding the test session). Test sessions were the same as two-lever training sessions except that no reinforcers were delivered during the test session, and 30 consecutive responses on either single lever resulted in the session ending and a single food pellet to be delivered 2 seconds later. Control tests with the drug vehicle were conducted prior to testing each test compound. Cumulative dosing techniques were used for each test compound, in which supplemental doses were administered in addition to previous treatment in order to reach the desired effective dose (e.g., if the low dose were 1.0 mg/kg and the next dose were 5.0 mg/kg, then the next amount given of the drug would be 4.0 mg/kg) (Wenger, 1980). Drugs were tested in a pseudo-random order (see Appendix A). All training sessions were 15 minutes long, and test sessions lasted until an FR 30 schedule had been completed on a single lever or 15 minutes' time had elapsed.

Data Analysis

Percent lever responding for drug and non-drug levers, responses per minute (RPM), and the lever on which the first FR 30 schedule was completed were collected for each training and test session. Percent gabapentin-appropriate responding and RPM were reported as means (+/- the standard error of the mean [SEM]) in dose-effect curves. Full-substitution was defined as 80% or greater gabapentin-appropriate responding, and partial substitution was defined as 60%

or greater and less than 80% gabapentin-appropriate responding. Because these procedures were designed to evaluate test compounds for gabapentin-like stimulus effects, criterion-based assessment (i.e., full, partial, or no substitution), rather than statistical assessment, will be used for percent gabapentin-appropriate responding results. This method of analysis is standard for drug discrimination research (Glennon & Young, 2011). For drugs that produced full substitution (i.e., ≥ 80% gabapentin-appropriate responding), ED_{50} values were obtained for the dose–response curves (with 95% confidence levels) using a least-squares linear regression analysis (Goldstein, 1964). If an animal's response rate falls below 5 RPM, its percent lever-responding data was included in either the dose–effect curve or the ED_{50} calculations. A one-factor repeated-measures analysis of variance (ANOVA) was conducted to assess changes in response rates. When appropriate, Dunnett's multiple comparison tests were conducted to identify significant changes in response rates for drug doses relative to vehicle.

RESULTS

Gabapentin Drug Discrimination Training

Of the initial 10 animals obtained for this study, all 10 of these subjects met the training criteria. Discrimination training and subsequent time course analysis were initially conducted 60 minutes after drug administration. However, this was later reduced to 30 minutes based on cumulative dosing procedure, post-injection times found in a previous study (Pan, Eisenach, & Chen, 1999), and empirical time course analysis data (i.e., full substitution produced in the 30.0 mg/kg training dose 30 minutes post drug administration). These 10 subjects met the two-lever discrimination criteria after a mean of 40.8 (+/- SEM = 3.38) sessions (Figure 1).

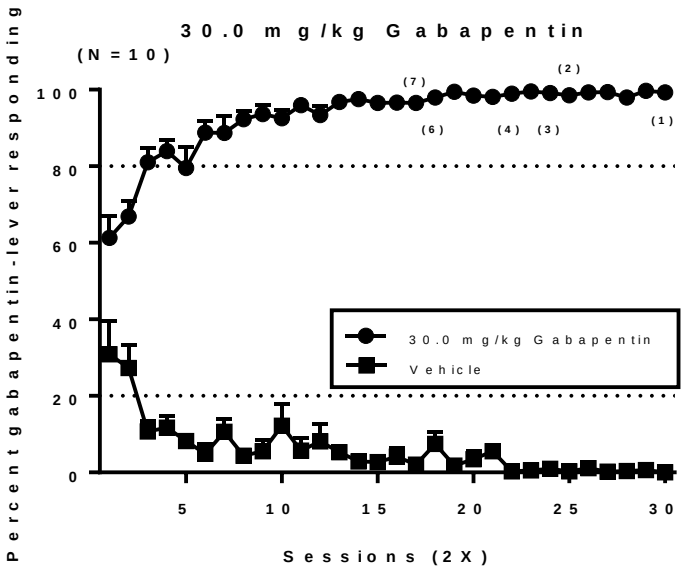
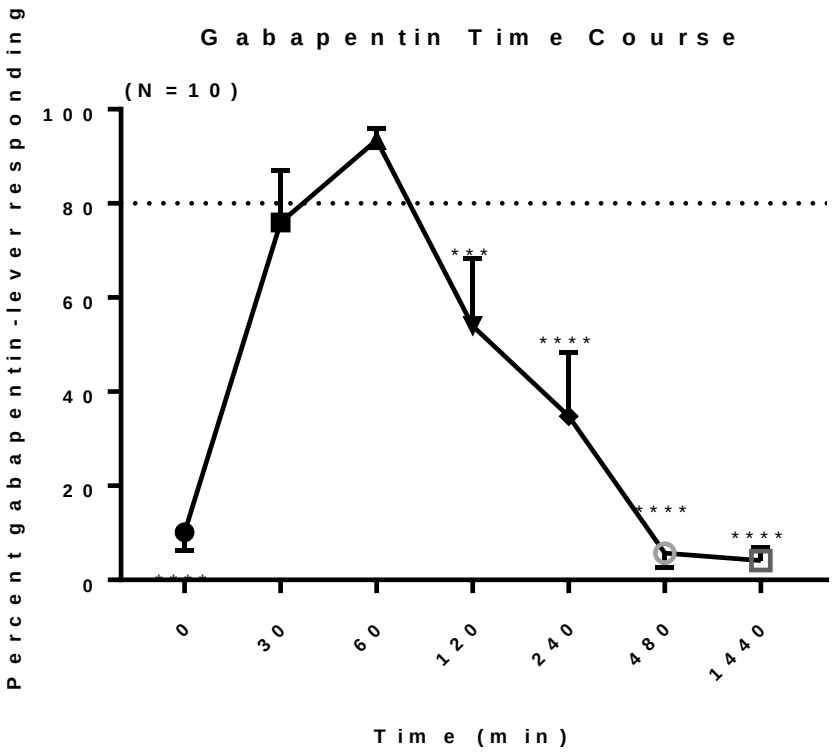


Fig 1. Mean percent gabapentin-appropriate responding during two-lever discrimination training for 30.0 mg/kg gabapentin versus vehicle. The ordinate axis indicates percent gabapentin-lever responding. The abscissa indicates the number of training sessions for either gabapentin or vehicle. Each session number represents both a gabapentin and vehicle training session. The

number in parentheses indicates the number of rats that had not met criteria at that point in training; the number of subjects was otherwise equal to N.

Gabapentin Time Course

Results from time course analysis generalization testing with gabapentin are shown in Figure 3. Gabapentin produced full generalization at a 30.0 mg/kg dose 60 minutes after drug administration. Gabapentin also produced partial generalization (75.95 [SEM = +/- 11.12%] gabapentin-lever responding) at 30 minutes after drug administration. However, it was not uncommon for gabapentin to produce ≥ 80% gabapentin-lever responding at 30 minutes post drug administration during training sessions. A significant increase in response rates occurred 60 and 120 minutes after drug administration ($F[6, 54] = 5.17$, $p < 0.001$).


Gabapentin Time Course
(N=10)
Percent gabapentin-lever responding
100
80
60
40
20
0
****
***
****
****
****
0
30
60
120
240
480
1440
Time (min)

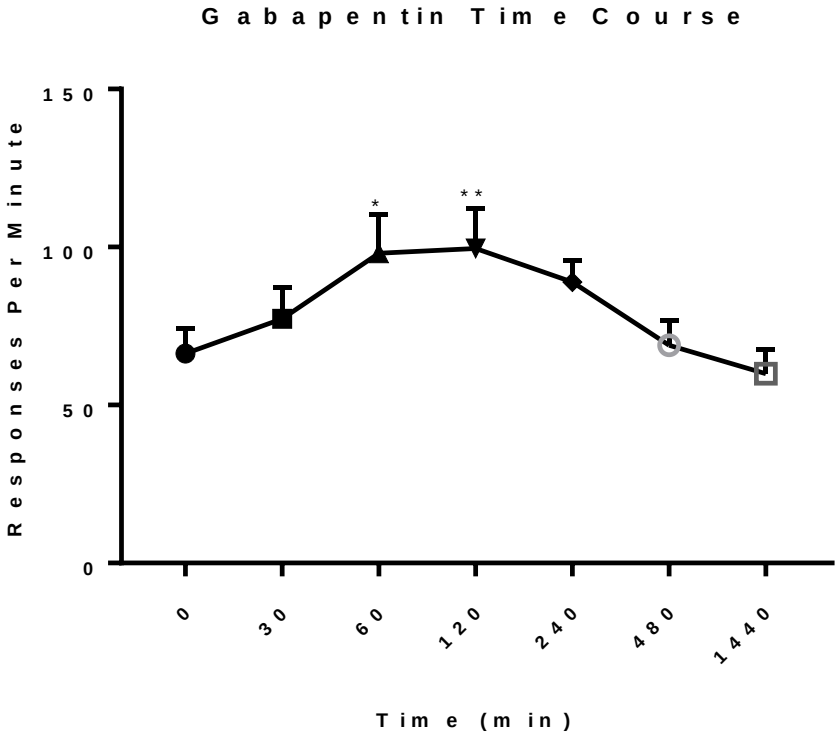
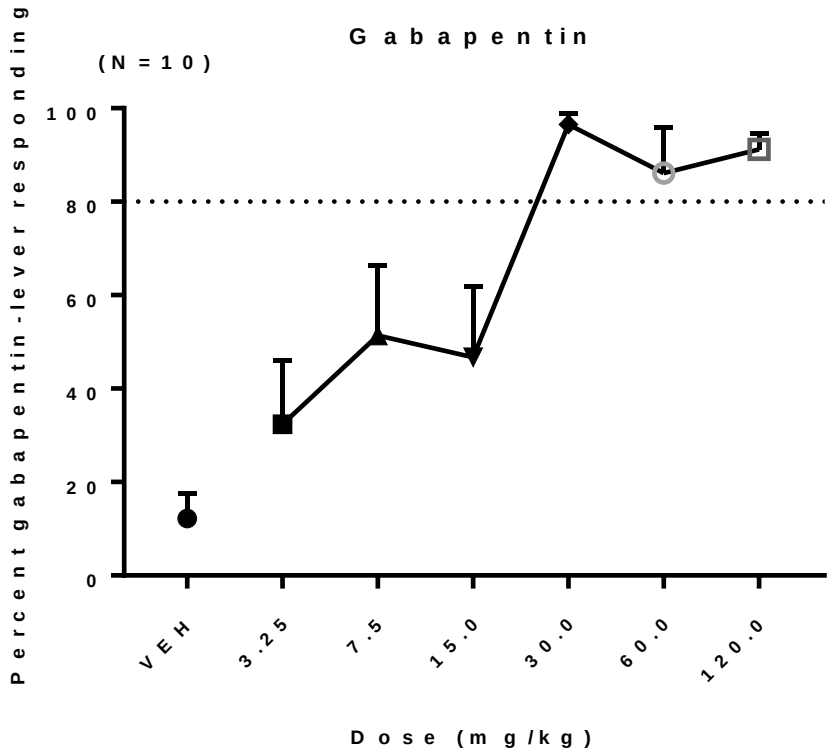

