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Glial Cell Modulators and Associated Anti-Inflammatory Activity: Implications in Reducing Methamphetamine Abuse-Related Behaviors in Rodents

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Glial Cell Modulators and Associated Anti-Inflammatory Activity: Implications in Reducing Methamphetamine Abuse-Related Behaviors in Rodents

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

by

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Abstract

GLIAL CELL MODULATORS AND ASSOCIATED ANTI-INFLAMMATORY ACTIVITY: IMPLICATIONS IN REDUCING METHAMPHETAMINE ABUSE-RELATED BEHAVIORS IN RODENTS

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Methamphetamine is a psychomotor stimulant that produces hyperactivity and euphoria and can lead to drug-seeking and abuse. An estimate from 2005 put the cost of methamphetamine abuse to society at an estimated 23.4 billion dollars. One of methamphetamine's effects is activation of glial cells and associated neuroinflammatory activity in the periphery and CNS. Glia are responsible for maintaining calcium homeostasis, neuroplasticity, immune activity, and cell signaling. Activation of glia and neuroinflammation are becoming recognized as links in drug abuse-related behavior. The goal of the present work was to assess the ability of ibudilast, AV1013, and minocycline, three glial cell modulating compounds, to attenuate responding in rodent procedures that model different aspects of methamphetamine abuse-related behavior. First, Ibudilast (1.8, 7.5, 13 mg/kg) and AV1013 (10, 30, 56 mg/kg) were examined for their effects on methamphetamine-induced (3 mg/kg) locomotor activity and sensitization in mice, the

latter thought to involve neurocircuitry common with drug relapse. Ibudilast and AV1013 dose-dependently attenuated methamphetamine-induced locomotor activity and its sensitization. Second, ibudilast (1, 7.5, 10 mg/kg), AV1013 (1, 10, 30 mg/kg), and minocycline (10, 30, 60 mg/kg) were examined for their effects on methamphetamine self-administration (0.001, 0.03, and 0.1 mg/kg/inf) in rats. All three compounds significantly reduced methamphetamine (0.03 mg/kg/inf) self-administration. Results suggested baseline self-administration rate as a possible determinant of these effects; however, follow-up tests with ibudilast while controlling for baseline response rate dismissed this possibility. Additional follow-up tests identified whether the attenuation of operant response rates was specific to methamphetamine-maintained behavior. Using a behavioral economic approach, all three test compounds were determined to also affect non-nutritive banana pellet-maintained responding when the baseline strengths maintained by methamphetamine and banana pellet delivery were matched. Finally, ibudilast was evaluated for its effects on methamphetamine discrimination in rats, a procedure thought to model clinical subjective effects. Ibudilast (1, 7.5, 10 mg/kg) did not significantly modify methamphetamine's discriminative stimulus effects when trained at either 1 or 0.56 mg/kg. These results support the hypothesis that attenuation of glial cell activity and neuroinflammation may be linked to some abuse-related behaviors of methamphetamine, reinforcing their examination as novel targets for treating methamphetamine abuse.

Chapter I: INTRODUCTION

Background and Significance

Methamphetamine abuse

Methamphetamine is a psychomotor stimulant and is classified as a Schedule II controlled substance in the United States (Control, 2012). Methamphetamine abuse is widespread and detrimental to both society and the individual. In 2010, amphetamine type stimulant abuse prevalence was ranked second only to cannabis worldwide with an estimated 14.3 to 52.5 million users (Crime, 2012). Methamphetamine use continues to be consistently high in Asia and the market for methamphetamine is increasing in parts of Europe (Norway, Germany, Sweden, Finland, and other Scandinavian countries) (Crime, 2012). Although estimates for current users in the United States were down from 731,000 in 2006 to 353,000 in 2010, there still were approximately 105,000 young users who reportedly tried methamphetamine for the first time (SAMHSA, 2010). Additionally, with the increase in methamphetamine seizures in the US, new methods for methamphetamine production by clandestine laboratories has been increasing rapidly (Crime, 2012). Geographically, methamphetamine abuse in the US is most prevalent in the western states in country/farm

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regions (Johnston, 2011), whereas methamphetamine clandestine laboratory incidents almost exclusively occur in Midwestern states (i.e. Kentucky, Tennessee, Missouri, Indiana) (DEA, 2012). In 2005, methamphetamine abuse cost society an estimated 23.4 billion dollars, largely due to premature death and productivity losses, drug treatment and health care, criminal justice, and cleanup of toxic chemicals from methamphetamine production (Nicosia *et al.*, 2009; Watanabe-Galloway *et al.*, 2009)

Centrally, methamphetamine increases motor activity, libido, alertness, and feelings of euphoria (Peachey *et al.*, 1976; MacKenzie & Heischober, 1997; Winslow *et al.*, 2007) by interacting with the monoaminergic dopamine, serotonin, and norepinephrine neurotransmitter systems (Creese, 1983; Cho & Segal, 1994). Peripherally, dopaminergic, alpha- and beta-adrenergic stimulation leads to increased heart rate, blood pressure, force contraction, and arterial and venous vasoconstriction (Lynch & House, 1992; MacKenzie & Heischober, 1997). The perceived positive effects of elevated mood states and increased physical output, can lead to drug-seeking and chronic abuse (Everitt & Robbins, 2005; Vanderschuren & Everitt, 2005). Chronic methamphetamine abuse is associated with a range of centrally and peripherally mediated toxicities (Lynch & House, 1992; Chuck *et al.*, 1996; MacKenzie & Heischober, 1997; Hamamoto & Rhodus, 2009).

Central nervous system (CNS) toxicity associated with chronic methamphetamine use include agitation, insomnia, psychosis, paranoia, and visual and tactile hallucinations (MacKenzie & Heischober, 1997). Also, the spontaneous recovery of methamphetamine psychosis and paranoid hallucinatory states (i.e. flashbacks) in the absence of drug, can be elicited by mild stressors and are associated with increased plasma norepinephrine levels underscoring methamphetamine's ability to sensitize noradrenergic hyperactivity (Yui *et al.*, 1997; Yui *et al.*, 2000).

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Peripherally, cardiac problems as a result of chronic methamphetamine abuse are wide-ranging. Dysrhythmias, myocardial infarcts, hypertension, congestive heart failure, vasculitis, cardiomyopathy, and cardiac muscle lesions have all been cited as outcomes of chronic use (Lynch & House, 1992; He *et al.*, 1996). Dental disease is also a significant outcome, as methamphetamine abusers commonly have tooth decay, wear from grinding, and decreased salivary flow (Hamamoto & Rhodus, 2009). Interestingly, dental disease is not a product of smoking the drug because intravenous methamphetamine users are more likely to have missing teeth than users who smoked (Shetty *et al.*, 2010). Corneal ulceration, known as keratitis, has been associated with chronic methamphetamine usage as well (Chuck *et al.*, 1996; Poulsen *et al.*, 1996). Finally, methamphetamine's immune suppression peripherally increases abusers' vulnerability to pathogens and infection (Harms *et al.*, 2012). Methamphetamine-related hepatitis has been reported although most cases of hepatitis observed in methamphetamine users are contracted virally (Davis *et al.*, 1970). Similarly, methamphetamine abuse has high rates of co-morbidity with human immunodeficiency virus (HIV) and an exacerbation of its effects, including increasing viral load and neurocognitive impairment (Carey *et al.*, 2006; Theodore *et al.*, 2006; Toussi *et al.*, 2009; Wang *et al.*, 2012b; Wires *et al.*, 2012; Blackstone *et al.*, 2013).

Methamphetamine-related deaths are most often caused by coronary heart disease and subarachnoid hemorrhage (Karch *et al.*, 1999), but may also be caused by seizure, stroke, liver or lung disease (MacKenzie & Heischober, 1997; Karch *et al.*, 1999). Thus, it is clear that methamphetamine's widespread abuse is costly to both society and to the user's health underscoring the need for a treatment approach for methamphetamine stimulant abuse.

Current treatments

Some behavioral intervention programs can reduce methamphetamine abuse. The Matrix Model integrates a variety of treatment approaches including individual therapy, relapse prevention and family education groups, urine testing, and participation in a 12-step program (Rawson *et al.*, 1995) and is successful in reducing methamphetamine usage and sustaining abstinence for up to two years (Obert *et al.*, 2000). Contingency Management (CM) programs in which the participant receives monetary or prize rewards for sustained abstinence also decreases positive methamphetamine urine samples from methamphetamine abusers (Roll *et al.*, 2006). Furthermore, CM alone and in combination with cognitive behavioral therapy (CBT) significantly reduces methamphetamine use and sexual behaviors in gay and bi-sexual men when compared to standard CBT or gay-specific social support therapy alone (Shoptaw *et al.*, 2005; Shoptaw *et al.*, 2008). Other interventions such as Acceptance and Commitment Therapy, which emphasizes observation, thinking, and mindfulness/acceptance exercises, as opposed to avoidance, reduce methamphetamine abuse to a similar degree as CBT (Smout *et al.*, 2010) and presenting repeated within-session multi-modal (picture, video, in-vivo) drug-related cues diminish methamphetamine cue-elicited craving (Price *et al.*, 2010). While these treatments can be successful in time, they require individualized therapy and close monitoring by the counselor as well as knowledgeable personnel and adequate funding (Rawson *et al.*, 2002; Kay-Lambkin, 2008). As such, discovering an effective pharmacotherapy to reduce methamphetamine abuse may serve as a vital complement to behavioral interventions to increase methamphetamine abstinence.

In order to identify potential targets for an effective pharmacotherapy, a close examination of methamphetamine's neurochemical effects is required. While most research efforts have focused on targeting the bioamine receptors to develop pharmacotherapeutic

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agents, for they are the *direct* mediators of methamphetamine's abuse-related effects (see below), those efforts have not been successful (Karila *et al.*, 2010). For instance, compounds involving varied bioamine receptor activity such as bupropion, modafinil, and aripiprazole, have had poor efficacy (Brackins *et al.*, 2011). Thus, while an understanding of methamphetamine's classical neuro-receptor mechanisms is important, an expanded examination of its non-receptor targets, such as the neuroglia, and how that interaction affects behavior may lead to fresh approaches in drug development.

Methamphetamine's Neurochemical Effects

Monoamines

Methamphetamine is best known for interacting with the monoaminergic dopamine, serotonin, and norepinephrine neurotransmitter systems (Creese, 1983; Cho & Segal, 1994). Amphetamine and methamphetamine work in several ways to increase the concentration of monoamines in the synapse promoting increased signaling. First, amphetamines cause a reversal of vesicular dopamine and monoamine transporter systems causing non-exocytotic (i.e. calcium independent) efflux of dopamine and other monoamines out of vesicles and then out of the cell (Liang & Rutledge, 1982; Creese, 1983; Cho & Segal, 1994; Brown *et al.*, 2001; Schmitz *et al.*, 2001; Khoshbouei *et al.*, 2003). Amphetamines also reduce dopamine transporter function (Fleckenstein *et al.*, 2000) and cause cell-surface dopamine transporter endocytosis, likely through a clathrin-mediated pathway (Saunders *et al.*, 2000), which impedes dopamine re-uptake. Serotonin (5-HT) transporter function is rapidly decreased following acute methamphetamine exposure as well, likely through a similar mechanism to dopamine transporter down-regulation (Fleckenstein *et al.*, 1999). Conversely, while the norepinephrine

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transporter is structurally similar to the other two, it is less vulnerable to oxidative inactivation via reactive oxygen species (Haughey *et al.*, 1999); instead the norepinephrine transporter's reduction in re-uptake activity is a proposed to be a direct effect of methamphetamine's (or its metabolites') action on the transporter itself (Haughey *et al.*, 2000). However, the result is similar in that there is decreased uptake.

Under normal physiological conditions, dopamine (D2) auto-receptors, located extra-synaptically, sense dopamine and its metabolites (i.e. DOPAC and homovanillic acid) and decrease dopamine production and release (Cooper *et al.*, 2003). Amphetamines interrupt this negative feedback mechanism and enhance dopamine synthesis (Cooper *et al.*, 2003). Enhanced dopamine synthesis occurs via methamphetamine-induced increases in tyrosine hydroxylase activity to promote the production of dopamine (Mandell & Morgan, 1970; Cooper *et al.*, 2003), while concurrently inhibiting monoamine oxidase (MAO), the enzyme that degrades monoamines (Green & El Hait, 1980). Thus, methamphetamine's neurochemical actions promote the release of monoamines, prolong the time they are present in the synapse by inhibiting re-uptake, and stimulate continued synthesis which results in increased excitation and signaling.

Repeated administrations and/or high doses of methamphetamine are neurotoxic to the same monoaminergic systems. At high doses, methamphetamine decreases tyrosine hydroxylase and tryptophan hydroxylase activity, 5-HT concentrations, and dopamine and 5-HT transporter function (Buening & Gibb, 1974; Hotchkiss *et al.*, 1979), which is likely due to axonal and/or terminal injury (Kogan *et al.*, 1976; Fleckenstein *et al.*, 2000). While the primary mechanism of methamphetamine-induced neurotoxicity is still unclear, the presence of excessive cytoplasmic and extracellular dopamine levels appears to be necessary to induce neurotoxicity (Wagner *et al.*, 1983). Dopamine metabolism produces reactive oxygen species

and dopamine quinones which can cause detrimental effects to the nerve terminals (Cadet & Brannock, 1998; Yamamoto & Zhu, 1998), inactivate the dopamine transporters (Fleckenstein *et al.*, 2000), and induce distinct behavioral responses (Kita *et al.*, 2009). Oxidative inactivation of transporters and subsequent nerve injury is also exacerbated by methamphetamine-induced hyperthermic effects produced by the drug alone and in conjunction with environmental conditions like ambient temperature (Fleckenstein *et al.*, 2000; Kiyatkin & Sharma, 2012). In sum, methamphetamine causes a profound effect on the monoaminergic system both acutely and chronically, and following repeated or high dose administration methamphetamine can cause severe terminal damage and neurotoxicity.

While most pharmacotherapeutic approaches have focused on these direct mechanisms affecting bioamine receptor activity, methamphetamine also has additional, less understood, neurochemical effects including those on intracellular cAMP levels and of glial cell activation that have both been suggested to play a role in modulating methamphetamine-induced behavior.