Gabapentin Time Course
Responses Per Minute
150
100
50
0
*
**
0
30
60
120
240
480
1440
Time (min)

Fig. 2. Generalization results for gabapentin time course analysis in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For generalization and response rate data, significant differences (from 60 minutes post injection in generalization testing; from vehicle in response rate) (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

Substitution Testing

Results for generalization testing with gabapentin are shown in Figure 2. Gabapentin produced full substitution (≥ 80% gabapentin-lever responding) at the 30.0 mg/kg training dose and at the 60.0 mg/kg and 120.0 mg/kg doses (ED_{50} = 8.15 mg/kg, 95% C.I. = 5.17-12.86 mg/kg). A significant increase in response rates (relative to vehicle) occurred at the 15.0 mg/kg, 30.0 mg/kg, 60.0 mg/kg, and 120.0 mg/kg doses ($F[6, 54] = 6.27$, $p < 0.0001$).


Gabapentin
(N=10)
Percent gabapentin-lever responding
100
80
60
40
20
0
VEH
3.25
7.5
15.0
30.0
60.0
120.0
Dose (mg/kg)

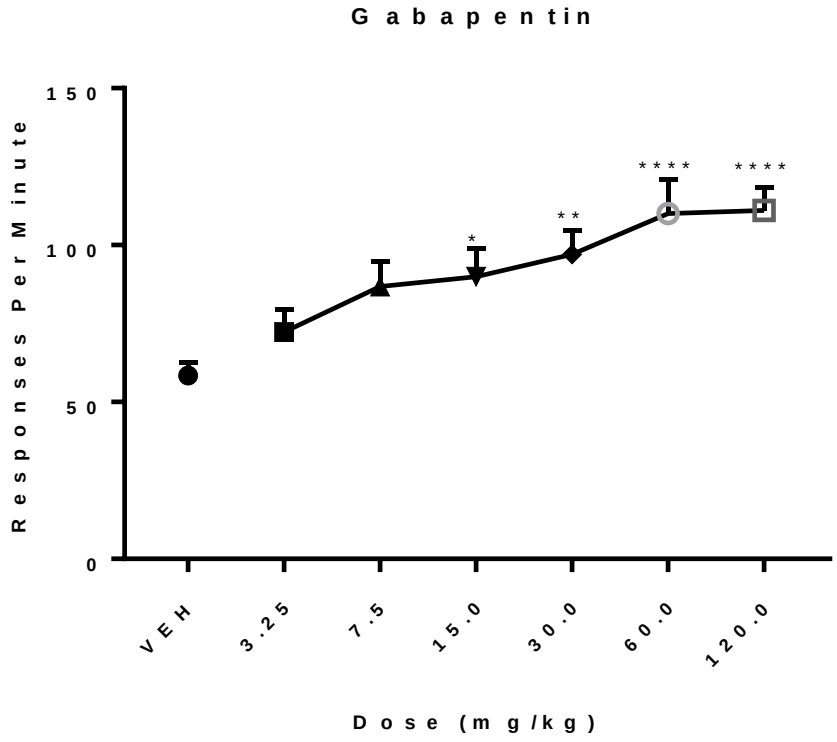
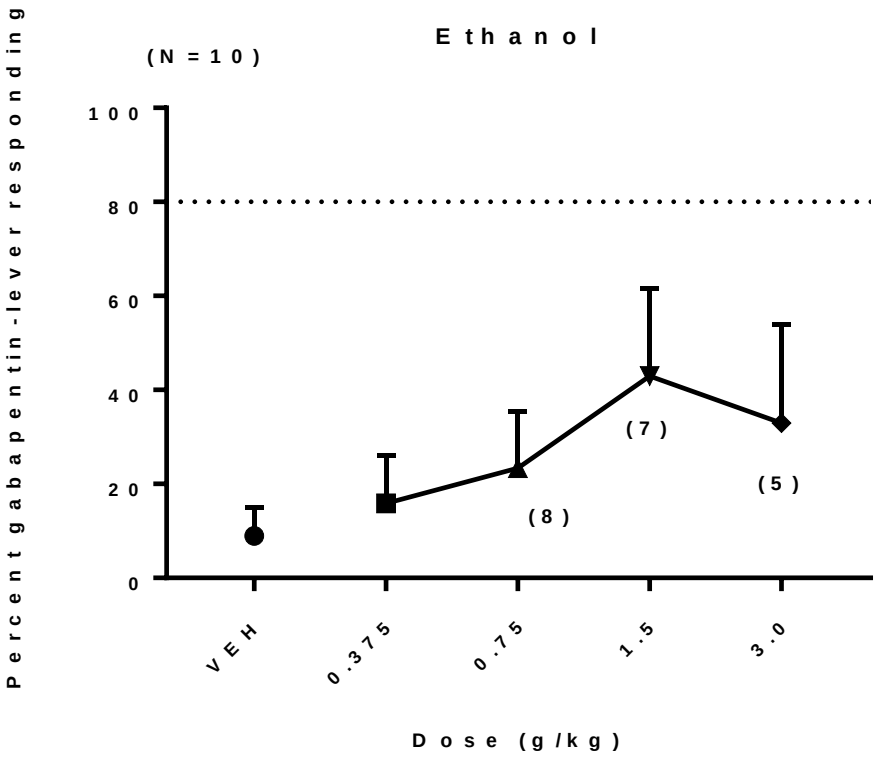

Gabapentin
Responses Per Minute
150
100
50
0
*
**
****
****
VEH
3.25
7.5
15.0
30.0
60.0
120.0
Dose (mg/kg)

Fig. 3. Generalization results for gabapentin in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

Ethanol

Results for generalization testing with ethanol are shown in Figure 4. Ethanol did not substitute for the 30.0 mg/kg gabapentin training dose up to rate suppressant doses. However, three of six rats displayed full substitution (95.24% gabapentin-lever responding) at the 1.5 g/kg dose. A significant decrease in response rates (relative to vehicle) occurred at the 0.375 g/kg, 0.75 g/kg, 1.5 g/kg, and 3.0 g/kg doses ($F[4, 36] = 15.76$, $p < 0.0001$).


Ethanol
(N = 10)
Percent gabapentin-lever responding
100
80
60
40
20
0
(8)
(7)
(5)
VEH
0.375
0.75
1.5
3.0
Dose (g/kg)

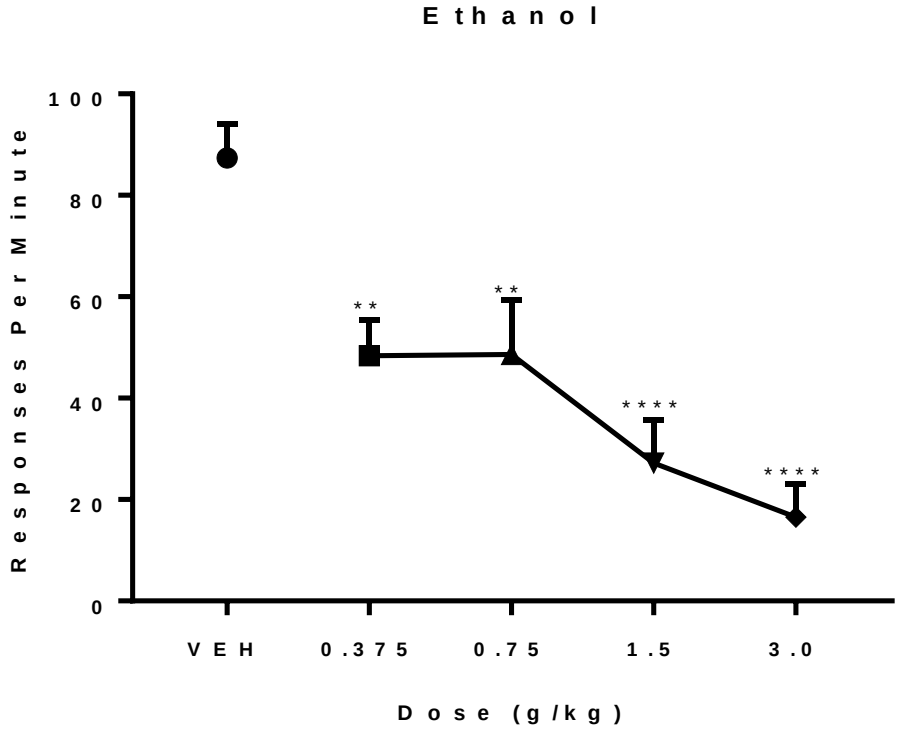
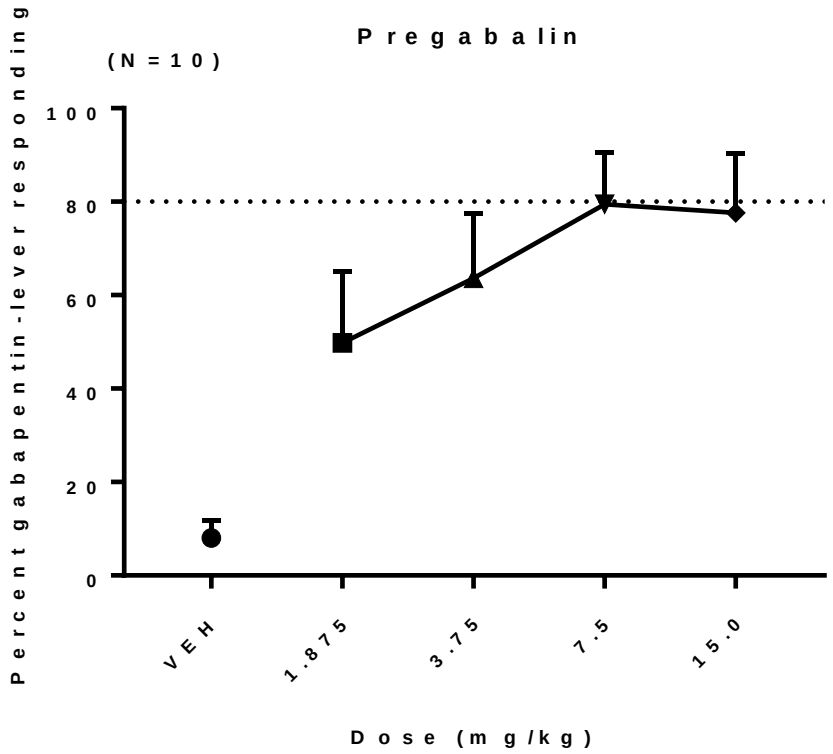

Ethanol
Responses Per Minute
100
80
60
40
20
0
**
**
****
****
VEH
0.375
0.75
1.5
3.0
Dose (g/kg)

Fig. 4. Generalization results for ethanol in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (**$p < 0.01$; ****$p < 0.0001$). Note: the administration route for all doses of ethanol was intragastric (oral gavage).

Pregabalin

Results for generalization testing with pregabalin are shown in Figure 5. Pregabalin produced partial substitution at the 3.75 mg/kg (63.33 [SEM = +/- 13.90%] gabapentin-lever responding), 7.5 mg/kg (79.45 +/- 11.09% gabapentin-lever responding), and 15.0 mg/kg (77.62 [SEM = +/- 12.68%] gabapentin-lever responding) doses. However, six of ten rats displayed full substitution at the 3.75 g/kg dose, and seven of ten rats displayed full substitution at the 7.5 mg/kg and 15.0 mg/kg doses. A significant increase in response rate (relative to vehicle) occurred at the 3.75 mg/kg, 7.5 mg/kg, and 15.0 mg/kg doses ($F[4, 36) = 7.08$, $p < 0.001$).


Pregabalin
(N = 10)
Percent gabapentin-lever responding
100
80
60
40
20
0
VEH
1.875
3.75
7.5
15.0
Dose (mg/kg)

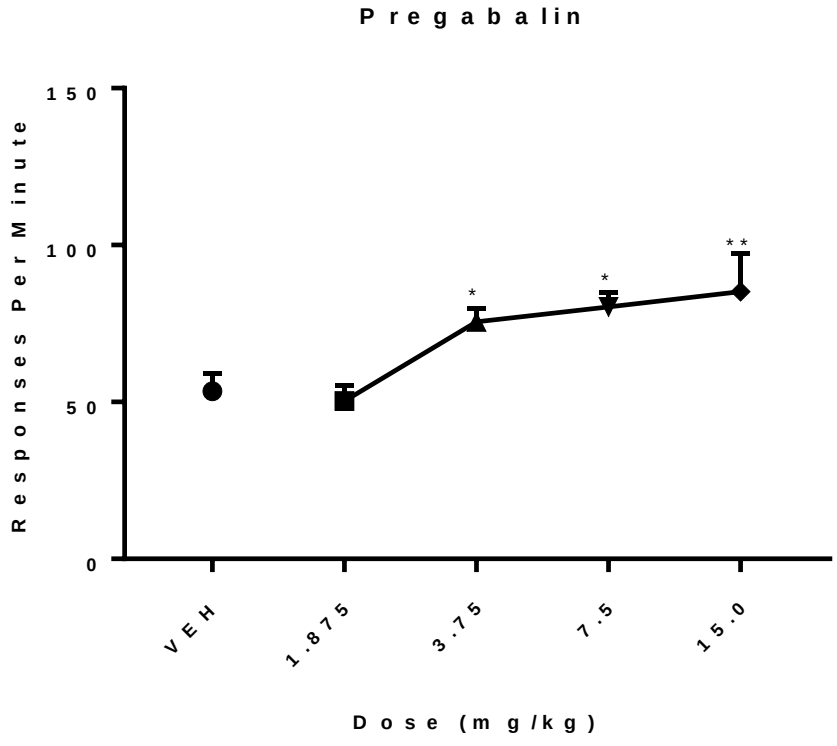
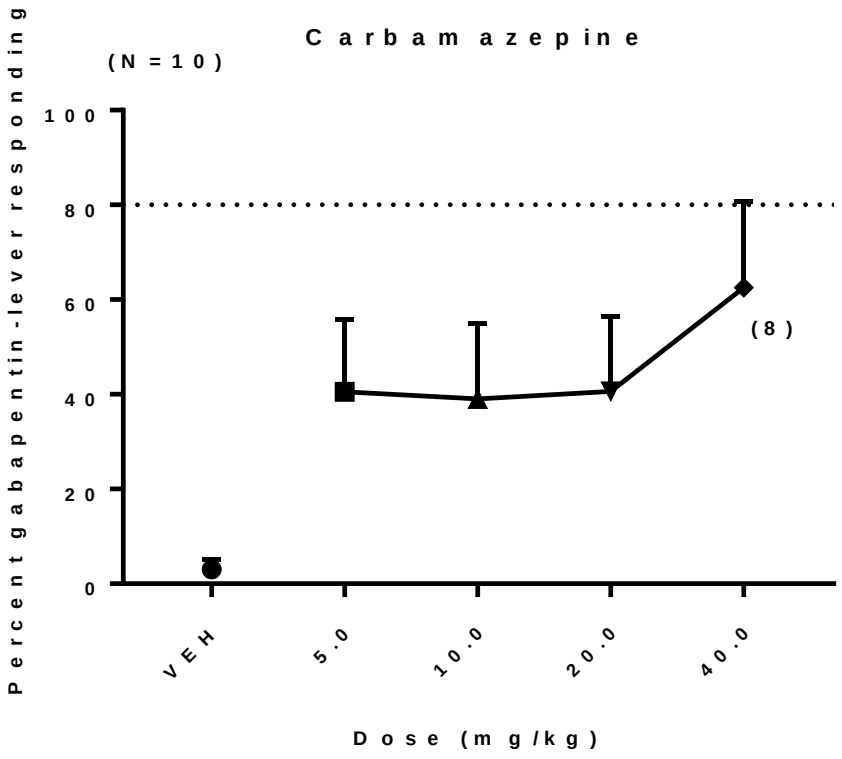

Pregabalin
Responses Per Minute
150
100
50
0
*
*
**
VEH
1.875
3.75
7.5
15.0
Dose (mg/kg)

Fig. 5. Generalization results for pregabalin in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (*$p < 0.05$; **$p < 0.01$).

Carbamazepine

Results for generalization testing with carbamazepine are shown in Figure 6. Carbamazepine produced partial substitution at the 40.0 mg/kg dose (62.50 [SEM = +/- 16.37%] gabapentin-lever responding). A significant increase in response rate (relative to vehicle) occurred at the 5.0 mg/kg, 10.0 mg/kg, and 20.0 mg/kg dose ($F[4, 36) = 5.49$, $p = 0.0015$). There was no significant decrease in response rates at any dose tested, although the 40.0 mg/kg dose produced rate disrupting effects in two of ten rats.


Carbamazepine
(N=10)
Percent gabapentin-lever responding
100
80
60
40
20
0
(8)
VEH
5.0
10.0
20.0
40.0
Dose (mg/kg)