Cyclic adenosine monophosphate (cAMP, cyclic AMP or 3'-5'-cyclic adenosine monophosphate)

Methamphetamine-induced increases in synapse monoamine neurotransmitters lead to dopamine D1 receptor (D1R) and D2 receptor (D2R) activation. D1R activation is associated with an increase in adenylyl cyclase activity, which catalyzes the conversion of ATP to cAMP, producing a subsequent increase in cAMP levels; whereas, activation of D2R is associated with a decrease in cAMP (Kebabian *et al.*, 1984). As cAMP is a second messenger molecule, these alterations in cAMP levels may lead to modulations of downstream signal transmission and behavioral effects. Although the activation of the two dopamine receptors work in apparently

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opposing directions, it has been hypothesized that activation of both D1R and D2R are required to produce methamphetamine sensitization and reinforcing effects (Kelly *et al.*, 2008).

Specifically, with an increase in cAMP levels (through D1R), cAMP-dependent protein kinase (PKA) phosphorylates dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) at one of several phosphorylation sites (Hemmings *et al.*, 1984) . Phosphorylation of DARPP-32 at the Thr34 residue converts DARPP-32 into a potent inhibitor of another protein inhibitor, protein phosphatase-1 (PP-1) (Hemmings *et al.*, 1984). Phosphorylated Thr34-DARPP-32 dependent signaling is associated with modulating many drug-induced actions including behavioral responses mediated through the dopaminergic pathways (Svenningsson *et al.*, 2005). For example, DARPP-32 knockout mice have an attenuated response to amphetamine-induced increases in repetitive movements and pre-pulse inhibition (Svenningsson *et al.*, 2003) suggesting that DARPP-32 is partially responsible for these behaviors. Interestingly, acute administration of amphetamine upregulates Thr34-DARPP-32 phosphorylation (Svenningsson *et al.*, 2003), while repeated administration of methamphetamine produces sensitization and a decrease in Thr34-DARPP-32 phosphorylation (Chen & Chen, 2005; Borgkvist & Fisone, 2007). Methamphetamine also induces an increase in the phosphorylation of the transcription factor cAMP response element-binding protein (CREB). While CREB plays several roles in behavioral activity as well, an enhancement of CREB seems to play a protective role against addiction-like behaviors (Carlezon *et al.*, 2005). For example, cocaine-induced up-regulation of CREB in the nucleus accumbens is suggested to counter-act the primary effects of cocaine and limit sensitization (Carlezon *et al.*, 2005; Dong *et al.*, 2006).

Because PP-1, the enzyme inhibited by phosphorylated Thr34-DARPP-32, inhibits CREB, Thr-34-DARPP-32 and CREB seem to be linked. Furthermore, the previously mentioned

studies showing a decrease in Thr34-DARPP-32 phosphorylation following methamphetamine sensitization are consistent with CREB's protective activity role. That is, with repeated administration of methamphetamine, the decrease in phosphorylated Thr34 DARPP-32 increases PP-1 inhibition activity, thus decreasing CREB's protective activity to counter-act sensitization.

While the D2R works in the opposite direction at adenylyl cyclase to decrease cAMP concentration, D2R stimulation also leads to activation of phospholipase Cβ (PLCβ) via its coupling to G_q protein (Yan *et al.*, 1999). PLC promotes the production of IP3 which in turn releases Ca^{+2} stores (Yan *et al.*, 1999; Hernandez-Lopez *et al.*, 2000) to excite downstream targets, suggesting a mechanism for D2R activation to affect behavioral activity.

Finally, D1R and D2R activation are both necessary to alter synaptic plasticity in the striatum which can affect behavioral outcomes such as locomotor activity (Calabresi *et al.*, 1992; Pollack, 2004). In fact, heteromerization of D1R and D2R has been shown to lead to the activation of Gq protein, PLC, and IP3 inducing a Ca^{+2} signal (Hasbi *et al.*, 2010). Ca^{+2} signaling activates CaMKII in the rat striatum contributing to synaptic plasticity (Rashid *et al.*, 2007), and potentiating amphetamine self-administration (Loweth *et al.*, 2008). Interestingly, psychostimulants' up-regulation of medium spiney neuron spine density in the nucleus accumbens is more stable and sustained longer in the D1 receptors over the D2 receptors (Lee *et al.*, 2006) implying the downstream effects of D1 activation may be longer lasting. Thus, modulation of cAMP levels may be sufficient to attenuate methamphetamine-induced abuse-like behaviors through D1's DARPP-32 and CREB protective actions.

In addition to its effects on cAMP, methamphetamine also induces glial cell activation and pro-inflammatory responses.

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Glial Cells

While neuroinflammation is commonly associated with neurodegenerative conditions, decades of evidence indicate that some CNS-active drugs can induce neuroinflammatory processes via activation of glial cells as well. Glial cells can be separated into two main groups, macroglia and microglia. One sub-type of macroglia, astrocytes, are the most prevalent cell type in the CNS and have a variety of functions including the response to injury by stimulating pro-inflammatory cytokine release and immune function activity (Benveniste, 1992). Microglia work as macrophages to degrade foreign debris and are also associated with immune response by responding to and secreting inflammatory cytokines (Benveniste, 1992). Following methamphetamine administration, activated microglia and astrocytes release pro-inflammatory cytokines (Yamaguchi *et al.*, 1991; Nakajima *et al.*, 2004a; Goncalves *et al.*, 2008; Loftis *et al.*, 2011). Methamphetamine increases levels of cytokines and inflammatory factors, such as tumor necrosis factor (TNFα), interleukin 6 (IL-6), interleukin 1β (IL-1β) mRNA levels, monocyte chemo-attractant protein 1 (MCP-1), and cellular adhesion molecule (ICAM-1) (Yamaguchi *et al.*, 1991; Nakajima *et al.*, 2004b; Goncalves *et al.*, 2008).

How methamphetamine promotes neuroinflammation is not yet known. As mentioned above, dopamine quinones and reactive oxygen species contribute to methamphetamine-induced cellular damage and apoptosis (Fleckenstein *et al.*, 1997; Zhu *et al.*, 2006). As a result of the damaged cells and neurotoxicity, astrocytes and microglia become activated and elicit an immune response and increase pro-inflammatory cytokine production (Kita *et al.*, 2008; Clark *et al.*, 2012). Environmental factors such as ambient temperature and hyperthermic state of the subject can exacerbate the methamphetamine-induced effects of glial activation and damage to the blood-brain barrier (Kiyatkin & Sharma, 2012; Kousik *et al.*, 2012).

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While cell damage and death is a common catalyst for inflammation induction, methamphetamine's effects on inflammatory pathways can also temporally occur before dopamine cell terminal pathology (LaVoie *et al.*, 2004). Thus, methamphetamine-induced inflammation can occur at non-neurotoxic levels and independently of cell damage. A mechanism has been proposed for methamphetamine-induced inflammation via the nuclear transcription factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Shah *et al.*, 2012). Methamphetamine's release of excitatory neurotransmitters activates the metabotropic glutamate receptor, mGluR5. The mGluR5 receptor is described to activate the intracellular signaling pathway, AKT/PI3K, that downstream induces the release of NF-κB, which, in turn, translocates to the nucleus to promote transcription of inflammatory cytokine proteins such as TNFα, IL-6 and IL-8 (Shah *et al.*, 2012).

Interestingly, cAMP levels also modulate microglial cell activation (Ghosh *et al.*, 2012), and glutamate derived from glial cells preferentially can act on NMDA extrasynaptic receptors to de-phosphorylate CREB and inhibit its protective action (Hardingham *et al.*, 2002). These reports indicate the potential for these two methamphetamine-induced mechanisms (i.e. glial cell activation and cAMP modulation) to be associated with one another providing our first links among cAMP, glial cell signaling, and drug abuse behavior (D'Ascenzo *et al.*, 2007).

In sum, methamphetamine's effects on classic neuronal signaling systems has been well defined, however, methamphetamine also causes other neurochemical effects including those on cAMP and glial cell activation. While the importance of glial cells in the CNS may have been initially overlooked by the scientific community, there is recent evidence to suggest that glia and their activation play a significant role in neurotransmission and drug-induced behavior.

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Importance of Glia

Nervenkitt or neuroglia, literally "nerve-glue" in English, were merely considered connective tissue that served only as scaffolding between neurons when first identified in the early 1900s (Somjen, 1988). However, in the 1980s one subtype of glial cells, astrocytes, were shown to exhibit voltage-gated channels and neurotransmitter receptors leading to increased interest in their function (Volterra & Meldolesi, 2005). Glial cells, specifically astrocytes and microglia, are now described as having a significant role in homeostatic processes, synaptogenesis and guiding neuronal development, neuroplasticity, and regulating the immune responses in the CNS by releasing pro-inflammatory cytokines and chemokines (Volterra & Meldolesi, 2005). Glial cells can elicit their own signals, termed gliotransmission, and regulate synapse formation and strength via the release of pro-inflammatory cytokines by astrocytes and microglia (Lawrence *et al.*, 2007). Activated glial cells have been correlated with altering synaptic transmission and drug abuse behavior (Haydon *et al.*, 2009) and an examination of glial cell function should highlight the importance of glial cell activity in behavior and suggest a unique target for novel drug abuse pharmacotherapies.

Gliotransmission

Glial cells can elicit both excitatory and inhibitory signals, known as gliotransmission, giving rise to the idea of the tripartite synapse (Araque *et al.*, 1999) in which neurotransmitters released from the pre-synaptic neuron not only bind and affect the post-synaptic neuron, but also glial cells, which in turn can release their own gliotransmitters or neutralize synaptic neurotransmitters. First, glial cells are activated as they exhibit many ionic and metabotropic receptor complexes on their membranes such as those for norepinephrine, glutamate, GABA, acetylcholine, histamine, adenosine, and ATP (Haydon & Carmignoto, 2006). They do not

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produce action potentials, but rather signal via oscillations in intracellular Ca^{+2} (Volterra & Meldolesi, 2005). Following activation, glial cells may also release neurotransmitters, known as gliotransmitters, glutamate, D-serine, and ATP which may modulate synaptic transmission and neuronal excitability (Haydon & Carmignoto, 2006). For example, release of glutamate from glial cells can excite glutamatergic NMDA and AMPA receptors on the post-synaptic neuron (Haydon & Carmignoto, 2006; Eroglu & Barres, 2010). Furthermore, glial cells also play an active role in maintaining the extracellular glutamate concentration to prevent excitotoxicity to the neurons (Barbour *et al.*, 1988). Thus, neurotransmitter signaling is no longer isolated to neuronal receptors. Further, as a function of chronic gliotransmission signaling, glial cells also have the capability to modulate synapses.

Regulation of synaptic strength by cytokines

The main processes thought to regulate synaptic plasticity are the cell surface delivery and retention of the glutamatergic receptors, NMDA and AMPA (Eroglu & Barres, 2010). Interestingly, an up-regulation of the pro-inflammatory cytokine tumor necrosis factor (TNFα) elicited from activated astrocytes increases the expression of AMPA receptors on the cell surface and increases NMDA receptor and AMPA receptor mediated synaptic currents (Beattie *et al.*, 2002; Stellwagen & Malenka, 2006) which subsequently improves synaptic efficacy and signal strength. Conversely, the blockade of TNFα and an increase in brain-derived neurotrophic factor (BDNF) will have the opposite effect (Beattie *et al.*, 2002). A prolonged change in this synaptic regulation is known as synaptic scaling which involves changes in the synaptic strength based on activity levels. Importantly, these mechanisms in which AMPA/NMDA ratios induce synaptic plasticity are important because they are associated with stimulant-induced behavioral responses such as sensitization (Wolf, 1998).

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Thus, glial cells are capable of much more than providing scaffolding and debris removal. Activated glial cells may transmit their own signals as well as regulate synaptic strength suggesting that the innate immune system can modulate behaviors that define addiction (Crews *et al.*, 2011). While methamphetamine's effects on glial cells were mentioned above, it is not the only drug of abuse that does affect the glia.

Effects of other drugs on glia

Of the 12 most widely recognized classes of abused drugs worldwide, half can be categorized as producing an anti-inflammatory and the other half an inflammatory profile in the CNS (see Table 1). Inflammation is operationally defined here as the induction of CNS microglial and/or astrocyte activation as well as the production and release of pro-inflammatory cytokines or chemokines. While it cannot be concluded that neuroinflammation is a global indicator of drug abuse behavior, an in depth examination of the mechanism by which these drugs affect glial activity may reveal a pattern and potentially a mechanistic target.