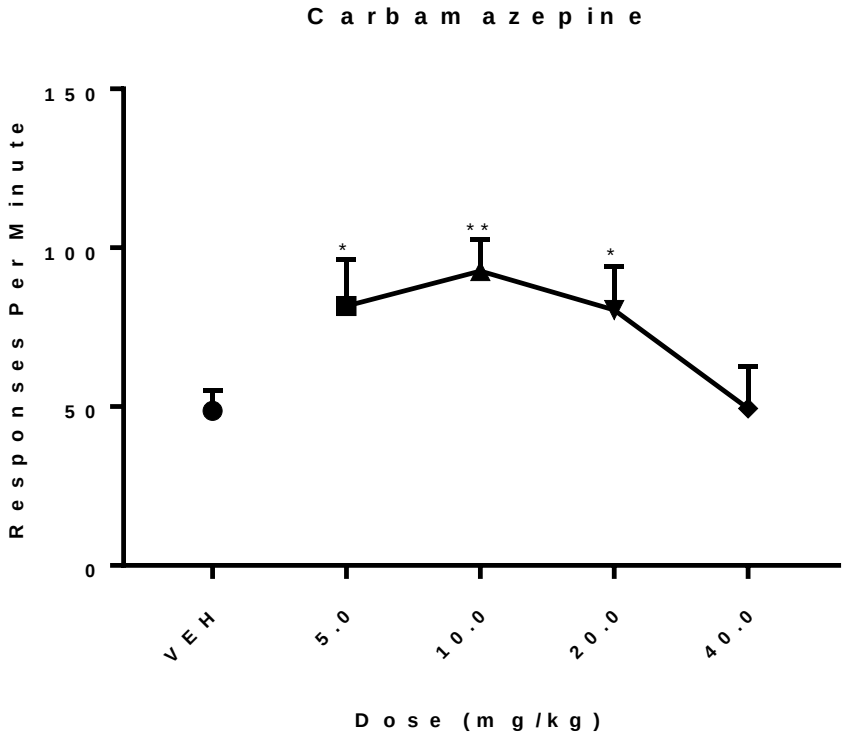
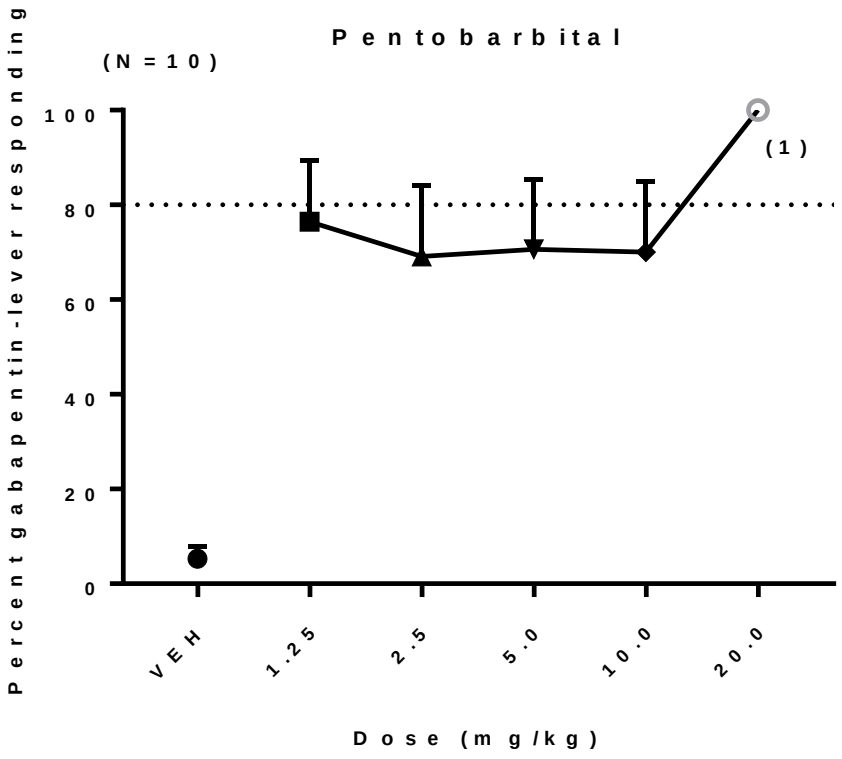

Carbamazepine
Responses Per Minute
150
100
50
0
*
**
*
VEH
5.0
10.0
20.0
40.0
Dose (mg/kg)

Fig. 6. Generalization results for carbamazepine in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (*$p < 0.05$; **$p < 0.01$).

Pentobarbital

Results from generalization testing with pentobarbital are shown in Figure 7. Pentobarbital produced partial substitution at the 1.25 mg/kg (76.44 [SEM = +/- 12.91%] gabapentin-lever responding), 2.5 mg/kg (69.15 [SEM = +/- 14.90%] gabapentin-lever responding), 5.0 mg/kg (70.59 [SEM = +/- 14.77%] gabapentin-lever responding), and 10.0 mg/kg (70.00 [SEM = +/- 14.84%] gabapentin-lever responding) doses. A significant increase in response rate (relative to vehicle) occurred at the 1.25 mg/kg, 5.0 mg/kg, and 10.0 mg/kg doses. A significant decrease in response rate (relative to vehicle) occurred at the 20.0 mg/kg dose ($F[5, 45) = 21.03$, $p < 0.0001$). Because of rate suppression at the 20.0 mg/kg pentobarbital dose, only one of ten rats met the response rate criteria (≥ 5 RPM) to be included in the calculation for percent gabapentin-lever responding. However, the rat displayed full substitution at the 20.0 mg/kg dose (ED_{50} = 1.67 mg/kg, 95% confidence interval (C.I.) = 0.80-3.50 mg/kg).


Pentobarbital
(N = 10)
Percent gabapentin-lever responding
100
80
60
40
20
0
(1)
VEH
1.25
2.5
5.0
10.0
20.0
Dose (mg/kg)

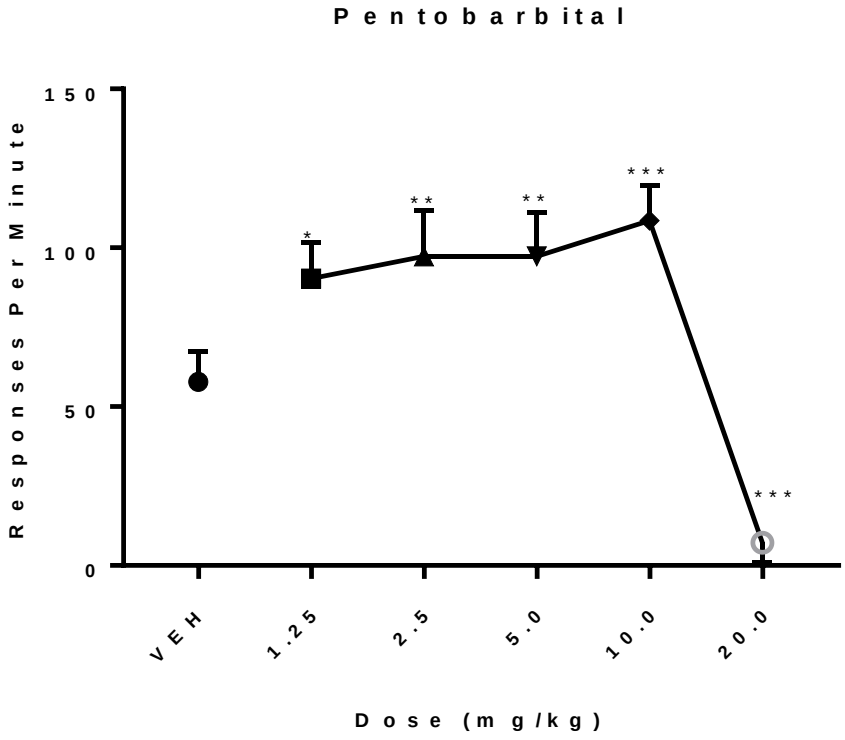
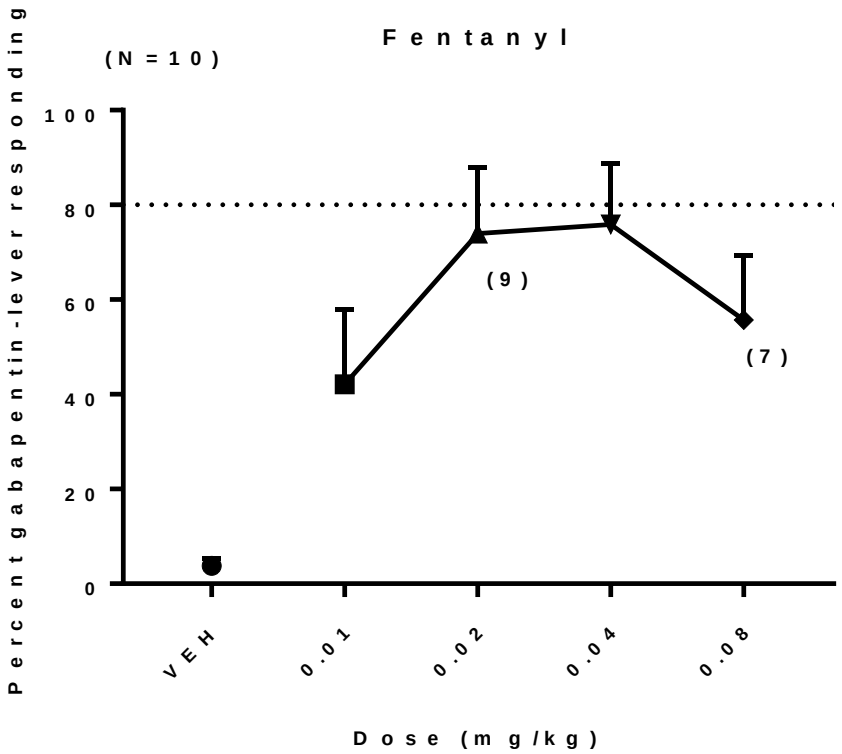

Pentobarbital
Responses Per Minute
150
100
50
0
*
**
**
***
***
VEH
1.25
2.5
5.0
10.0
20.0
Dose (mg/kg)

Fig. 7. Generalization results for pentobarbital in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Fentanyl

Results for generalization testing with fentanyl are shown in Figure 8. Fentanyl produced strong partial substitution at the 0.02 mg/kg (73.93 [SEM = +/- 13.34%] gabapenting-lever responding) and 0.04 mg/kg (75.92 [SEM = +/- 12.83%] gabapentin-lever responding) doses. However, seven of nine rats displayed full substitution at the 0.02 mg/kg dose and seven out of ten rats at the 0.04 mg/kg dose. A significant increase in response rate (relative to vehicle) occurred at the 0.02 mg/kg and 0.04 mg/kg doses ($F[4, 36) = 5.37$, $p = 0.0017$). There was no significant change in response rate relative to vehicle at any dose tested, although the 0.08 mg/kg dose produced rate disrupting effects in three of ten rats.


Fentanyl
(N = 10)
Percent gabapentin-lever responding
100
80
60
40
20
0
(9)
(7)
VEH
0.01
0.02
0.04
0.08
Dose (mg/kg)

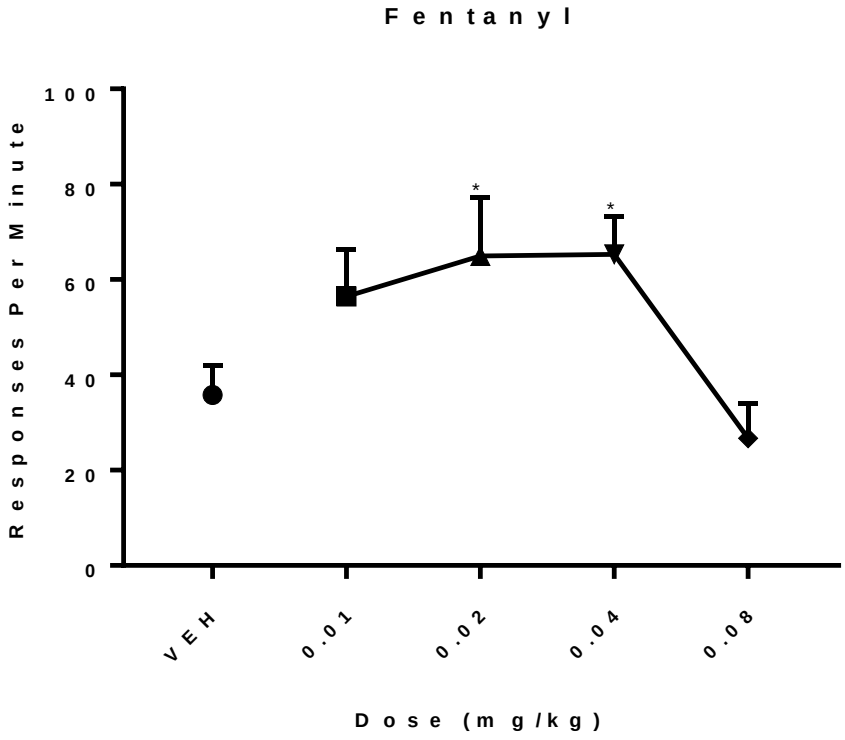
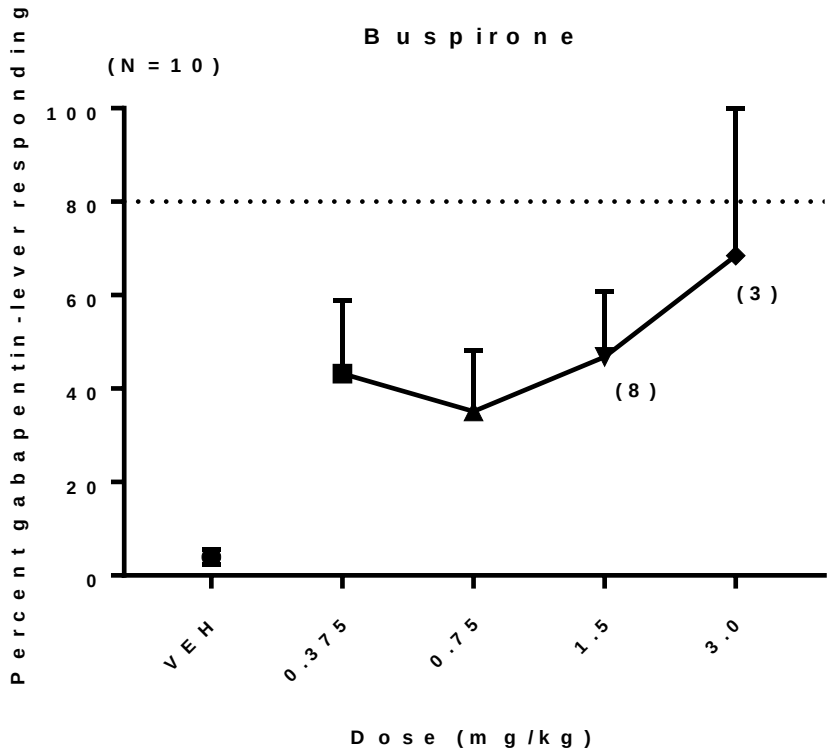

Fentanyl
Responses Per Minute
100
80
60
40
20
0
*
*
VEH
0.01
0.02
0.04
0.08
Dose (mg/kg)

Fig. 8. Generalization results for fentanyl in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (*$p < 0.05$).

Buspirone

Results for generalization testing with buspirone are shown in Figure 9. Because of the rate suppression at the 3.0 mg/kg buspirone dose, only three of ten rats met the response rate criteria (≥ 5 RPM) to be included in the calculation for percent gabapentin-lever responding. However, those three rats displayed partial substitution for 30.0 mg/kg gabapentin (68.42 [SEM = +/- 17.30%] gabapentin-lever responding) at the 3.0 mg/kg dose. A significant decrease in response rate (relative to vehicle) occurred at the 3.0 mg/kg dose ($F[4, 36] = 10.74$, $p < 0.0001$).


Buspirone
(N=10)
Percent gabapentin-lever responding
100
80
60
40
20
0
(3)
(8)
VEH
0.375
0.75
1.5
3.0
Dose (mg/kg)

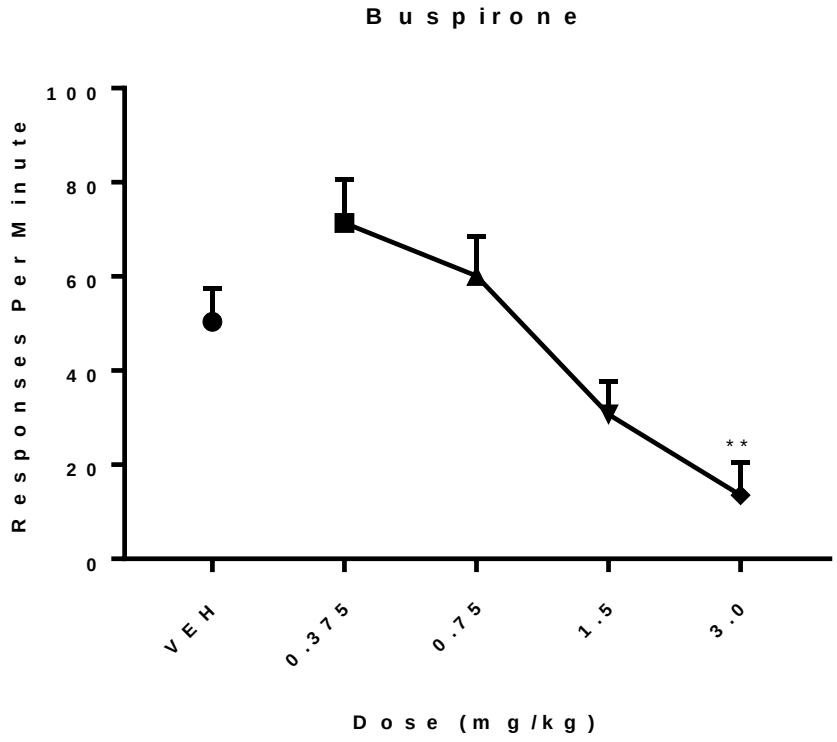
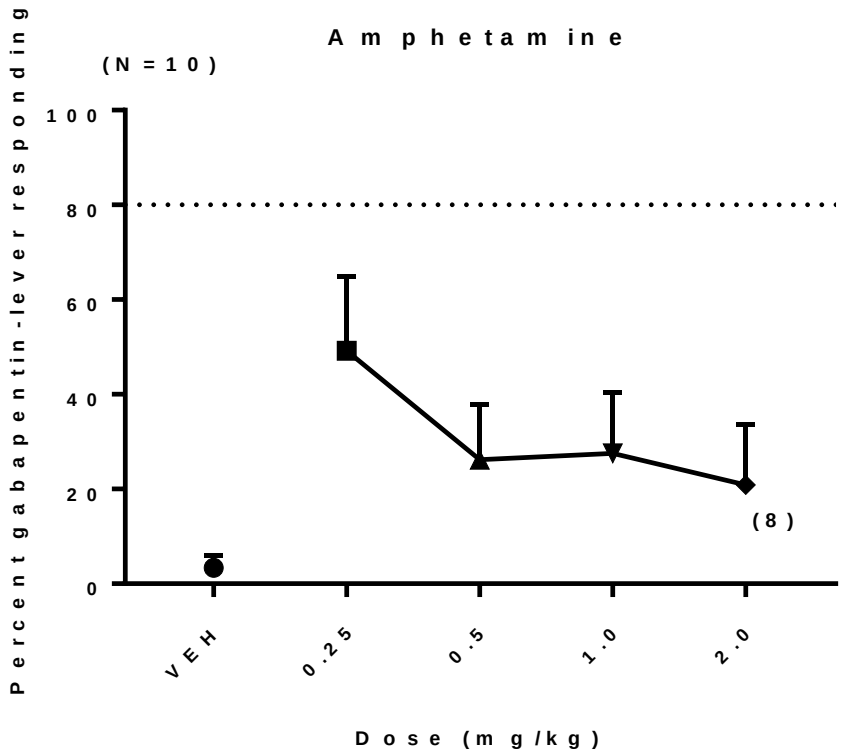

Buspirone
Responses Per Minute
100
80
60
40
20
0
**
VEH
0.375
0.75
1.5
3.0
Dose (mg/kg)

Fig. 9. Generalization results for buspirone in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (**$p < 0.01$).

Amphetamine

Results for generalization testing with amphetamine are shown in Figure 10. Amphetamine did not substitute for the 30.0 mg/kg gbapentin training dose at any of the doses tests. However, the 0.25 mg/kg dose amphetamine fully substituted in five of ten rats. There was no significant change in response rate relative to vehicle at any dose tested, although the 2.0 mg/kg dose produced rate disrupting effects in two of ten rats.