Table 1. Effects of common abused drugs on glial cell activity and inflammatory markers

Drug of Abuse	Effect on Glial Cells	Cytokines/Chemokines Affected
Inflammatory		
Methamphetamine		↑ *TNF-a, IL-6, IL-1b, MCP-1, ICAM-1* ↓ *IL-2, IFN-y* *(Yamaguchi et al., 1991; Nakajima et al., 2004b; Goncalves et al., 2008; Loftis et al., 2011)*
MDMA	*Increases astrocyte/microglia activation* *(Orio et al., 2004; Gekker et al., 2006; Bruce-Keller et al., 2008; Kita et al., 2008; Sharma & Ali, 2008; Yao et al., 2010; Clark et al., 2012; Zhang et al., 2012; Frau et al., 2013)*	↑ *IL-1b* *(Thomas et al., 2004b; Orio et al., 2010)*
Morphine		↑ *TNFa, IL-1b* *LPS-induced ↑ in TNFa, IL-6, iNOS, and NO* *(Sawaya et al., 2009; Berta et al., 2012; Chen et al., 2012)*
Cocaine		↑ *TNFa, IL-6, IL-1b, MCP-1* ↓*IL-10* *(Gan et al., 1999; Lin et al., 2011; Fox et al., 2012)*
Ethanol	*Increases astrocyte activation* *(Miguel-Hidalgo, 2005; 2006)*	↑ *TNFa, IL-6, IL-1b, MCP-1, IFN-y, TGF-b, IL-8, IL-10, IL-12, iNOS, COX2* *(Blanco et al., 2004; Laso et al., 2007; He & Crews, 2008)* ↓ *GDNF in dependent users* *(Heberlein et al., 2010)*
PCP	*Induces microglia (only because high doses are neurotoxic)* *(Nakki et al., 1996a; Fattorini et al., 2008)*	↑ *TNFa (with non-toxic PCP doses)* *(Paterson et al., 2006)*
Anti-inflammatory		
THC	*Activates CB2 receptors on microglia* *(Puffenbarger et al., 2000; Chung et al., 2011)*	↓ *LPS-induced TNFa, IL-6, IL-1b, IL-1a* *(Puffenbarger et al., 2000)*
LSD	*Suppresses proliferation of B-and T-lymphocytes*	↓ *IL-2, IL-4, IL-6* *(House et al., 1994)*

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	(House et al., 1994; Yu et al., 2008)	
Benzodiazepines	*Decreases proliferation of activated microglia Increases neuroprotection (Wilms et al., 2003)*	*↓ TNFa and NO (Wilms et al., 2003)*
Nicotine	*Inhibits microglial and astrocyte activation and inflammation Increases neuroprotection (Ohnishi et al., 2009; Liu et al., 2012b)*	*↓ TNFa and iNOS (De Simone et al., 2005; Ohnishi et al., 2009)*
Pentobarbital		*↓ TNFa mRNA (Yang et al., 2007)*
Caffeine	*Inhibits LPS- and ageing-induced microglia activation (Brothers et al., 2010)*	

Anti-inflammatory Drugs of Abuse

Δ⁹-tetrahydrocannabinol (THC)

THC, an agonist at CB1 and CB2 receptors, has several reported anti-inflammatory mechanisms. CB1 and CB2 receptor activation both work to reduce inflammation. A specific CB1 agonist (WIN55, 212-2) protects against MPTP-induced dopamine neurotoxicity, microglial activation, cytokine release, and oxidative damage (Chung *et al.*, 2011) and CB2 receptors on microglia inhibit inflammatory processes downstream (Puffenbarger *et al.*, 2000). THC can also bind to peroxisome proliferated activated receptor (PPAR) receptors, nuclear receptors which act as transcription factors to affect gene expression which could potentially affect inflammation (Granja *et al.*, 2012). Agonists of PPAR-y reduce lipopolysaccharide (LPS)-induced microglial activation, cytokine, and prostaglandin E2 (PGE2) release that would activate astrocytes for a secondary reaction (Granja *et al.*, 2012). Furthermore, THC can directly affect transcription factors and genes, such as STAT1, STAT3, and Socs3 to cause even more anti-inflammatory signal (Kozela *et al.*, 2010). Finally, because THC is highly lipophilic, THC is hypothesized to inhibit LPS-induced inflammation and cytokine release by disrupting the plasma membrane fluidity and thereby inhibiting the activation of TLR-4 receptor by LPS (Puffenbarger *et al.*, 2000). Thus, THC's anti-inflammatory activity is multi-modal and works at several levels of the cell to inhibit the glial activation.

Nicotine

Nicotine's effects are also anti-inflammatory. Nicotine decreases immune cell responses both peripherally and centrally (Shi *et al.*, 2009). Peripherally, nicotine decreases inflammation by altering the activity of T-cells (Shi *et al.*, 2009). Centrally, neurons, astrocytes, and microglia have all been shown to exhibit nicotinic acetylcholine receptors (Shi *et al.*, 2009), and activation

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by nicotine can suppress TNFα production by microglia and exert a neuroprotective role against thrombin cytotoxicity (Ohnishi *et al.*, 2009). Similarly, nicotine pretreatment significantly suppresses LPS-induced microglial activation and TNFα mRNA expression in vivo and in vitro through α7 nicotinic receptors and protects dopamine neurons (De Simone *et al.*, 2005; Park *et al.*, 2007). Nicotine also suppresses LPS and MPP+- induced astrocyte activation in vitro via a reduction in TNFα release, extracellular regulated kinase 1/2 (Erk1/2) and p38 activation (Liu *et al.*, 2012b).

As far as mechanism, nicotine is proposed to attenuate CNS inflammation by reducing NF-κB and c-myc activation by inhibiting MAP kinases through the α7 nAChR (Liu *et al.*, 2007), and that stimulation of the α7 nAChR is necessary for the inhibition of astrocytes and inflammation by cholinergic agonists (Liu *et al.*, 2012b).

Benzodiazepines

The translocator protein (18 kDa)(TSPO), also known as peripheral benzodiazepine receptor, is present in the CNS and its expression increases upon injury or inflammation (Chen & Guilarte, 2008). Specific ligands for TSPO (Ro-5-4864 and R-PK11195) increase reactive oxygen species (ROS) and IL-1β production, however both ligands inhibit ATP-induced inflammation indicating that TSPO ligands have anti-inflammatory properties in the presence of activated microglia (Choi *et al.*, 2011). Bezodiazepines bind to TSPO and decrease the proliferation of activated microglia, the release of NO, and TNFα in vitro which is thought to also increase neuroprotection (Wilms *et al.*, 2003).

Barbiturates

While there is an apparent absence of reports regarding the barbiturates' direct effects on astrocytes and microglia activation, pentobarbital has been shown to suppress TNFα mRNA

expression in vivo and protect cells from TNFα-induced apoptosis in vitro (Yang *et al.*, 2007). Additionally, thiopental inhibits NF-kB by suppressing IkB kinase activity (Loop *et al.*, 2003), however the "thio" group (sulfur atom at the C2 position) was likely a structural requirement for NF-kB inhibition (Loop *et al.*, 2003) qualifying the potential for a common effect among all barbiturates.

Lysergic acid diethylamide (LSD)

As a strong partial agonist at the 5-HT$_{2A}$ receptor, LSD suppresses proliferation of B-lymphocytes, production of IL-2, IL-4, and IL-6 cytokines, and the induction of cytotoxic T-lymphocytes in vitro (House *et al.*, 1994). Similarly, activating the 5-HT$_{2A}$ receptor with the receptor-specific agonist, (R)-1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane ((R)-DOI) inhibits TNFα and its pro-inflammatory markers ICAM-1, VCAM-1, IL-6, NO synthase, and NF-κB (Yu *et al.*, 2008) indicating that 5-HT$_{2A}$ receptors are involved in an anti-inflammatory response. However, these reports are limited to peripheral inflammation, and LSD has not yet been linked to microglial or neuroinflammatory signaling.

Caffeine

Caffeine antagonizes both the adenosine A$_1$ and A$_{2A}$ receptors pre-synaptically, which increases glutamate signaling (Solinas *et al.*, 2002), and attenuates LPS- and aging-induced microglial activation (Brothers *et al.*, 2010). Caffeine enhances astrocyte and microglial reactivity following exposure to MDMA, however it was proposed that the gliosis was likely due to hyperthermia as a result of MDMA neurotoxicity that was exacerbated by caffeine (Khairnar *et al.*, 2010).

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In sum, while it seems THC, nicotine, benzodiazepines, barbiturates, LSD, and caffeine all exhibit some anti-inflammatory effects and most attenuate glial activation, there is currently not a common mechanism by which these drugs work. Conversely, there are a number of drugs that cause inflammatory effects similar to methamphetamine's effects on glial cells.

<u>Inflammatory Drugs of Abuse</u>

The following drugs of abuse all exhibit neuroinflammatory profiles as reported in the literature, and the body of evidence to support the hypothesis that glial cell activation and inflammation play a role in addiction-like behaviors is growing rapidly (Miguel-Hidalgo, 2009; Coller & Hutchinson, 2012). Thus, an examination of the effects on CNS inflammation induced by these drugs, as well as the potential mechanisms involved, may provide insight for common targets for potential pharmacotherapies.

MDMA (3, 4-methylenedioxy-N-methylamphetamine)

MDMA induces microglial activation and the release of IL-1β (Orio *et al.*, 2004; Thomas *et al.*, 2004a; Orio *et al.*, 2010; Frau *et al.*, 2013). MDMA has also been shown to induce astrocyte activation, however, this was only after MDMA first induced hyperthermia, cellular injury, and disruption of the blood-brain barrier (BBB), suggesting that astrocyte activation is a consequence of earlier MDMA induced toxic events (Sharma & Ali, 2008). Conversely, while microglial activation was correlated with MDMA hyperthermia (Frau *et al.*, 2013), it was not dependent upon it, indicating independent mechanisms (Orio *et al.*, 2004).

MDMA's potential mechanism may be linked to the A_{2A} adenosine receptor system. The A_{2A} receptor antagonist, SCH 58261, attenuates MDMA-induced increases in NF-κB mRNA and protein levels (Kermanian *et al.*, 2013) indicating an anti-inflammatory effect. Furthermore, A_{2A} receptor knock-out mice exhibit decreases in microglial and astrocyte activation following

MDMA administration and less MDMA self-administration compared to their wild-type littermates (Ruiz-Medina *et al.*, 2011). Interestingly, CB1 and CB2 receptor systems have also been shown to modulate MDMA-induced microglia and astrocytes and protect against MDMA neurotoxicity. For example, a CB2 agonist as well as THC significantly inhibits MDMA-induced microglial activation and IL-1β release (Torres *et al.*, 2010; Tourino *et al.*, 2010). THC's neuroprotective role against MDMA requires both CB1 and CB2 receptors to be present (Tourino *et al.*, 2010), as CB1 receptors attenuate MDMA hyperthermia and CB2 receptors attenuate the inflammatory response (Tourino *et al.*, 2010).

Phencyclidine (PCP)

Acute, non-neurotoxic doses of PCP produce a regionally specific (prefrontal cortex only) decrease in TNFα levels (Paterson *et al.*, 2006). In contrast, high doses of ketamine (80 mg/kg), PCP (10 and 50 mg/kg), and MK-801 (5-10 mg/kg) all induce microglial activation, however this effect is likely due to neuronal injury, damage, and cell death (Nakki *et al.*, 1995; Nakki *et al.*, 1996b; Nakki *et al.*, 1996c). Glutamatergic NMDA receptors are present on microglial cells and which can be activated with kainic acid (Eriksson *et al.*, 2000). Further, the resulting cytokine mRNA production may be attenuated by MK-801 (Eriksson *et al.*, 2000) suggesting that NMDA receptor activation may be an important player in glial cell activation and inflammatory actvity.

Ethanol

Chronic ethanol up-regulates iNOS, COX2, and IL-1β in cultured astrocytes, and up-regulates NF-κB and AP-1 signaling (Blanco *et al.*, 2004; Valles *et al.*, 2004). In humans, ethanol produces increases in IL-1β, IL-6, TNFα, and IFN-γ cytokine levels (Laso *et al.*, 2007), and elevation of MCP-1 levels in the VTA, substantia nigra, hippocampus, and amygdala were

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found in post-mortem alcoholics' brain tissue (He & Crews, 2008) indicative of increased pro-inflammatory signaling. Alcohol's major metabolite, acetaldehyde, also induces NF-κB mediated IL-1β and TNFα expression indicating that the pro-inflammatory response is most likely a combined effect of ethanol and its metabolites (Hsiang *et al.*, 2005). Furthermore, serum levels of the neurotrophic factor GDNF are decreased in alcohol dependent subjects (Heberlein *et al.*, 2010), reducing neuroprotection.

While ethanol withdrawal increases astrocyte proliferation in the prefrontal cortex (Miguel-Hidalgo, 2005; 2006), some neuroprotective factors are also up-regulated. In humans GDNF and BDNF serum levels become escalated in early withdrawal and are associated with lower withdrawal scores and lower alcohol tolerance scores (Heberlein *et al.*, 2010) indicative of some recovery.

Ethanol-induced inflammatory signaling in astrocytes is a consequence of up-regulating p65 activity, a transcription factor involved in the NF-κB signaling pathway (Davis & Syapin, 2004). Ethanol also activates the TLR4 receptor by inducing its translocation to lipid rafts in astrocytes, which enhances the same NF-κB signaling pathway downstream (Blanco & Guerri, 2007; Szabo *et al.*, 2007; Blanco *et al.*, 2008). In microglia, ethanol induces this activation and translocation of TLR4 as well as TLR2 receptors to lipid rafts (Fernandez-Lizarbe *et al.*, 2013). The hetero-dimerization of TLR4 to TLR2 potentiates ethanol's inflammatory effect (Fernandez-Lizarbe *et al.*, 2013) suggesting that microglia enhance the inflammatory signal induced by astrocytes.

Finally, LPS-induced inflammation, via TLR4 receptor activation, increases alcohol consumption in mice (Blednov *et al.*, 2011). Genetic knockouts of the TLR4 receptor, on the other hand, are protected against ethanol-induced inflammation, locomotor activity, memory and anxiety, and epigenetic changes (Pascual *et al.*, 2011), indicating that the TLR4 receptor and its

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downstream signaling pathways are vital components of ethanol's inflammatory and behavioral effects.

Cocaine

Cocaine's inflammatory profile does not seem to be nearly as extensive as the other drugs of abuse. Cocaine up-regulates IL-1β from astrocytes, IL-6, and TNFα expression, and down-regulates IL-10 (Gan *et al.*, 1999; Cearley *et al.*, 2011; Lin *et al.*, 2011; Fox *et al.*, 2012). Cocaine also increases BBB permeability, enhancing monocyte migration across the barrier inducing ICAM-1 and VCAM-1 expression (Fiala *et al.*, 1998). Cocaine's induction of MCP-1 and enhancement of HIV are regulated through the sigma-1 receptor which is present on microglia (Gekker *et al.*, 2006; Yao *et al.*, 2010). Also, withdrawal from cocaine has an effect on astrocytes themselves as suggested by the induction of GFAP following three weeks of abstinence, indicative of astroglial plasticity (Bowers & Kalivas, 2003).

Mechanistically, there is no direct evidence linking cocaine's effect on glial cells or inflammation and the TLR4 receptor as with alcohol and morphine. However, chronic cocaine increases NF-κB induction in the nucleus accumbens (Ang *et al.*, 2001) and has been implicated in playing a direct role in cocaine's abuse and sensitization by inducing increases in dendritic spine densities to alter neuronal plasticity (Russo *et al.*, 2009). Another proposed mechanism for cocaine's effect on glial cells involves the glutamatergic systems. mGluR5 receptors, which are present on astrocytes, become activated by extracellular glutamate released by cocaine administration. Then, astrocytes generate Ca^{+2} signaling cascades inducing a prolonged stimulus associated with selectively activated NR2B-containing extrasynaptic neuronal NMDA receptors (D'Ascenzo *et al.*, 2007; Fellin *et al.*, 2007) implicating gliotransmission's role in altering cocaine-induced behaviors. Furthermore, mice lacking the mGluR5 gene do not exhibit cocaine-induced locomotor activity increases nor do they reliably

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self-administer cocaine (Chiamulera *et al.*, 2001). Interestingly, the activation of astrocytic mGluR5 receptors and of NF-kB pathways is similar to the proposed mechanism of methamphetamine's action and it is linked to abuse-like behaviors.

Morphine

Similar in breadth to methamphetamine's neuroinflammatory activity, morphine activates astrocytes and microglia and up-regulates the production of many pro-inflammatory cytokines in the CNS (Bruce-Keller *et al.*, 2008; Zhang *et al.*, 2012). Morphine up-regulates cytokines such as TNF-α, IL-1β, IL-6 mRNA levels in the nucleus accumbens, medial pre-frontal cortex, and dorsal root ganglia (Sawaya *et al.*, 2009; Berta *et al.*, 2012; Chen *et al.*, 2012) and increases LPS-induced expression of the same cytokines via the mu opioid receptor located on microglial cells and the protein kinase C (PKC) pathway (Merighi *et al.*, 2013). Furthermore, one of morphine's metabolite's, morphine-3-glucoronide, activates TLR4 and causes microglial activation and the release of IL-1β (Lewis *et al.*, 2010) indicating that opioids' metabolites contribute to their inflammatory action as well.

Mechanistically, similar to ethanol, morphine has been suggested to require TLR2 receptors for its inflammatory action, as TLR2 receptor knock-out mice do not exhibit morphine-induced microglial activation, cytokine release, and have attenuated morphine withdrawal symptoms relative to wild types (Zhang *et al.*, 2011). Also, a study utilizing a small interfering RNA (siRNA) directed against p65, an element of the NF-kB signaling pathway, indicated that morphine-induced TNFα release was NF-κB pathway dependent (Sawaya *et al.*, 2009). Activation of the PKC pathway stimulates AKT upstream of ERK 1/2 and iNOS (Merighi *et al.*, 2013) which is the same AKT pathway that stimulates NF-kB induction in the proposed mechanism of methamphetamine-induced glial cell activation (Shah *et al.*, 2012; see discussion). Interestingly, CB2 receptor activation attenuates morphine-induced inflammation by

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interfering with the AKT-ERK 1/2 pathway suggesting that activation of CB2, and the inhibition of microglial activation and subsequent downstream inflammatory effects, may increase the clinical efficacy of opioids (Merighi *et al.*, 2013).

Also similar to methamphetamine's effects, opioids are also involved in increasing the phosphorylation of DARPP-32 and DARPP-32's link to behavioral effects and synaptic plasticity (Mahajan *et al.*, 2009). Finally, morphine's glial cell activation and pro-inflammatory action is also associated with its behavioral effects. Astrocyte-conditioned medium (i.e. activated astrocytes) increases morphine-induced CPP when injected into the nucleus accumbens (Narita *et al.*, 2006).

In sum, the drugs of abuse that have exhibited neuroinflammatory profiles share some aspects of their proposed mechanisms of action. For example, both ethanol and morphine have been shown to require both TLR2 and TLR4 receptor activation to induce NF-kB to promote cytokine production (Zhang *et al.*, 2011; Fernandez-Lizarbe *et al.*, 2013), and cocaine, morphine, and methamphetamine have all been linked to activation of mGluR5 receptor and AKT pathway activation to induce NF-kB (D'Ascenzo *et al.*, 2007; Shah *et al.*, 2012; Merighi *et al.*, 2013). Thus, perhaps a common mechanism of action preludes a common mechanistic target for attenuating the downstream effects of these drugs and the behaviors they induce.

Novel approaches to attenuate drug abuse-like behavior

As mentioned above, methamphetamine's regulation of cAMP levels in the brain are implicated in mediating many of its behavioral effects such as methamphetamine-induced hyperactivity, sensitization, and drug discrimination. Phosphodiesterase (PDE) degrades cAMP (Beavo, 1995); so compounds that block PDE, will increase cAMP levels and could,

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consequentially, cause a change in methamphetamine-induced behaviors. Rolipram (a PDE inhibitor) increases cAMP levels in the brain and dose dependently attenuates methamphetamine-induced hyperlocomotion (Iyo *et al.*, 1995; Iyo *et al.*, 1996a; Iyo *et al.*, 1996b; Mori *et al.*, 2000). In addition, rolipram and nifiracetam (another PDE inhibitor) attenuate methamphetamine discrimination in mice (Yan *et al.*, 2004; Yan *et al.*, 2006). Thus, compounds that influence cAMP levels may be potential methamphetamine pharmacotherapies that should be tested in animal models. Methamphetamine also influences glial cells, suggesting another therapeutic target.

Glial cell attenuating compounds have been shown to prevent increases or cause decreases in up-regulated cytokines and chemokines in brain regions associated with opioid withdrawal such as the ventral tagmental area and nucleus accumbens (Hutchinson *et al.*, 2009a), block morphine-induced CPP (Narita *et al.*, 2006; Hutchinson *et al.*, 2009a; Hutchinson *et al.*, 2009b), and block morphine and oxycodone spontaneous and precipitated withdrawal signs (Hutchinson *et al.*, 2009a). Developing evidence indicates that attenuating glial activation can reduce methamphetamine-induced behavioral effects (Narita *et al.*, 2006; Zhang *et al.*, 2006; Fujita *et al.*, 2012). Correspondingly, an enhancement of neuroprotective growth factors, like glial cell derived neurotrophic factor (GDNF), blocks cocaine (Green-Sadan *et al.*, 2003; Green-Sadan *et al.*, 2005) and methamphetamine self-administration and vulnerability towards reinstatement and sensitization (Niwa *et al.*, 2007c; Yan *et al.*, 2007) and attenuates morphine CPP and morphine sensitization (Niwa *et al.*, 2007a; Niwa *et al.*, 2007b).

Finally, the initial component of methamphetamine's proposed mechanism of glial cell activation is the activation of the mGluR5 receptor. Activation of mGluR5 receptors, which are present on glial cells (Miller *et al.*, 1995), can lead to inflammatory downstream processes (Shah *et al.*, 2012), while mGluR5 antagonism attenuates on-going cocaine and

methamphetamine self-administration as well as cue-and drug-induced reinstatement (Chiamulera *et al.*, 2001; Gass *et al.*, 2009). Thus, both PDE inhibition, up-regulation of neuroprotective neurotrophic factors, and anti-inflammatory activity all attenuate methamphetamine-induced abuse-like behaviors.

Compounds of Interest

Ibudilast (AV411)

Ibudilast (3-isobutyryl-2-isopropylpyrazolo-[1, 5-a] pyridine) is a pyrazole-pyridine small molecule which has a broad range of functions. Ibudilast is approved clinically to treat asthma and post-stroke dizziness in Japan and is well tolerated in humans (Rolan *et al.*, 2008; Rolan *et al.*, 2009). Ibudilast is a non-selective phosphodiesterase (PDE) inhibitor for PDEs 3, 4, 10, and 11 (Kishi *et al.*, 2001; Gibson *et al.*, 2006) and is a glial cell modulator and anti-inflammatory agent which attenuates LPS-induced nitric oxide release, reactive oxygen species, TNF-a, IL-1b, and IL-6 production (Suzumura *et al.*, 1999; Mizuno *et al.*, 2004; Rolan *et al.*, 2009). Ibudilast is also a potent inhibitor of macrophage migration inhibitory factor (MIF) (Cho *et al.*, 2010) and it attenuates LPS-induced microglial production of the chemokine MCP-1 (Ledeboer *et al.*, 2007).

Ibudilast enhances neuroprotective function by increasing the production of the anti-inflammatory cytokine, IL-10, and neurotrophic factors such as GDNF, nerve growth factor (NGF), and neurotrophin (NT-4) (Mizuno *et al.*, 2004). Ibudilast is also neuroprotective against glutamate toxicity by reducing Ca^{+2} influx (Tominaga *et al.*, 1996). Separately, PDE inhibition and glial cell modulation have already been reported to reduce drug abuse activity, as described above. Thus, ibudilast's effects could be a result of any or a combination of all these mechanisms. Ibudilast has been reported to decrease opioid dependence and withdrawal signs

(Ledeboer *et al.*, 2007; Hutchinson *et al.*, 2009a), attenuate morphine-induced dopamine release in the nucleus accumbens (Bland *et al.*, 2009), and CPP reinstatement in rats (Schwarz *et al.*, 2011). Importantly, ibudilast attenuates prime- and cue-induced reinstatement of methamphetamine-maintained responding (Beardsley *et al.*, 2010) further supporting the hypothesis that PDE inhibition and/or glial cell modulation can alter methamphetamine-induce behaviors.

AV1013

AV1013 ((R)-2-amino-1-(2-isopropylpyrazolo[1,5-a]pyridin-3-yl)propan-1-one hydrochloride) is an amino analogue of ibudilast which exhibits similar glial attenuating actions as ibudilast, but is impotent at inhibiting PDE (Cho *et al.*, 2010). A characterization of AV1013's effects on methamphetamine-induced behaviors could suggest whether modulation of glial cell activity is sufficient to have target effects without PDE inhibition.

Minocycline Hydrochloride

Minocycline (7-dimethylamino-6-dimethyl-6-deoxytetracycline), a second generation, semi-synthetic tetracycline derivative, is indicated as a treatment for numerous infections due to gram-positive and gram-negative micro-organisms including severe acne, some sexually transmitted diseases, respiratory tract infections as well as some more serious conditions including syphilis, anthrax, and plague (FDA, 2010). In addition to minocycline's anti-microbial action, its anti-inflammatory and neuroprotective functionality have implicated it as a potential therapeutic for disorders including dermatitis, periodontal disease, rheumatoid arthritis, CNS pathologies, neuropathic pain, inflammatory bowel disease, and allergic asthma (Garrido-Mesa *et al.*, 2013). Minocycline significantly attenuates microglial activation (Sriram *et al.*, 2006; Zhang *et al.*, 2006) and suppresses LPS-induced TNFα, IL-6, IFN-γ and chemokines such as

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IL-8, MCP-1, and interferon inducible protein (IP)-10 via inhibition of IKKα/β phosphorylation, a component of the NF-κB signaling cascade (Tai *et al.*, 2013). Minocycline also attenuates the biochemical effects of some drugs of abuse as it decreases NF-κB, IL-1β, and microglial activation induced by MDMA (Orio *et al.*, 2010) and significantly attenuates ethanol-induced up-regulation of IκBα protein levels (Wu *et al.*, 2011).

Minocycline attenuates drug-induced *behavioral* effects as well. Minocycline suppresses methamphetamine and cocaine-induced hyperlocomotion and sensitization (Zhang *et al.*, 2006; Chen *et al.*, 2009a) and prevents methamphetamine conditioned place preference (CPP) (Fujita *et al.*, 2012). Minocycline reduces morphine-induced respiratory suppression, attenuates morphine CPP, enhances morphine analgesia (Hutchinson *et al.*, 2008; Zhang *et al.*, 2012), attenuates tolerance to morphine analgesia (Cui *et al.*, 2008) and decreases alcohol consumption in mice (Agrawal *et al.*, 2011). Finally, psychotic symptoms following methamphetamine use are improved by minocycline in humans (Tanibuchi *et al.*, 2010). Thus, there is some previous evidence that PDE inhibition and glial cell modulation is related to drug abuse-related behavior. Given that ibudilast, AV1013, and minocycline alter several of these novel molecular targets, it is important to assess whether they could attenuate methamphetamine induced abuse-like behaviors in several animal models of drug abuse.

Animal Models of Drug Abuse

There are a number of laboratory animal procedures that have face and/or predictive validity for clinical drug abuse disorders that can be useful when evaluating potential pharmacotherapies. While there is not one model that alone predicts drug abuse or its blockade by a compound, studying the effects of compounds on several animals models of

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methamphetamine abuse provides a fuller profile of their ability to attenuate abuse-like behaviors.

Locomotor Activity

First, methamphetamine, as a stimulant, elicits hyperactivity following administration (Peachey *et al.*, 1977). There are several ways to measure methamphetamine-induced hyperactivity in rodents. Methods include recording stereotypies such as repeated rearing, continuous sniffing or head bobbing, or tracking ambulatory behavior by measuring an animal's total distance traveled in an open field (Hall, 1934; Iyo *et al.*, 1995; Kuribara, 1997; Buccafusco, 2001; Tatsuta *et al.*, 2006). For example, acute methamphetamine-induces hyperactivity. When wistar rats are administered 4 mg/kg methamphetamine (i.p.) it produces a significant increase of distance traveled measured by almost 40,000 locomotor counts/hr compared to less than 300 counts/hr in the vehicle group (Iyo *et al.*, 1995). Importantly, following repeated methamphetamine administration its hyperlocomotion effect becomes more pronounced (i.e. sensitized) (Nishikawa *et al.*, 1983; Hirabayasi *et al.*, 1991; Iyo *et al.*, 1996b). For example, animals challenged with 2 mg/kg methamphetamine following once-daily injections of 4 mg/kg methamphetamine for 5 days show twice as great an increase in locomotor activity counts compared to those who had received saline vehicle for the 5 days (Iyo *et al.*, 1996a; Iyo *et al.*, 1996b).