Amphetamine
(N=10)
Percent gabapentin-lever responding
100
80
60
40
20
0
(8)
VEH
0.25
0.5
1.0
2.0
Dose (mg/kg)

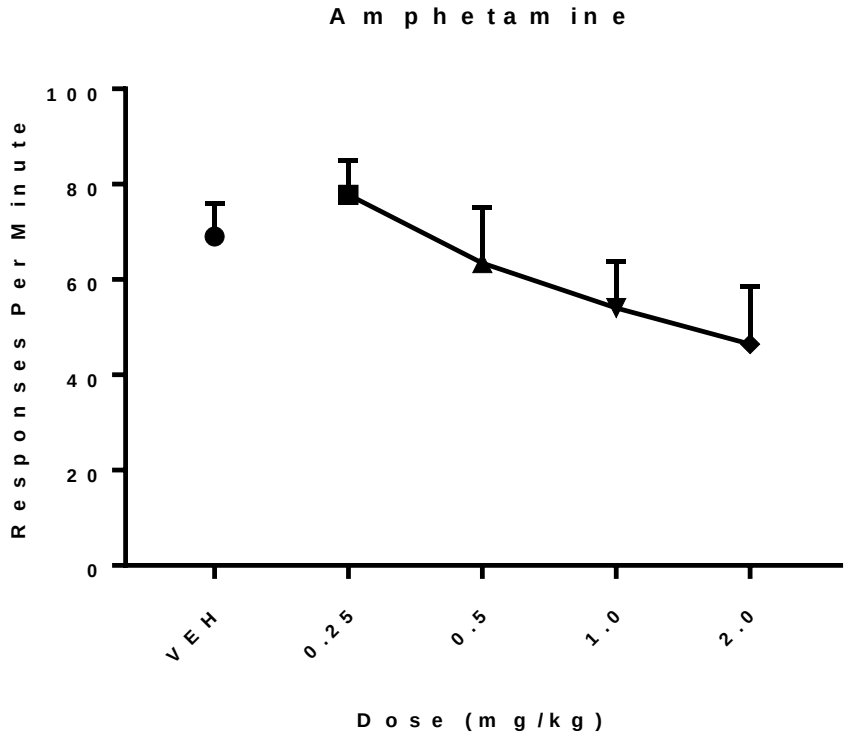
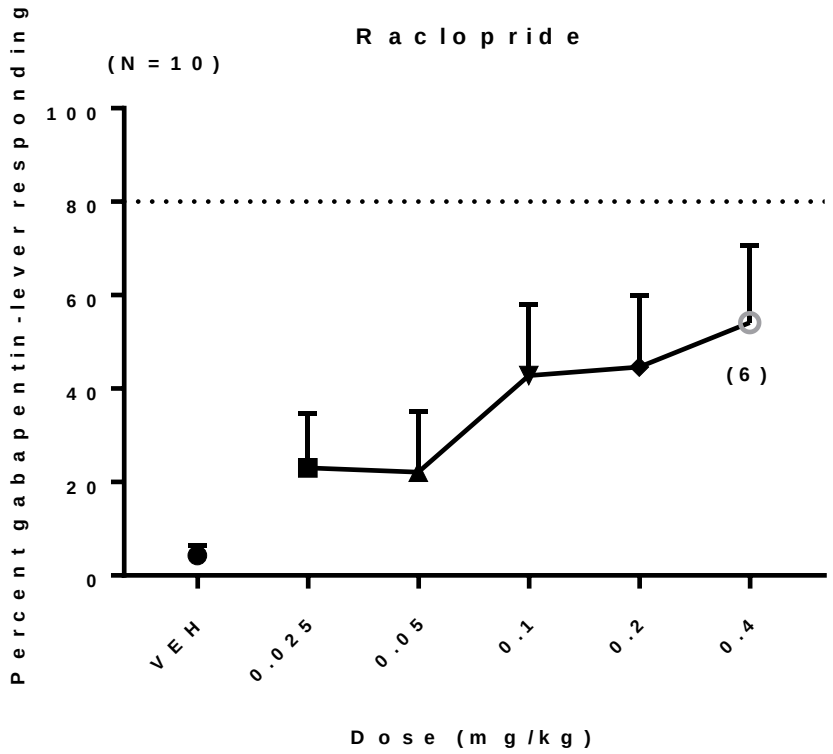

Amphetamine
Responses Per Minute
100
80
60
40
20
0
VEH
0.25
0.5
1.0
2.0
Dose (mg/kg)

Fig. 10. Generalization results for amphetamine in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks.

Raclopride

Results for generalization testing with raclopride are shown in Figure 11. Raclopride did not substitute for 30.0 mg/kg gabapentin at any of the tested doses. A significant increase in response rates occurred at the 0.05 mg/kg dose, and a significant decrease occurred at the 0.4 mg/kg dose ($F[5, 45] = 10.90$, $p < 0.0001$).


Raclopride
(N=10)
Percent gabapentin-lever responding
100
80
60
40
20
0
(6)
VEH
0.025
0.05
0.1
0.2
0.4
Dose (mg/kg)

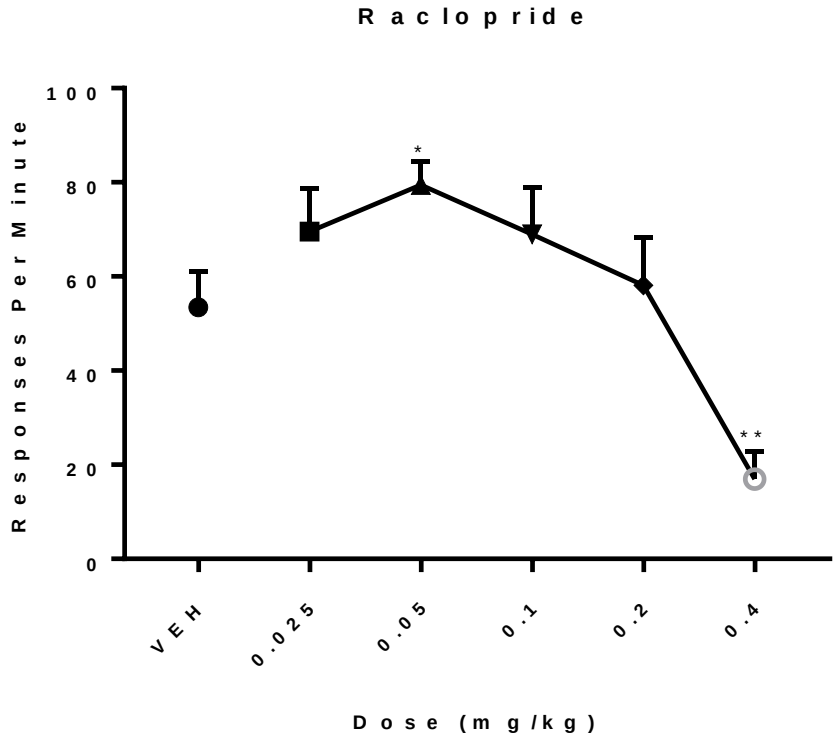

Raclopride
Responses Per Minute
100
80
60
40
20
0
*
**
VEH
0.025
0.05
0.1
0.2
0.4
Dose (mg/kg)

Fig. 12. Generalization results for raclopride in rats trained to discriminate 30.0 mg/kg (N = 10) gabapentin from vehicle in a two-choice drug discrimination task. Mean percent gabapentin-lever responding is shown in the upper panel, and mean responses per minute (RPM) are shown in the lower panel. The dashed line at 80% indicates full generalization to the gabapentin-lever. Prior to generalization testing, control tests were conducted with the appropriate gabapentin training dose and vehicle. Rats with response rates below 5.0 RPM were not included in the % gabapentin-lever data (the number of rats included is indicated in parentheses). For response rate data, significant differences from vehicle (calculated using Dunnett's multiple comparisons tests) are indicated by asterisks (*$p < 0.05$; **$p < 0.01$).

DISCUSSION

Gabapentin 30.0 mg/kg (i.p.) was successfully established as a discriminative cue in all 10 rats. Furthermore, gabapentin fully generalized (≥ 80% gabapentin-lever responding) during dose-response testing for itself. The barbiturate pentobarbital (20.0 mg/kg) produced full substitution. Pregabalin (7.5 and 15.0 mg/kg doses), carbamazepine (40.0 mg/kg dose), fentanyl (0.02 and 0.04 mg/kg), and buspirone (3.0 mg/kg) all produced partial substitution (≥ 60% gabapentin-lever responding) for gabapentin. Ethanol and raclopride did not substitute for gabapentin. The psychostimulant amphetamine also did not produce substitution; however, the 0.25 mg/kg dose D-amphetamine fully substituted in five of ten rats. Based on these findings, gabapentin appears to produce subjective effects similar to those exerted by many of the GABAergic compounds tested in this study.

Gabapentin

Although several studies have used the anticonvulsant gabapentin as a test compound in drug discrimination studies, the present study is the first to demonstrate that gabapentin can be established as a discriminative cue. Of the initial 10 animals obtained for this study, all 10 of these subjects passed the two-lever discrimination criteria and were able to discriminate gabapentin 30.0 mg/kg dose from vehicle. In a similar drug discrimination study where the anticonvulsant tiagabine (acts by inhibiting reuptake at GABA GAT-1) was used as a training compound, only 25 of 40 rats were successful in discriminating 30.0 mg/kg tiagabine from vehicle (McDonald et al., 2008). These findings show that not all drugs are capable of being readily established as a discriminative cue and suggest the ability of gabapentin to produce robust subjective effects.

Gabapentin produced full generalization at the 30.0 mg/kg dose 60 minutes after drug administration. Gabapentin also produced partial substitution at 30 minutes after drug administration; however, 8 of 10 rats displayed full substitution at this time point. Although it is typical to test 60 minutes after the administration of gabapentin (Radulovic et al., 1995), time course analysis of drug effects revealed that it was not uncommon for gabapentin to produce ≥ 80% gabapentin-lever responding at 30 minutes post drug administration during training sessions. Additionally, a Dunnett's multiple comparisons test revealed no significant difference between gabapentin generalization at 30 and 60 minutes post injection. Coinciding with previous literature, gabapentin has been shown to have no effect on locomotor activity at 30 and 60 minutes post injection (Tanabe et al., 2005). In this study, a significant increase in response rates occurred 120 minutes after drug administration.