Sensitization has been postulated to play a key role in drug addiction in humans (Strakowski & Sax, 1998; Sax & Strakowski, 2001; Chen *et al.*, 2009b), and the adaptations of specific brain regions implicated in the process of sensitization have been associated with reward pathways linked to drug-seeking and addiction (Robinson & Berridge, 1993). Furthermore, sensitization phenomena interacts with similar neurocircuitry, neurotransmitter and

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receptor systems as those activated during reinstatement in models of drug abuse (Steketee & Kalivas, 2011) implicating its association with craving and drug relapse. Thus, perhaps if sensitization may be attenuated pharmacologically, the potential for relapse may been attenuated as well. In sum, hyperactivity after acute methamphetamine administration and sensitization after repeated administration can be modeled in rodents, and provide a valuable tool for the study of drug addiction.

Self-administration

Drug use may be generated in laboratory animals using drug self-administration procedures in which responses (such as lever pressing) result in drug administration (Schuster & Thompson, 1969; Thompson & Pickens, 1970). Intravenous (i.v.) methamphetamine self-administration in rats is well-established in our laboratory (e.g. Shelton & Beardsley, 2008; Beardsley *et al.*, 2010). Aside from the assay's strong face validity for modeling drug-taking behavior, self-administration procedures may be used to measure the ability of test compounds to alter the reinforcing efficacy of an abused drug (Mello & Negus, 1996). In fact, several compounds have been reported to reduce methamphetamine self-administration in rats (Ranaldi & Poeggel, 2002; Neugebauer *et al.*, 2007; Reichel *et al.*, 2009). Thus, methamphetamine i.v. self-administration is a well-established model of drug-taking behavior in rats, and should provide a strong model of drug abuse-related behavior to test the effects of glial cell modulators.

Given the results obtained with self-administration studies alone, sometimes the identification of the determinants and range of conditions for observing self-administration effects is also important. Two potential variables that may influence the effects of test compounds on drug self-administration are the response rate and response strength of the drug-maintained baselines.

<u>Response Rate</u>

Response rate dependency holds that a drug's effects may differentially affect behavior based on initial baseline rates of behavior. For example, Dews (1955) reported that a dose of pentobarbital can increase low rates, but the same dose can decrease high rates of schedule controlled behavior. Thus, a drug's effect on behavior is a function of the control response rate. A response rate control procedure controls for and determines whether a treatment compound's effects are rate dependent by maintaining the response rate for all conditions equal to determine a change in behavior following test compound administration.

<u>Behavioral Economics</u>

The behavioral economic approach applies consumer demand theory to behavioral psychology (Hursh, 1984). Consumer demand theory examines the relationship between price and demand for a commodity, while behavioral economics examines the relationship between an operant, such as lever presses, and total consumption of a reinforcer. The equation $\log Q = \log Q_0 + k(e^{-\alpha P} - 1)$ developed by Hursh (1984) includes several variables, Q, Q_0, k, P, and α, used to generate a demand curve. Variable Q indicates total or normalized consumption of the reinforcer, Q_0 is consumption of the reinforcer when the price is set to zero, k is a constant that specifies the range of the dependent variable in logarithmic units, P is the price of the commodity, and α is a rate constant indicating the rate of change in elasticity of the demand curve (Hursh & Silberberg, 2008). The demand curve itself indicates total consumption of a particular reinforcer as a function of increasing price. The elasticity of the curve's descent with increasing price indicates the amount to which the subject will defend responding for the particular reinforcer at higher prices (Hursh, 1984). Thus, when examining the demand curve for a particular reinforcer, the rate of change in elasticity of the demand curve (α level) becomes an important dependent variable. "Less elasticity", and a small α level, can be interpreted as "more

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reinforcing" per this approach because consumption behavior continues even at high prices (Hursh, 1984; Hursh & Winger, 1995; Hursh *et al.*, 2005; Hursh & Silberberg, 2008). In order measure and compare the reinforcing efficacy of several reinforcers to one another, the behavioral economic approach engenders several advantages over response rate, behavioral momentum, choice, and breakpoint methodologies.

Why behavioral economics?

In contrast to measuring response rates for a self-administration of a commodity to assess its reinforcing strength, the dependent measure of behavioral economics is total consumption. While response rate analyses are conventionally used in self-administration studies, the inverted U-shaped curve in which response rates decline at higher doses of self-administered drugs inhibits a comparison or reinforcing efficacies between different reinforcers (Hursh *et al.*, 2005). Furthermore, response rate measures are incapable of comparing reinforcing strengths because the behavioral schedules and local contingencies set by the experimenter can affect response rate responding (Hursh & Silberberg, 2008). There are other approaches to measuring reinforcing strength. Behavioral momentum, the notion that the relative strength of reinforcers may be measured by their relative resistance to change responding following an experimenter-introduced disruption (Nevin, 1992), is again not completely independent of schedule effects on rate of responding (Hursh & Silberberg, 2008). Choice tasks maintain face-validity, but they are not independent of environmental factors such as income and price (Hursh & Silberberg, 2008). Finally, many researchers employ progressive ratio schedules to obtain a breakpoint measure of reinforcing strength. To obtain a breakpoint, the ratio size incrementally increases with every reinforcer delivery during the session until the subject no longer earns reinforcement. While breakpoints could be informative, they omit information regarding behavior that occurs at different ratio sizes (Hursh & Silberberg, 2008),

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and the breakpoint may co-vary with experimenter set criteria (Stafford & Branch, 1998). Breakpoints determined via within session progressive ratio tests are also vulnerable to satiation effects (Giordano *et al.*, 2001).

Hursh and others have effectively argued that traditional measures of reinforcement (i.e. peak response rate, breakpoint, and preference) are all encompassed by and represented in different portions of the demand curve (Bickel *et al.*, 2000; Hursh & Silberberg, 2008) and that measuring unit price is the most parsimonious method to studying reinforcing strength (DeGrandpre *et al.*, 1993). Thus, the demand curve and the behavioral economic approach is currently the most appropriate method of comparing the reinforcing efficacy of different reinforcers.

Drug Discrimination

Drug discrimination using laboratory animals is a procedure that is used to model the subjective effects of a drug experienced by humans (Schuster, 1976; Brady & Balster, 1981). A discrimination procedure reinforces a response dependent upon the stimulus conditions prevailing during training sessions (Overton, 1979). In a drug discrimination procedure, the discriminative stimuli are the interoceptive effects that occur following drug and saline administration, and reinforcement is typically a food pellet delivery, access to sweetened milk, or avoidance of shock reinforced by pressing a lever or a nose poke. The drug discrimination procedure is viewed as a valuable method for studying the abuse liability of drugs of abuse (Berkley & Stebbins, 1990) as it may be used to assess the extent to which a novel test drug reproduces the "subjective effects" of a drug of abuse used as the training drug, or whether the test drug attenuates those subjective effects when the two drugs are given concurrently (Schuster, 1976; Solinas *et al.*, 2006). Methamphetamine drug discrimination is well

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established, and methamphetamine is known to substitute for other stimulants such as ephedrine, cocaine, methylphenidate, and d-amphetamine (Schechter, 1997b; Bondareva *et al.*, 2002; Sevak *et al.*, 2009). In sum, a methamphetamine drug discrimination assay can determine whether the interoceptive stimuli produced by methamphetamine administration are attenuated when a potential pharmacotherapy is administered, and by inference, whether the subjective effects of methamphetamine and the control they exert on behavior is weakened.

Rationale

Locomotor activity and its sensitization, self-administration, and drug discrimination each are related to different aspects of drug abuse-like behavior as modeled in rodents. Thus, utilizing all three of these assays in combination to assess the effects of the glial cell modulators would help identify their effectiveness in attenuating different aspects of methamphetamine abuse. First, examining effects on locomotor activity in mice allows for a measure of drug effects on methamphetamine-induced acute hyperactivity and the development of sensitization, which potentially tracks the effects of methamphetamine-induced synaptic plasticity as well as the likelihood of relapse. Comparing ibudilast's effects to those of AV1013 on methamphetamine-induced locomotor activity also helps identify whether glial cell modulation alone, without inhibition of PDE, is sufficient to affect this behavior and its sensitization.

Second, assessing ibudilast, AV1013, and minocycline's effects on self-administration behavior in rats determines whether PDE inhibition and/or glial cell modulation can attenuate on-going drug-taking behavior and again allows for a comparison between these drugs' effects. If the test compounds did effect on-going methamphetamine self-administration, identification of the behavioral determinants and range of conditions for observing these effects would be important. Two potential variables that may influence the effects of test compounds on drug self-

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administration are the response rate and strength of the drug-maintained baselines. Controlling for potential response rate determinants of ibudilast's effects is crucial to ensure that the drug's effects are not exclusively rate dependent. Behavioral economics can be used to help in the evaluation of the non-specific effects of the test compounds, such as testing for reductions in responding maintained by alternate reinforcers (i.e. food pellets) when baseline strength is equated.

Finally, drug discrimination is thought to model the subjective effects of a drug. Thus, evaluating a compound's effects on methamphetamine's discriminative stimulus effects would help identify the importance of its mechanism(s) as involved in occasioning episodes of drug taking in human abusers. In summary, examining the test compounds ibudilast, AV1013, and minocycline in these behavioral assays, accompanied by control procedures, clarify their ability to attenuate different components of methamphetamine abuse-related behaviors, as well as the underlying mechanisms of those behaviors.

Hypothesis

Previous literature suggests that inhibiting PDE function and modulating glial cell activity can alter drug abuse-related behavior (Iyo *et al.*, 1995; Yan *et al.*, 2006; Miguel-Hidalgo, 2009; Crews *et al.*, 2011). Ibudilast, AV1013 and minocycline all modulate glial cell activation and reduce inflammatory processes (Kishi *et al.*, 2001; Mizuno *et al.*, 2004; Cho *et al.*, 2010; Tai *et al.*, 2013). Furthermore ibudilast and minocycline have been reported to attenuate some drug-induced behavioral effects including those of methamphetamine (Zhang *et al.*, 2006; Hutchinson *et al.*, 2009a; Beardsley *et al.*, 2010; Agrawal *et al.*, 2011; Schwarz *et al.*, 2011; Fujita *et al.*, 2012). Thus, the present study hypothesizes that ibudilast, AV1013 and minocycline will

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attenuate the locomotor activity and sensitization, self-administration, and the discriminative stimulus effects of methamphetamine.

Research Approach

1) Determine ibudilast and AV1013's effects on the locomotor activity and its sensitization induced by methamphetamine in mice

2) Evaluate ibudilast, AV1013, and minocycline's ability to modulate methamphetamine self-administration in rats
    a. Control for rate-dependent effects
    b. Control for methamphetamine-specific effects using a behavioral economic approach

3) Determine if the discriminative stimulus effects of methamphetamine are modulated by ibudilast

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Introduction

This purpose of the following study was to examine the ability of ibudilast to attenuate the acute and chronic effects of methamphetamine-induced hyperactivity and sensitization in mice. Additionally, the amino analog of ibudilast, AV1013, which retains ibudilast's ability to inhibit glial cell activation but has minimal PDE inhibitory effects (Cho et al., 2010), was also tested to determine whether PDE inhibition was essential for the initial effects observed with ibudilast.

Methods

Subjects

Male adult C57BL/6J mice were obtained at approximately 8 weeks of age (The Jackson Laboratory, Bar Harbor, ME) and were allowed to acclimate to the vivarium for approximately one week prior to commencement of testing. The mice were housed at a maximum of four per

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[1] Some content of Chapter II is adapted from *Euro. J. Pharmacol,* 2012, 679: 75-80

cage in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited animal facility with food (7012 Teklad LM-485 Mouse/Rat Sterilizable Diet, Harlan Laboratories, Inc., Indianapolis, IN) and water available ad libitum under a 12-h/12-h light/dark cycle (lights illuminated from 0600-h to 1800-h) with all testing occurring during the light phase. All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996) and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Apparatus

Locomotor activity tests were conducted in eight commercially obtained, automated activity monitoring devices each enclosed in sound- and light-attenuating chambers that recorded distance traveled in cm in 10-min bins via computer-controlled circuitry (AccuScan Instruments, Columbus OH). The interior of each device was divided into separate 20 × 20 × 30 cm arenas permitting the independent and simultaneous measurement of two mice. Sixteen photobeam sensors per axis were spaced 2.5 cm apart along the walls of the chamber and were used to detect movement.

Locomotor activity procedure

One hundred and twenty-eight mice were randomly assigned into 16 groups of eight mice each. Eight groups were treated b.i.d. for 7 days with subcutaneous (s.c.) injections of either 0 (vehicle; VEH1), 1.8, 7.5, or 13 mg/kg ibudilast, with two groups of eight at each dose. Eight other groups were similarly treated but with 0 (vehicle; VEH2), 10, 30, or 56 mg/kg AV1013. Both ibudilast and AV1013 injections occurred twice daily separated approximately 7 hours apart (0900-h and 1600-h). During the last five days of these seven-day regimens (Days

3–7), the mice were given locomotor activity tests. Two, 1-h locomotor activity sessions (Baseline and Test) were given on Days 1 and 5. Single locomotor activity sessions were given on Days 2–4 to minimize the occurrence of extinction of any conditioned locomotor activity effects in methamphetamine treated mice. On days when locomotor activity sessions were administered (Days 3–7), morning ibudilast and AV1013 injections were given 1 h prior to the first session. Immediately prior to Baseline and Test sessions on Days 1 and 5, all mice were injected intraperitoneally (i.p.) with saline or 3 mg/kg methamphetamine, respectively. On Days 2–4, half of all mice in the ibudilast and AV1013 groups received 3 mg/kg i.p. methamphetamine (METH) before all locomotor activity sessions (IBUD + METH and AV1013 + METH groups), while the other half received saline injections (IBUD + SAL and AV1013 + SAL groups). Thus, the mice were distributed across groups as shown in Table 2 and treated as shown in Table 3.

Drugs

(±)-Methamphetamine (National Institute on Drug Abuse, Rockville, MD) was prepared in 0.9% saline stock solutions sterilized by filtration through 0.2 µm filtration disks. Working methamphetamine solutions were dissolved in sterile 0.9% saline and injected i.p. Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine) and AV1013 ((R)-2-amino-1-(2-isopropylpyrazolo[1,5-a]pyridin-3-yl)propan-1-one hydrochloride) were received as a gift from MediciNova, Inc., San Diego, CA). Ibudilast was prepared in 35% polyethylene glycol (PEG) in saline vehicle and administered s.c. (referred to below as "VEH1"). Doses of AV1013 were administered s.c. and prepared in sterile 0.9% saline (referred to below as "VEH2"), with the exception of the highest dose (56 mg/kg) that was solubilized in a 35% PEG in saline vehicle (i.e., VEH1) because of its incomplete dissolution in 0.9% saline. All injections were given in a volume equivalent to 10 ml/kg body weight.

Data analysis

Distance traveled (cm) was subjected to analysis by a mixed-model ANOVA (repeated measures on Testday test and between comparisons on drug condition) for the chronically administered methamphetamine and vehicle groups separately for each drug (i.e., 2 drugs × 2 methamphetamine treatment conditions = 4 ANOVAs). Comparisons between ibudilast or AV1013-treated mice to their respective vehicle condition were made using Bonferroni Multiple Comparisons Tests. AD50 (CI) values for attenuating methamphetamine hyperactivity by 50% relative to vehicle controls were estimated by first converting distance traveled scores for each mouse to percent of its respective mean vehicle control, logarithmically transforming dose, and using nonlinear regression assuming a normalized response. All statistical tests were conducted using computer software (Prism 5d for Macintosh, GraphPad Software, Inc., San Diego, CA), and all types of comparisons were considered statistically significant if $P < 0.05$.

<u>Results</u>

Ibudilast and chronic methamphetamine

Fig. 1 shows the effects of ibudilast on chronic methamphetamine administration (upper frame) and on chronic vehicle administration (lower frame). ANOVA results indicated that drug [$F_{(3, 28)} = 7.093$; $p = 0.0011$] and time [$F_{(6,168)} = 56.64$; $P < 0.0001$] and their interaction [$F_{(18,168)} = 2.479$; $p = 0.0013$] significantly affected activity. Methamphetamine induced a significant increase in total distance traveled of over 2900 cm during the Testday 1 test relative to Baseline 1 levels in the VEH1 + METH group ($t = 3.735$, $df = 7$, $P < 0.05$) (Fig. 1, upper frame; significance not indicated by asterisks). Methamphetamine also induced increases in distance traveled during the Testday 1 test from Baseline 1 levels in the IBUD + METH treated

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groups, but their levels were non-significantly different, and increases were less than those of the VEH1 + METH group. Distance traveled progressively increased in the VEH1 + METH group following each subsequent day of methamphetamine administration and was significantly ($t = 4.325$, df = 7, $P < 0.01$) greater during the Testday 5 test relative to the Testday 1 test indicative of sensitization. Distance traveled on Testday 5 was significantly greater relative to Testday 1 in the 1.8 IBUD + METH ($t = 6.316$, df = 7, $P < 0.0001$) and 7.5 IBUD + METH ($t = 5.2000$, df = 7, $P < 0.0001$) groups, but not the 13 IBUD + METH group, indicating that 13 mg/kg ibudilast blocked the induction of sensitization. Ibudilast reduced distance traveled during all test sessions following methamphetamine administration relative to the VEH1 + METH treatment group, and significantly so during Testday 2–5 tests at 13 mg/kg ibudilast and during Testday 3 and 4 tests at 7.5 mg/kg ibudilast.

Ibudilast and acute methamphetamine

Distance traveled did not differ between the VEH1 + SAL group and any of the ibudilast groups following saline administration indicating that ibudilast did not affect locomotor behavior in mice without methamphetamine histories (Fig. 1, lower frame). However, ibudilast significantly reduced distance traveled following methamphetamine administration during the Testday 5 test, relative to the VEH1 + SAL group, at all doses of ibudilast (1.8 mg/kg ibudilast: $t = 3.278$, df = 7, $P < 0.05$; 7.5 mg/kg ibudilast: $t = 6.944$, df = 7, $P < 0.0001$; 13 mg/kg ibudilast: $t = 6.374$, df = 7, $P < 0.0001$) indicating its ability to blunt the acute challenge by methamphetamine.

AV1013 and chronic methamphetamine

Fig. 2 shows the effects of AV1013 on chronic methamphetamine administration (upper frame) and on chronic vehicle administration (lower frame). ANOVA indicated a significant effect

of drug [$F_{(4, 34)} = 5.947$; $p = 0.0010$], time [$F_{(6,204)} = 92.28$; $P < 0.0001$, and their interaction [$F_{(24,204)} = 2.282$; $p = 0.0010$]. Methamphetamine induced a significant increase in total distance traveled during the Testday 1 test relative to Baseline 1 levels in the VEH1 + METH group ($t = 4.341$, $df = 7$, $P < 0.001$). Methamphetamine induced a non-significant mean increase in total distance traveled in the VEH2 + METH group during the Testday 1 test relative to Baseline 1 levels, which further increased to significant levels during Testday 2 tests ($t = 4.530$, $df = 7$, $P < 0.001$) (Fig. 2, upper frame). Methamphetamine induced increases in total distance traveled by all AV1013 treatment groups during the Testday 1 test relative to Baseline 1 levels; however, these were non-significant increases and were always less than respective control vehicle groups. Total distance traveled generally increased in all groups following each subsequent day of methamphetamine administration and were significantly greater during the Testday 5 test relative to the Testday 1 test in all chronically-treated methamphetamine groups (VEH2 + METH: $t = 4.181$, $df = 7$, $P < 0.001$; VEH1 + METH: $t = 5.027$, $df = 7$, $P < 0.001$, 10 AV1013 + METH: $t = 5.618$, $df = 7$, $P < 0.001$; 30 AV1013 + METH: $t = 8.095$, $df = 7$, $P < 0.001$, 56 AV1013 + METH: $t = 4.154$, $df = 7$, $P < 0.001$). Total distance traveled was significantly reduced following 56 mg/kg AV1013 administrations relative to its vehicle control group (VEH1 + METH) following methamphetamine treatment on all days. (Testday 1: $t = 3.357$, $df = 7$, $P < 0.01$; Testday 2: $t = 3.681$, $df = 7$, $P < 0.01$; Testday 3: $t = 5.089$, $df = 7$, $P < 0.001$; Testday 4: $t = 5.434$, $df = 7$, $P < 0.001$; Testday5: $t = 4.009$, $df = 7$, $P < 0.01$).

AV1013 and acute methamphetamine

Total distance traveled did not differ between the VEH2 + SAL group and either the 10 AV1013 + SAL or 30 AV1013 + SAL groups during all test sessions that were preceded by saline administration, indicating that AV1013 did not affect locomotor behavior on its own (Fig.

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2, lower frame). Similarly, distance traveled did not differ between the VEH1 + SAL and 56 AV1013 + SAL group indicating that 56 mg/kg AV1013 did not affect locomotor behavior in mice without a methamphetamine history. However, following methamphetamine challenge during Testday 5 tests, 30 mg/kg AV1013 significantly reduced levels of total distance traveled relative to its vehicle control group, VEH2 + SAL ($t = 4.683$, df = 7, $P < 0.001$), as did 56 mg/kg AV1013 relative to its vehicle control group, VEH1 + SAL ($t = 4.900$, df = 7, $P < 0.001$).

Ibudilast vs. AV1013

The AD50 (CI) for ibudilast to reduce the hyperactivity effects of acute methamphetamine challenge on Testday 1 was 7.146 (3.763–13.57) mg/kg for groups chronically-treated with methamphetamine. By Testday 5 the AD50 (CI) increased to 23.23 (9.660–55.86) mg/kg in these groups. In groups whose first exposure to methamphetamine was on Testday 5, but which had received chronic ibudilast up to Testday 5, the AD50 (CI) was 7.092 (3.420–14.71) mg/kg. This AD50 value was non-significantly different from that on Testday 1 in the chronically treated methamphetamine group (i.e., vs. 7.146 mg/kg).

AV1013 attenuated methamphetamine's effects with an AD50 (CI) of 43.88 (19.40–99.27) mg/kg on Testday 1 that increased to 201.2 (51.49–786.0) mg/kg on Testday 5 in the chronically treated methamphetamine groups. In groups whose first exposure to methamphetamine was on Testday 5 but which had received chronic AV1013 up to Testday 5 the AD50 (CI) was 48.13 (19.05–121.7) mg/kg. This AD50 value was non-significantly different from that on Testday 1 in the chronically treated methamphetamine group (i.e., vs. 43.88 mg/kg).

When compared to each other, ibudilast produced significantly lower AD50 values than AV1013 on both Testday 1 [$F(1,61) = 11.32$; $p = 0.0013$] and on Testday 5 [$F(1,61) = 6.978$; $p =$

0.0105] in groups chronically-treated with methamphetamine, as well when comparing groups chronically-treated with saline and challenged for the first time on Testday 5 [$F_{(1,62)} = 10.90$; p = 0.0016].

Summary

Ibudilast dose-dependently reduced both chronically and acutely administered methamphetamine-induced locomotor activity. Chronic treatment with methamphetamine provided evidence of sensitization as subsequent administrations elicited greater increases in distance traveled. The highest dose of ibudilast (13 mg/kg) tested significantly attenuated these methamphetamine-induced sensitization effects. Ibudilast's analog, AV1013, which lacks its potency for inhibiting PDE, but retains its ability to suppress activated glial activity, similarly dose-dependently attenuated methamphetamine's chronic and acute locomotor activity effects, but was ~ 6–9 fold less potent in doing so. These later observations suggest that the ability to modulate glial activity is sufficient to attenuate methamphetamine's locomotor activity effects, although PDE inhibition likely can additionally contribute if present.

Table 2. Distribution of mice in chronically and acutely treated methamphetamine (METH) groups.

Chronic METH	Acute METH
Ibudilast Groups	
VEH1+METH	VEH1+SAL
1.8 IBUD+METH	1.8 IBUD+SAL
7.5 IBUD+METH	7.5 IBUD+SAL
13 IBUD+METH	13 IBUD+SAL
AV1013 Groups	
VEH2+METH	VEH2+SAL
10 AV1013+METH	10 AV1013+SAL
30 AV1013+METH	30 AV1013+SAL
56 AV1013+METH	56 AV1013+SAL

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Table 3. Treatment procedures

	Day 1	Day 2	Day 3	Day 4	Day 5
[1]**Chronic METH groups**					
Injection #1	Saline				Saline
Session	*Baseline 1*				*Baseline 5*
Injection #2	METH	METH	METH	METH	METH
Session	*Test 1*	*Test 2*	*Test 3*	*Test 4*	*Test 5*
[2]**Acute METH groups**					
Injection #1	Saline				Saline
Session	*Baseline 1*				*Baseline 5*
Injection #2	Saline	Saline	Saline	Saline	METH
Session	*Test 1*	*Test 2*	*Test 3*	*Test 4*	*Test 5*

[1] Groups include: IBUD+METH, AV1013+METH, VEH1+METH, VEH2+METH
[2] Groups include: IBUD+SAL, AV1013+SAL, VEH1+SAL, VEH2+SAL

Chapter II: Figure Legends

Fig. 1. <u>Upper frame:</u> Results on distance traveled (cm) by mice treated b.i.d. for seven days with ibudilast (IBUD) or its vehicle (VEH1), beginning two days before five days of treatment with 3 mg/kg methamphetamine. Ibudilast was administered at 1.8, 7.5, or 13 mg/kg. Data points represent group means (± S.E.M.) obtained during 1-h experimental sessions. Filled data points represent sessions preceded by 3 mg/kg i.p. methamphetamine injections. Unfilled data points represent sessions preceded by i.p. saline injections. N = 8 for each treatment group. *P < 0.05 with respect to mice treated with ibudilast's vehicle. <u>Lower frame</u>: Results on distance traveled (cm) by mice treated b.i.d. for seven days with ibudilast (IBUD) or its vehicle (VEH1), beginning two days before four days of saline administration and acute treatment with 3 mg/kg methamphetamine on the fifth day. Ibudilast was administered at 1.8, 7.5, or 13 mg/kg. Data points represent group means (± S.E.M.) obtained during 1-h experimental sessions. Other details are as in the upper frame.

Fig. 2. <u>Upper frame</u>: Results on distance traveled (cm) by mice treated b.i.d. for seven days with AV1013 or its vehicle (VEH2 for 10 and 30 mg/kg and VEH1 for 56 mg/kg), beginning two days before five days of treatment with 3 mg/kg methamphetamine. AV1013 was administered at 10, 30, or 56 mg/kg. *P < 0.05 with respect to mice treated with AV1013's vehicle. Other details are as in Fig. 1. <u>Lower frame</u>: Results on distance traveled (cm) by mice treated b.i.d. for seven days with AV1013 or its vehicle (VEH2 for 10 and 30 mg/kg and VEH1 for 56 mg/kg), beginning two days before four days of saline administration and acute treatment with 3 mg/kg methamphetamine on the fifth day. Other details are as in the upper frame.

Figure 1.

Figure 2.