Ethanol

The CNS depressant ethanol did not produce substitution for gabapentin in the present study, as gabapentin-appropriate responses were below the substitution threshold (i.e., < 60% gabapentin-lever responding). However, three of six rats displayed full substitution at the 1.5 g/kg ethanol dose. Additionally, a significant decrease in response rates (relative to vehicle) occurred at all doses leading up to the 3.0 g/kg dose, which precluded tested higher doses of ethanol. In a previous study by Besheer, Frisbee, Randall, Jaramillo, & Masciello (2016), gabapentin (120.0 mg/kg) displayed partial substitution (defined as >40% ethanol-lever responding in Besheer's study) as a test compound in rats trained to discriminate ethanol 1.0 g/kg (training dose) from vehicle. However, this may be due to the test dose (120.0 mg/kg gabapentin) being larger than the training dose (30.0 mg/kg gabapentin) used in this study. Gabapentin's involvement with the production of subjective drug effects likely involves indirect modulation of

GABAergic pathways or through inhibition of voltage-gated Ca^{2+} channels. Although ethanol and gabapentin both inhibit L-type voltage-gated Ca^{2+} channels, ethanol's interaction with these channels produce non-reinforcing objective effects (i.e., increase in urination, lower blood pressure, increased aggression) (Walter & Messing, 1999). The reinforcing effects of ethanol are more likely thought to involve ethanol's indirect modulation of neurotransmitters in the nucleus accumbens (i.e., $GABA_A$, 5-HT, and endocannabinoid modulation) (Harris et al., 1997; Yoshimoto et al., 1992; Hungund et al., 2003), which may explain ethanol's lack of substitution with gabapentin. Additionally, ethanol is known to produce a complex discriminative cue, composed of distinct components that are mediated by different neurotransmitter systems (Grant, 1999). As such, multiple features of the complex cue could serve as the discriminative basis for the drug's effect (Grant, 1999). Thus, it is difficult to draw conclusions between the subjective effects of gabapentin and ethanol without knowing the discriminative basis for ethanol's cue in a particular animal.

Pregabalin

In the present study, the anticonvulsant and analgesic pregabalin produced partial substitution at the 7.5 mg/kg and 15.0 mg/kg doses, with many of the subjects emitting full generalized responding. Pregabalin shares a novel mechanism of action with gabapentin, binding selectively to the α2δ subunit of voltage-gated Ca^{2+} channels, ultimately reducing excitatory neurotransmitter release. A thorough dose-response spectrum of pregabalin (i.e., increase dose until stimulus generalization or rate disrupting effects occur) should be further explored in order to conclude the potency of pregabalin in relation to the training drug (30.0 mg/kg gabapentin). Higher doses of pregabalin would be expected to produce full substitution relative to gabapentin.

Carbamazepine

Generalization testing with the anticonvulsant carbamazepine produced partial substitution at the 40.0 mg/kg dose. A significant increase in response rate (relative to vehicle) occurred at the 10.0 mg/kg dose. There was no significant decrease in response rates at any dose tested, although the 40.0 mg/kg dose produced rate disrupting effects in two of ten rats. The range in which carbamazepine and gabapentin exert their pain attenuating effects (carbamazepine: ED_{50} = 42.2 mg/kg; gabapentin 50.0 mg/kg) (De Vry, Kuhl, Franken-Kunkel, & Eckel, 2004) relate closely to the doses that produced substitution in this study. Carbamazepine is thought to act through the blockade of Na+ channels (Rogawski, Loescher, & Rho, 2016; MacDonald, 1995; Czapinski, Blaszczyk, & Czuczwar, 2005) and, more recently, $Na_v1.8$-like sodium channels (Cardenas et al., 2006). However, it has been hypothesized that, similar to the proposed mechanisms of gabapentin, carbamazepine is able to modulate K^+ currents and GABAergic pathways (Olpe, Kolb, Hausdorf, & Haas, 1991; Waldmeier et al., 1995). This hypothesis would serve to explain the analogous antiallodynic effects of each drug within a similar dose range.

Pentobarbital

In this study, the barbiturate pentobarbital produced partial substitution at the 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, and 10.0 mg/kg doses. Because of rate suppression at the 20.0 mg/kg pentobarbital dose, only one of ten rats met the response rate criteria (≥ 5 RPM) to be included in the calculation for percent gabapentin-lever responding. However, that rat displayed full substitution at the 20.0 mg/kg dose. Although not yet replicated in a rodent model, previous studies have shown the ability of gabapentin to produce significant increases in GABA concentrations in human neocortical slices (Errante, Williamson, Spencer, & Petroff, 2002), similar to the $GABA_A$ agonism of pentobarbital. Gabapentin may modulate GABA concentration

by inhibiting GABA transaminase inhibitor (GABA-T; responsible for GABA degradation) or by modulating non-vesicular GABA release via GAT-1 (GABA transporter) (Loscher, Honack, & Taylor, 1991; Sills, 2006; Goldlust et al., 1995; Leach et al., 1997). Additionally, the anticonvulsant properties of gabapentin and pentobarbital (Akula, Dhir, & Kulkarni, 2009) may be produced by the shared mechanism of inhibiting voltage-gated Ca^{2+} channels (Werz & Macdonald, 1982; Barker & Rogawski, 1993; Schoeber, Sokolova, & Gingrich, 2010). These shared interactions may serve to explain the shared discriminative and therapeutic effects of pentobarbital and gabapentin.

Fentanyl

In this study, the opioid fentanyl produced partial substitution at the 0.02 mg/kg and 0.04 mg/kg doses. There was no significant change in response rate relative to vehicle at any dose tested, although the 0.08 mg/kg dose produced rate-disrupting effects in three of ten rats. Fentanyl and gabapentin have been shown to share both antiallodynic and antihyperalgesic properties (Rode et al., 2007; Celerier et al., 2000). The doses used to produce these antinociceptive effects in previous studies (50.0-100.0 mg/kg gabapentin and 0.01-0.04 mg/kg fentanyl) closely resemble the range of doses tested in this study that produced generalizable subjective effects (30.0 mg/kg gabapentin training dose; 0.02-0.04 mg/kg fentanyl). Additionally, gabapentin-like compounds have been shown to block morphine-induced dopamine release in the nucleus accumbens (responsible for reinforcing drug effects), as well as reverse morphine-induced place preference in rodent models (Andrews et al., 2001). Based on these findings and gabapentin's known modulation of opioid-induced behavioral effects, gabapentin likely produces GABAergic effects similar to those elicited from μ opioid activation.

Buspirone

Because of the rate suppression at the 3.0 mg/kg buspirone dose, only three of ten rats met the response rate criteria (≥ 5 RPM) to be included in the calculation for percent gabapentin-lever responding. However, those three rats displayed partial substitution for gabapentin at the 3.0 mg/kg dose. A significant decrease in response rate (relative to vehicle) occurred at the 3.0 mg/kg dose. Although gabapentin does not modulate serotonin reuptake or concentration (Southam et al., 1998), previous studies have shown gabapentin and buspirone to share a similar profile, both producing significant anxiolytic-like effects within a similar dose range (30.0 mg/kg gabapentin; 5.0 mg/kg buspirone, s.c.) (Singh et al., 1996; Davis, Cassella, & Kehne, 1988). Additionally, studies assessing the anxiolytic effects of buspirone in a fear-potentiated startle paradigm have demonstrated that buspirone likely does not exert its behavioral effects through 5-HT_{1A} agonism (Davis, Cassella, & Kehne, 1988), but rather through antagonism of the dopamine D_2 receptor (Kamien & Woolverton, 1990; Ceretta et al. 2016; Ceretta et al. 2018). Thus, gabapentin and buspirone may act of similar mechanisms to exert their anxiolytic behavioral effects.

Amphetamine

In this study, the psychostimulant amphetamine did not substitute for gabapentin. However, the 0.25 mg/kg dose amphetamine fully substituted in five of ten rats. There was no significant change in response rate relative to vehicle at any dose tested, although the 2.0 mg/kg dose produced rate disrupting effects in two of ten rats. Gabapentin has been shown to produce counteractive effects to amphetamine (dopamine releaser), preventing hyperlocomotion, memory deficit, and social isolation is in rodent models of schizophrenia (Ceretta et al., 2016). Similarly, gabapentin has also been shown the reduce orofacial movements (animal model of tardive dyskinesia, a common adverse effect of chronic antipsychotic medication), induced by the D_2

antagonist haloperidol, and restore locomotor function in mice (Ceretta et al. 2018). However, the mechanisms by which gabapentin exerts its counteractive effects remain unclear, as gabapentin does not produce changes tyrosine hydroxylase (a dopaminergic marker) or monoamine levels in the striatum in mice (Ceretta et al. 2018). In this study, the lowest dose of amphetamine (0.25 mg/kg) produced full substitution of five of ten rats. In future studies, doses lower than 0.25 mg/kg should be tested for gabapentin substitution.

Raclopride

In this study, the D_2 antagonist raclopride did not produce substitution at any of the tested doses. A significant increase in response rates occurred at the 0.05 mg/kg dose, and a significant decrease occurred at the 0.4 mg/kg dose. Due to raclopride's high binding specificity to the dopamine D_2 receptor, the compound not been shown to produce substitution in drug discrimination paradigms that use a non-dopaminergic modulating compound as a discriminative cue. Based on the findings in this study, gabapentin does not generalize with raclopride, and therefore, does not likely modulate dopamine D_2 receptors.

Limitations

Several limitations exist that should be considered when drawing conclusions from this study. Although drug discrimination serves as a useful tool for deducing subjective drug effects, and pharmacological similarities can be surmised through stimulus generalization, this paradigm does not fully explain the mechanism by which gabapentin exerts its modulatory drug effects. Additionally, gabapentin may produce a complex discriminative cue, composed of multiple cues mediated by different neurotransmitter systems. In this case, test compounds that produce effects

similar to a single component of the compound cue may substitute for gabapentin while not emulating the full mechanism of gabapentin.

Directions for Future Research

Future research should attend to elucidate the mechanism by which gabapentin exerts its subjective effects. This may be achieved through the exploration of gabapentin's interaction with GABAergic, opioid, and dopaminergic neurotransmitter systems at the cellular level. Further elucidation of these interactions is critically important for fully explaining and predicting the subjective effects and drug interactions. Additionally, this would help explain the witnessed abuse potential of gabapentin, especially in combination with other prescription and illicit drugs.