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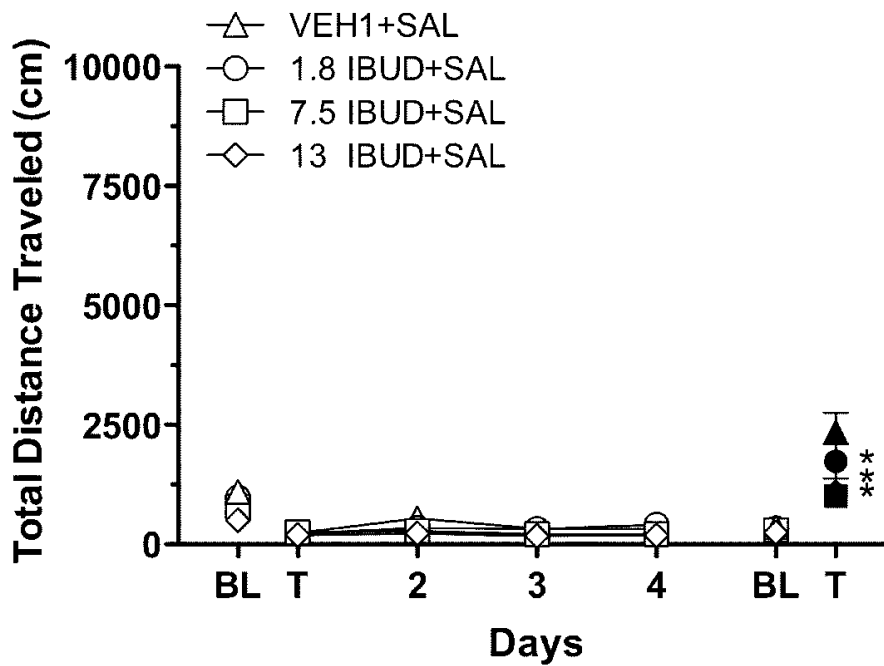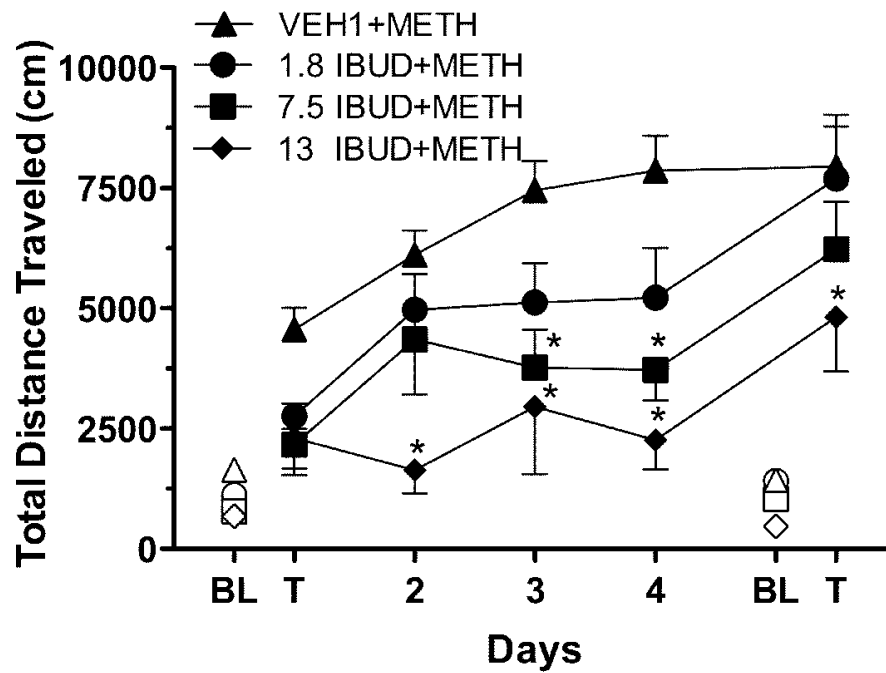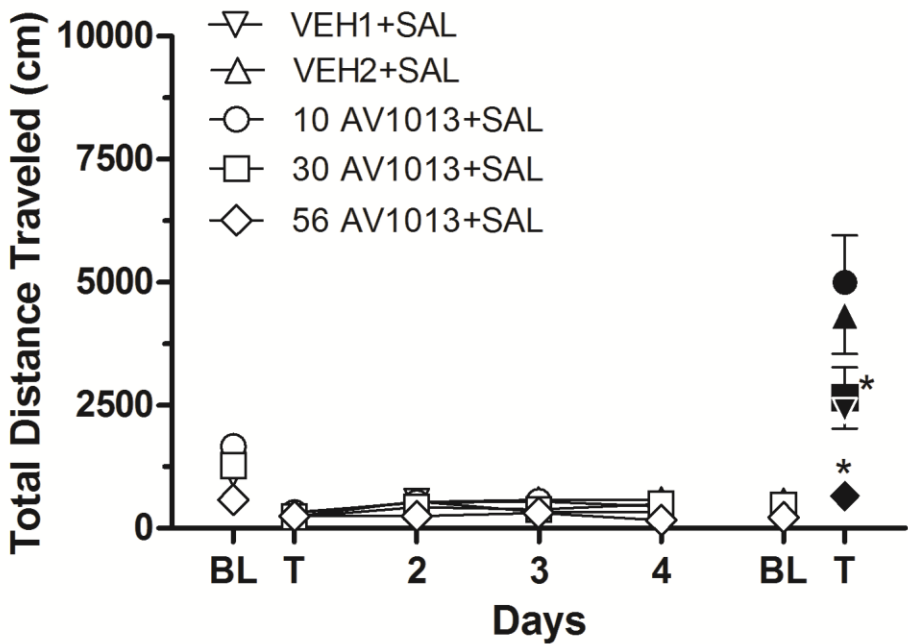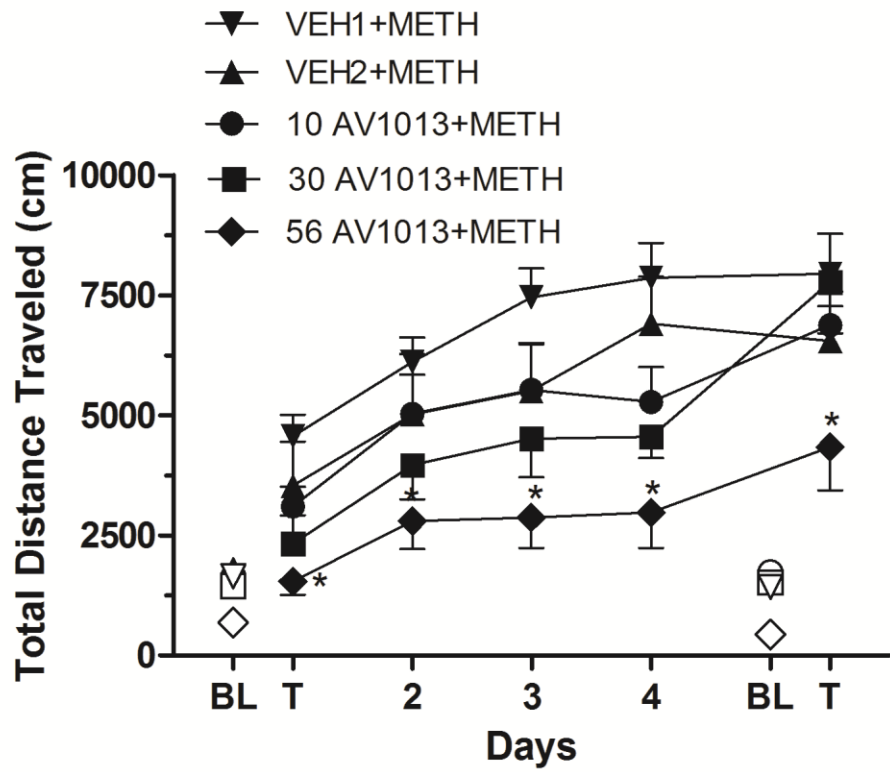
Chapter III: Glial cell modulators attenuate methamphetamine self-administration in the rat [2]

Introduction

The purpose of this study was to ascertain the effects of ibudilast, AV1013, and minocycline on on-going self-administration of methamphetamine in rats. It was previously reported that ibudilast attenuates stress- and prime-induced methamphetamine reinstatement in rats (Beardsley et al 2010), and the previously described locomotor activity study suggested that glial cell modulation and anti-inflammatory action is sufficient to attenuate methamphetamine locomotor activity in mice. Thus, it was hypothesized that ibudilast, AV1013, and minocycline, another compound that attenuates microglial activation, would attenuate on-going methamphetamine self-administration.

Methods

Subjects

Adult male Long-Evans hooded rats (Harlan, Indianapolis, IN) weighing 275-300 g at the start of studies were acclimated to the vivarium for at least one week prior to catheter implantation. When not in testing, rats were individually housed in standard plastic rodent cages in a temperature-controlled (22° C), AAALAC International-accredited facility in which they had *ad libitum* access to water. The rats were allowed *ad libitum* rat chow (7012 Teklad LM-485 Mouse/Rat Sterilizable Diet, Harlan Laboratories, Inc., Indianapolis, IN) for at least one week prior to commencement of training, after which they were maintained at 320 g by controlled feedings given after experimental sessions or at a comparable time of day if not tested. The rats were maintained on a reversed, 12 h/12 h light-dark cycle (0600-1800 h lights off) for the duration of the experiment, and they were trained and tested during the dark segment of this cycle.

All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996) and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Infusion assembly system

Catheters were constructed from polyurethane tubing (Access Technologies, Skokie, IL; 0.044" O.D. X 0.025" I.D.). The proximal 3.2 cm of the catheter was tapered by stretching following immersion in hot sesame oil. The catheters were prepared with a retaining cuff approximately 3 cm from the proximal end of the catheter. A second larger retaining cuff was positioned approximately 3.4 cm from the proximal end of the catheter. Mid-scapula cannula

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connectors were obtained from Plastics One (Roanoke, VA). The cannula connectors consisted of a threaded plastic post through which passed an "L" shaped section of 22 gauge stainless steel needle tubing. The lower surface of the plastic post was affixed to a 2 cm diameter disc of Dacron mesh. During sessions the exposed threaded portion of the infusion cannula was connected to an infusion tether consisting of a 35 cm length of 0.40 mm i.d. polypropylene tubing encased within a 30 cm stainless steel spring to prevent damage. The upper portion of the 0.40 polypropylene tubing was connected to a fluid swivel (Lomir Biomedical, Inc, Quebec, Canada) that was, in turn, attached via 0.40 polypropylene tubing to the infusion syringe.

Surgical procedure

Following acclimation to the laboratory environment, indwelling venous catheters were implanted into the right external jugular vein. Rats were administered 5 mg/kg carprofen s.c. (Rimadyl, Pfizer Animal Health, New York, NY) before surgery. Surgical anesthesia was induced with a combination of 50 mg/kg ketamine (KetaThesia, Butler Animal Health Supply, Dublin, OH) and 8.7 mg/kg xylazine (X-Ject E, Butler Animal Health Supply, Dublin, OH). The ventral neck area and back of the rat were shaved and wiped with povidone-iodine, 7.5% (Betadine, Purdue Products L.P., Stamford, CT) and isopropyl alcohol. The rat was placed ventral side down on the surgical table and a 3 cm incision was made 1 cm lateral from mid-scapula. A second 0.5 cm incision was then made mid-scapula. The rat was then placed dorsal side down on the operating table and a 2.5 cm incision was made longitudinally through the skin above the jugular area. The underlying fascia was bluntly dissected and the right external jugular vein isolated and ligated. A small cut was made into the vein using an iris scissors and the catheter was introduced into the vein and inserted up to the level of the larger retaining cuff. The vein encircling the catheter between the two cuffs was then tied with silk suture. A second

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suture was then used to anchor the catheter to surrounding fascia. The distal end of the catheter was passed subcutaneously and attached to the cannula connector that was then inserted subcutaneously through the larger incision. The upper post portion of the cannula connector exited through the smaller mid-scapula incision. All incisions were then sprayed with a gentamicin sulfate/betamethasone valerate topical antibiotic (Betagen, Med-Pharmex, Inc., Pomona, CA) and the incisions were closed with Michel wound clips. Five mg/kg oral carprofen (Rimadyl, Bio-Serv, Frenchtown, NJ) was administered 24 h after surgery, and 8 mg/kg oral enrofloxacin (Baytril, Bio-Serv, Frenchtown, NJ) was administered daily for three days following surgery.

Rats were allowed to recover from surgery for at least 5 days before self-administration training began. Periodically throughout training, ketamine (5 mg/kg) (KetaThesia, Butler Animal Health Supply, Dublin, OH) was infused through the catheters to determine patency as inferred when immediate anesthesia was induced. Between sessions the catheters were flushed and filled with 0.1 ml of a 25% glycerol (Acros, New Jersey)/75% sterile saline locking solution containing: 250 units/ml heparin (Abraxis Pharmaceutical Products, Schaumburg, IL) and 250 mg/ml ticarcillin/9 mg/ml clavulanic acid (Timentin, GlaxoSmithKline, Research Triangle Park, NC). If during the experiment a catheter was determined to be in-patent, the left external jugular was then catheterized and the rat was returned to testing.

Apparatus

Commercially-obtained test chambers equipped with two retractable levers, a 5-w house light, and a Sonalert® tone generator (MED Associates, Inc., St. Albans, VT) were used. Positioned above each lever was a white cue light. A syringe pump (Model PHS-100; MED Associates, Inc., St. Albans, VT) when activated, delivered a 6-sec, 0.2 ml infusion. Recording

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of lever presses and activation of lights, shockers, pumps, and Sonalerts were accomplished by a microcomputer, interface, and associated software (MED-PC® IV, MED Associates, Inc., St. Albans, VT).

Self-administration procedure

Methamphetamine self-administration training sessions were conducted seven days per week for 2 h daily. Each response (fixed ratio 1, FR1) on the right-side lever resulted in delivery of a 0.1 mg/kg methamphetamine infusion (0.2 ml/6 sec) followed by a 14-s timeout period. At the start of an infusion the house light was extinguished, the Sonalert® was sounded, and the cue lights above each lever flashed at 3 Hz. The Sonalert® and cue lights remained activated during the 6-s infusion. Twenty seconds following the onset of the infusion the house light was re-illuminated, and the opportunity to self-administer methamphetamine was again made available (i.e., each methamphetamine infusion initiated a 20 s period during which lever presses were recorded but were without scheduled consequences and further infusions could not be obtained). Active (right-side) lever presses during the infusions as well as all inactive (left-side) lever presses were recorded but were without scheduled consequences.

Training sessions occurred until stability criteria were met. Stability criteria were defined in which during the first and last session of at least 3 consecutive sessions neither the highest nor lowest number of infusions were obtained, and the number of infusions during each session was ±20% from the mean. Following training, ibudilast (1, 7.5 and 10 mg/kg) or AV1013 (1, 10 and 30 mg/kg) was administered i.p. twice daily, or minocycline (10, 30 and 60 mg/kg) was administered i.p. once daily, or their corresponding vehicles were administered for three consecutive days during self-administration of each of three doses of methamphetamine (0.1, 0.03, and 0.001 mg/kg/inf). Thus, a total of nine consecutive days of b.i.d. or once daily dosing

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of vehicle or dose of test drug was necessary to complete testing at each self-administered dose of methamphetamine. Between tests of vehicle or dose of test drug, rats were maintained under 0.1 mg/kg methamphetamine self-administration conditions with i.p. injections of the test drug's vehicle until training criteria were once again met.