Conclusion

Gabapentin, a synthetic analog of the neurotransmitter *gamma*-Aminobutyric acid (GABA), is an anticonvulsant primarily used for the treatment of epileptic seizures and neuropathic pain, as well as narcotic withdrawal and detoxification. Gabapentin's suspected mechanism of action involves interaction with the α2δ-1 subunit of voltage gated Ca^{2+} channels, ultimately reducing the release of excitatory neurotransmitters. Although the exact action of gabapentin remains somewhat unclear, there is increasing evidence that the drug possesses considerable abuse potential. Thus, there is much to learn about the pharmacological actions of gabapentin.

The present study presented evidence that rats can be successfully trained to discriminate 30.0 mg/kg gabapentin from vehicle in a two-lever drug discrimination task. Gabapentin produced full substitution (≥ 80% gabapentin-lever responding) for itself at 30.0 mg/kg (training dose), 60.0 mg/kg, and 120.0 mg/kg doses. The present study also supported conclusions from

previous studies that gabapentin exert its subjective effects either directly or indirectly through GABAergic pathways. Additionally, gabapentin's effect on GABAergic pathways may modulate other neurotransmitter pathways within the brain. The identification of pharmacological mechanisms that mediate the discriminative stimulus properties of gabapentin is important both to understand the stimulus properties responsible for stimulus generalization to drugs which exert positive subjective effects and to improve the ability of drug discrimination models to identify new compounds with abuse potential. New gabapentinoid compounds have been developed with a higher binding affinity to known receptors and a more liberal receptor pharmacology, suggesting greater abuse potential with the use of these compounds. For example, the relatively new gabapentinoid and anticonvulsant compound pregabalin has a greater binding affinity at the α2δ-1 subunit of voltage-gated Ca^{2+} channels. Pregabalin also partially substituted for gabapentin at much lower relative doses than the 30.0 mg/kg training dose.

Many of the compounds that produced substitution in this study are controlled substances that produce rewarding subjective effects through GABAergic. However, gabapentin has been shown to inhibit neuronal firing in the substantia nigra (Bloms-Funke & Loscher, 1996), inhibit dopamine and monoamine release of stimulated neurons (Pugsley, Whetzel, & Dooley, 1998), and block or reduce the reinforcing effects of opioids (Pugsley, Whetzel, & Dooley, 1998). Although compounds that serve to increase GABA concentrations have been shown to produce their reinforcing effects through interaction with the posterior ventral tegmental area (McBride, Murphy, & Ikemoto, 1999; Ikemoto, Murphy, & McBride, 1998; Ikemoto, Murphy, McBride, 1997), this effect on reinforcement is thought to be facilitatory (Bossert & Franklin, 2003; Seeger, Carlson, & Nazzaro, 1981). Studies suggest that reinforcement of GABAergic compounds may be mediated by opioid and dopaminergic mechanisms (Bossert & Franklin,

2001; Bossert & Franklin, 2003; Seeger, Carlson, & Nazzaro, 1981), which would explain the substitution of similar compounds to gabapentin.

The substitution demonstrated in this study is supported by reports of recreational or self-medicating poly-drug misuse, indicating the ability of gabapentin to modulate pathways involved in producing positive subjective effects. Similarly, because gabapentin is used to treat opioid, benzodiazepine, and alcohol detoxification and withdrawal, it is important for clinicians to monitor drug-seeking behaviors. Thus, the mechanisms of action surrounding gabapentin require further exploration, and the pharmacological modulatory effects of gabapentin should be considered by clinicians and researchers alike when working with the drug.

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APPENDIX A

Order of drug testing for each animal

Animal										
1	GTC	AMP	BUS	PGB	ETH	GPN	RAC	PNT	CBZ	FTN
2	GTC	GPN	AMP	BUS	PBG	ETH	RAC	PNT	CBZ	FTN
3	GTC	GPN	PGB	PNT	ETH	BUS	AMP	CBZ	FTN	RAC
4	GTC	AMP	PNT	BUS	PGB	GPN	ETH	RAC	CBZ	FTN
5	GTC	GPN	BUS	PNT	AMP	PGB	ETH	RAC	CBZ	FTN
6	GTC	AMP	PNT	GPN	BUS	PGB	ETH	RAC	CBZ	FTN
7	GTC	GPN	AMP	PNT	BUS	PGB	ETH	RAC	CBZ	FTN
8	GTC	PNT	PGB	ETH	GPN	RAC	AMP	CBZ	FTN	BUS
9	GTC	GPN	AMP	BUS	PNT	PGB	ETH	RAC	CBZ	FTN
10	GTC	PNT	ETH	GPN	RAC	AMP	CBZ	FTN	BUS	PGB

GTC = gabapentin time course, GPN = gabapentin, AMP = amphetamine, BUS = buspirone, PGB = pregabalin, ETH = ethanol, RAC = raclopride, PNT = pentobarbital, CBZ = carbamazepine, FTN = fentanyl

Substitution testing results for each animal

	Dose (mg/kg)	No Substitution	Partial	Full
Ethanol	375	1, 2, 3, 4, 5, 7, 8, 9, 10		6
	750	2, 3, 4, 5, 6, 7, 9		8
	1500	3, 4, 8, 9		2, 5, 6
	3000	4, 8, 9	2	6
Pregabalin	1.875	1, 2, 4, 9, 10	8	3, 5, 6, 7
	3.75	1, 3, 4, 9		2, 5, 6, 7, 8, 10
	7.5	1, 5, 9		2, 3, 4, 6, 7, 8, 10
	15.0	5, 8	4	1, 2, 3, 6, 7, 9, 10
Carbamazepine	5.0	1, 4, 6, 7, 8, 10		2, 3, 5, 9
	10.0	3, 4, 5, 8, 9, 10		1, 2, 6, 7
	20.0	4, 5, 6, 8, 9, 10		1, 2, 3, 7
	40.0	5, 8, 10		1, 3, 4, 6, 9
Pentobarbital	1.25	1, 5	8	2, 3, 4, 6, 7, 9, 10
	2.5	2, 9, 10		1, 3, 4, 5, 6, 7, 8
	5.0	2, 3, 5		1, 4, 6, 7, 8, 9, 10
	10.0	3, 8, 10		1, 2, 4, 5, 6, 7, 9
	20.0			9
Fentanyl	0.01	2, 4, 5, 6, 9, 10		1, 3, 7, 8
	0.02	3, 9		1, 2, 4, 5, 6, 7, 8
	0.04	5, 10	2	1, 3, 4, 6, 7, 8, 9
	0.08	1, 6, 8, 9	3	5, 7
Buspirone	0.375	1, 2, 5, 8, 9, 10		3, 4, 6, 7
	0.75	1, 3, 4, 6, 7, 8, 10	2	5, 9
	1.5	1, 3, 4, 9, 10	8	5, 6
	3.0	9		5, 6
Amphetamine	0.25	1, 2, 4, 8, 10		3, 5, 6, 7, 9
	0.5	1, 2, 3, 4, 6, 8, 9, 10		5, 7
	1.0	1, 3, 4, 5, 6, 8, 9, 10		2, 7
	2.0	1, 3, 4, 6, 8, 9, 10		5
Raclopride	0.025	2, 3, 4, 6, 7, 8, 9, 10		1, 5
	0.05	1, 2, 3, 5, 6, 7, 9, 10		4, 8
	0.1	2, 3, 4, 6, 7, 8		1, 5, 9, 10
	0.2	2, 4, 6, 8, 9, 10		1, 3, 5, 7
	0.4	8, 9, 10	2	5, 6
Gabapentin	3.75	1, 2, 5, 7, 8, 9, 10		3, 4, 6
	7.5	1, 2, 7, 8, 10		3, 4, 5, 6, 9
	15.0	1, 2, 4, 8, 9, 10		3, 5, 6, 7
	30.0			1, 2, 3, 4, 5, 6, 7, 8, 9, 10
	60.0	10		1, 2, 3, 4, 5, 6, 7, 8, 9

120.0	2, 5	1, 3, 4, 6, 7, 8, 9, 10

APPENDIX B

Institutional Animal Care and Use Committee approval form

SIGNATURE PAGE

IACUC #: **PROPOSAL TITLE (From cover page):** Discriminative stimulus effects of gabapentin

X. ACKNOWLEDGEMENT BY PRINCIPAL INVESTIGATOR

I acknowledge responsibility for this project. I have read the Northern Michigan University Principles for the Care and Use of Laboratory Animals and certify that this project will be conducted in compliance with those principles. I assure that I will obtain Institutional Animal Care and Use Committee approval prior to significant changes in the protocol. I assure that this project does not unnecessarily duplicate previous research or instructional projects. I assure that students, staff and faculty on the project are qualified or will be trained to conduct the project in a humane, safe, and scientific manner.

Signature: _____ 02/14/2017

Principal Investigator Date

XI. APPROVAL OF SCIENTIFIC MERIT (to be completed by the Department Head)

Before the project is initiated, it must be reviewed and approved on the basis of its scientific merit.

☐Review conducted by external agency.

☐Governmental Agency: Please specify the reviewing agency or board Federal agency (e.g., NIH, NSF, USDA, etc.) and evidence of approval

☐Nongovernmental agency (e.g., University review, specify if other):

☐Departmental Review: I assure that this project has been reviewed and approved for scientific or instructional merit by:

☐Expert reviewer (Name)

☐Departmental Committee Review (Committee Name and Chairperson):

☐Other (Describe):

Signature: _____ 02/14/2017

Department Head/Other Authorized Departmental Designee Date

XII. REVIEWED AND APPROVED BY THE IACUC

Signature: _____ 02/14/2017

Institutional Animal Care and Use Committee Chair Date

13

Revised June 19, 2014 Check the IACUC website to ensure you are using the most recent form.

Signature:  2/14/17

Institutional Animal Care and Use Officer Date

Following action on this application, copies of approval or denial letters will be sent to the applicant, Department Head, and appropriate College Dean.