Rate Dependency Analysis

Preliminary results had indicated that ibudilast reduced response rates maintained by 0.03 mg/kg/inf but not by 0.1 mg/kg/inf methamphetamine. In order to determine if the higher baseline response rates maintained by 0.03 mg/kg/inf methamphetamine were the sole determinants for the greater ibudilast-induced response rate reductions, relative to those maintained at 0.1 mg/kg/inf methamphetamine, response rates were matched across the two methamphetamine doses. Fixed-ratio requirements reinforced by 0.1 mg/kg/inf methamphetamine were increased to increase response rates, and ibudilast was then re-tested at its most influential dose of 10 mg/kg. Rats were trained to self-administer 0.1 mg/kg/inf methamphetamine reinforced according to a FR1 schedule, and they were required to complete the previously described stability criteria before proceeding. The fixed ratio requirement was then adjusted to between FR2- FR4 in individual rats in order to increase response rates reinforced by 0.1 mg/kg/inf methamphetamine to approximate, as a group mean response rate, those maintained by 0.03 mg/kg/inf methamphetamine. Once the response rates were stably matched between 0.03 and 0.1 mg/kg/inf methamphetamine groups, ibudilast (10 mg/kg) or its vehicle was then administered b.i.d. i.p. for three consecutive days. Between three-day sets of testing, animals were returned to training conditions and were required to meet stability criteria once again.

Drugs

(±)-Methamphetamine hydrochloride (National Institute on Drug Abuse, Rockville, MD) was prepared in sterile 0.9% saline. Methamphetamine stock solutions were sterilized by filtration through 0.2 µm filtration disks. Heparin (5 units/ml) was additionally added to methamphetamine and saline infusates. Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine) and AV1013 ((R)-2-amino-1-(2-isopropylpyrazolo[1,5-a]pyridin-3-yl)propan-1-one hydrochloride) were received as a gift from MediciNova, Inc. (San Diego, CA) and were dissolved in a 35% PEG400, 10% Cremophor® RH40 (BASF, Ludwigshafen, Germany) aqueous vehicle. Minocycline hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in saline and a few drops of 1 M hydrochloric acid. Immediately prior administration, the minocycline solution was adjusted to pH 3-4 using a few drops of sodium hydroxide. Ibudilast, AV1013, and minocycline were all administered i.p. in 1 ml/kg body weight volume, except for 60 mg/kg minocycline that was given at 2 ml/kg body weight volume due to insolubility at the lower volume.

Data Analysis

The number of infusions obtained on the third (and final) day of testing at each condition was used for data analysis because it was assumed it would most likely represent terminal behavior. Numbers of infusions comparing methamphetamine dose to saline under vehicle-treatment conditions were made with Dunnett's Multiple Comparison posttests following a one-way within-subjects ANOVA to determine if a dose of methamphetamine served as a reinforcer. Additionally, infusion numbers were analyzed using a two-way repeated measures ANOVA (repeated measures on treatment dose and between comparisons on methamphetamine dose), and comparisons of ibudilast, AV1013, or minocycline treatment on methamphetamine self-

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administration were assessed using Bonferroni Multiple Comparisons tests. AD50 (CI) values for attenuating methamphetamine self-administration by 50% relative to vehicle control conditions were estimated by first converting total infusions obtained to percent of their respective vehicle control infusions, logarithmically transforming dose, and using nonlinear regression assuming a normalized response.

For Rate Dependency Analysis, "response rate" was defined as the: (number of presses of the right-side lever - those occurring during time-out periods) ÷ (total session duration in sec - cumulative duration of all time-out periods). Response rates maintained by 0.03 mg/kg/inf methamphetamine at FR1 were considered matched to response rates for 0.1 mg/kg/inf methamphetamine at FR2-4 when there were no significant differences between group mean rates when compared by an unpaired t-test. During 10 mg/kg ibudilast and vehicle treatment, response rates at 0.03 mg/kg/inf methamphetamine administration were compared to those at 0.1 mg/kg/inf methamphetamine using a two-way analysis of variance (ANOVA) with repeated measures on ibudilast "dose" (i.e., 10 mg/kg or vehicle) followed by Sidak's Multiple Comparisons tests.

All statistical tests were conducted using commercial computer software (Prism 5d for Macintosh, GraphPad Software, Inc., San Diego, CA), and all types of comparisons were considered statistically significant if $p < 0.05$.

Results

Figure 3 illustrates the effects of ibudilast on methamphetamine self-administration. Under b.i.d. vehicle conditions, methamphetamine was self-administered characterized by an inverted U-shaped curve relating infusion numbers to dose, and the one-way within-subjects ANOVA on infusion numbers was significant [$F_{(3, 15)} = 32.75$; $p < 0.001$]. Dunnett's posttests

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revealed that infusions of 0.03 and 0.1 mg/kg/inf methamphetamine were self-administered significantly greater than those of saline (p<0.05) indicating that these doses were serving as positive reinforcers under baseline conditions.

There was a significant effect of methamphetamine dose [F (2, 27) =19.90; p= 0.0005] and ibudilast dose [F (3, 27) =3.44; p=0.0308]. Ibudilast did not systematically affect the number of 0.001 mg/kg methamphetamine infusions, which had not served as a positive reinforcer under vehicle conditions, nor the number 0.1 mg/kg methamphetamine infusions, which did serve as a positive reinforcer. At its two highest doses (7.5 and 10 mg/kg), ibudilast reduced the number of 0.03 mg/kg methamphetamine infusions, the methamphetamine dose that had maintained the greatest number of infusions above those of saline under baseline conditions, and significantly so at the 10 mg/kg ibudilast dose (p<0.05).

Response Rate Dependency analysis indicated that group mean response rate maintained by 0.03 mg/kg/inf methamphetamine at FR1 was not significantly different from the group mean response rate maintained by 0.1 mg/kg/inf methamphetamine in the matched response rate group (FR2-4) (t=0.1591, df=6, p=0.8788)(Fig. 4). Although 10 mg/kg ibudilast reduced infusion levels of 0.03 mg/kg/inf methamphetamine relative to vehicle control (t=3.998, df=6, p<0.05), and as described above, infusion rates of 0.01 mg/kg/inf methamphetamine in the matched response rate group were unaffected (t=1.324, df=6, p>0.05).

Figure 5 shows AV1013's effects on methamphetamine self-administration. Under b.i.d. vehicle conditions, methamphetamine was self-administered characterized by an inverted U-shaped curve relating infusion numbers to dose. The one-way within subjects ANOVA on infusion numbers was significant [F (3, 19) =214.9; p<0.0001]. Dunnett's posttest results indicated that 0.03 and 0.1 mg/kg/inf methamphetamine doses were self-administered

significantly above those of saline (p<0.05) indicating that these doses were serving as positive reinforcers.

There was a significant effect of methamphetamine dose [F (2, 36) =62.59; p<0.0001] and AV1013 dose [F (3, 36) =10.59; p<0.0001]. As with ibudilast, AV1013 did not systematically affect the number of 0.001 mg/kg methamphetamine infusions, which had not served as a positive reinforcer under vehicle conditions, nor the number 0.1 mg/kg methamphetamine infusions, which had served as a positive reinforcer. AV1013 did, however, significantly reduce the number of 0.03 mg/kg methamphetamine infusions at the 10 and 30 mg/kg AV1013 doses (p<0.05).

Minocycline's effects on methamphetamine self-administration are shown in Figure 6. During daily dosing conditions with minocycline's vehicle (saline), methamphetamine self-administration was characterized by an inverted U-shaped curve relating infusion numbers to dose. The one-way ANOVA on infusion numbers was significant [F (3, 19) =34.07; p<0.0001]. Dunnett's posttests indicated that the 0.03 mg/kg/inf methamphetamine dose (p<0.05), but neither the 0.001 mg/kg/inf nor the 0.1 mg/kg/inf methamphetamine doses, were self-administered significantly above those of saline (although the level of 0.1 mg/kg/inf methamphetamine self-administration infusions was similar to those obtained under baseline conditions during tests with ibudilast and AV1013, self-administered saline infusions were greater during minocycline testing).

There was a significant effect of methamphetamine dose [F (2, 36) =23.09; p<0.0001] and minocycline dose [F (3, 36) =6.907; p=0.0009]. Minocycline reduced infusion numbers of 0.03 mg/kg/inf methamphetamine, significantly so at the 60 mg/kg dose of minocycline (p<0.05), while infusion numbers of other self-administered doses of methamphetamine were non-systematically affected.

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The potency (AD50 value) relationship amongst the drugs for reducing total infusions obtained during 0.03 mg/kg/inf methamphetamine self-administration differed [$F_{(2,53)}=7.909$; $p=0.001$], and resulted in ibudilast being the most potent, followed by AV1013, and then minocycline with respective AD50 (CI) values of 10.67 (3.86-29.47), 60.80 (23.26-158.9) and 128.8 (57.14-290.3) mg/kg.

Summary

Methamphetamine was established as a positive reinforcer and was self-administered under vehicle pretreatment conditions characterized by an inverted U-shaped curve relating infusion numbers to dose, with significantly more methamphetamine infusions being obtained at the intermediate dose (0.03 mg/kg/inf) during testing of all drugs, and at the highest dose (0.1 mg/kg/inf) during testing of ibudilast and AV1013, relative to those obtained of saline. Ibudilast (10 mg/kg), AV1013 (10 and 30 mg/kg) and minocycline (60 mg/kg) significantly reduced total 0.03 mg/kg/inf methamphetamine infusions compared to vehicle pretreatment conditions. These results suggest that modulating glial cell activity and consequent neuroinflammatory processes, can, in turn, modulate abuse-related effects of methamphetamine.

Controlling for methamphetamine-specific effects

The purpose of this control study was to assess whether the test compounds' effects were specific to attenuating methamphetamine-induced behaviors. Knowing the degree of specificity of the effects of these glial modulators would better profile the freedom with which they could be used therapeutically, without affecting other behaviors. Relaxed specificity suggests that non-target behaviors could also be affected. Incomplete specificity, however,

should not preclude a test compound as a possible therapeutic, just as a potential cancer chemotherapeutic shouldn't be automatically excluded from clinical use if it affects some non-cancerous cells in addition to cancerous cells. Ibudilast, AV1013, and minocycline all reduced methamphetamine self-administration, however will these compounds reduce responding for another reinforcer such as food? It was hypothesized that in order to properly determine a test compound's effects on food- and drug-maintained behavior, that both food and methamphetamine should first be matched in terms of their relative reinforcing strength, otherwise effects might be seen on a weak baseline but not on a stronger baseline regardless if food or drug was the maintaining event. In order to match food- and methamphetamine-maintained baselines, a behavioral economics approach was used to ensure similar demand curves obtained by both reinforcers before testing. One assumption of a behavioral economic approach is that demand is calculated when the commodity in question is only available during the testing session, that is, provided within a closed economy (Hursh, 1984). Hursh (1984) showed that demand for a commodity is more elastic if it is available at alternative times outside the testing procedure, and in an open economy, as compared to a closed economy. Thus, controlling for this variable was an important component to consider prior to testing with a food-like reinforcer given animals must be maintained at healthy body weights throughout the study. Thus, non-nutritive banana flavored cellulose pellets were the closest approximation to a food-like reinforcer while avoiding open economy confounds during testing. Ibudilast, AV1013, and minocycline's effects on methamphetamine and non-nutritive banana pellet-maintained responding and consumption were then assessed under the conditions in which both reinforcers were inferred to have equal reinforcing strength.

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Methods

<u>Subjects</u>

Adult male Long-Evans hooded rats (Harlan, Indianapolis, IN) with a history of methamphetamine self-administration were allowed to acclimate to the vivarium for at least one week before training began. When not in testing, rats were individually housed in standard plastic rodent cages in a temperature-controlled (22°C), AAALAC International-accredited facility in which they had ad libitum access to water. Rats were assigned to one of two groups, to assess the demand for self-administration of either methamphetamine (METH group) or 45 mg calorie-free (non-nutritive) banana flavored cellulose pellets (TestDiet) (BANANA group). METH rats were maintained at 320 g by controlled feedings of rat chow (7012 Teklad LM-485 Mouse/Rat Sterilizable Diet, Harlan Laboratories, Inc., Indianapolis, IN) after daily sessions. Before training, BANANA rats were allowed ad libitum rat chow for at least six days in order to calculate each individual's free feeding body weight to be used throughout the study. BANANA rats were then maintained at 85% of their own free feeding body weight for remainder of the study. All rats were maintained on a reversed, 12 h/12 h light-dark cycle (0600-1800 h lights off) for the duration of the experiment, and they were trained and tested during the dark segment of this cycle. All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996) and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

<u>Procedure</u>

Methamphetamine training

      Methamphetamine self-administration training sessions were conducted seven days per week for 2 h daily. Each response (fixed ratio 1, FR1) on the right-side lever resulted in delivery of a 0.1 mg/kg methamphetamine infusion (0.2 ml/6 sec) followed by a 14-s timeout period. At the start of an infusion the house light was extinguished, the Sonalert® was sounded, and the cue lights above each lever flashed at 3 Hz. The Sonalert® and cue lights remained activated during the 6-s infusion. Twenty seconds following the onset of the infusion the house light was re-illuminated, and the opportunity to self-administer methamphetamine was again made available (i.e., each methamphetamine infusion initiated a 20-s period during which lever presses were recorded but were without scheduled consequences and further infusions could not be obtained). Active (right-side) lever presses during the infusions as well as all inactive (left-side) lever presses were recorded but were without scheduled consequences. Training sessions occurred until stability criteria were met. Stability criteria were defined in which during the first and last session of 3 consecutive sessions neither the highest nor lowest number of infusions were obtained, and the number of infusions during each session was ±20% from the mean.

BANANA pellet Training

      Banana pellet training sessions occurred seven days a week for 15 min daily. Rats were trained to respond on a fixed ratio 10 (FR10) schedule in which 10 consecutive left-lever responses resulted in a banana pellet delivery. Non-nutritive banana flavored cellulose pellets were used to best approximate a closed economy, in which the "food-like" reinforcer is only available during the session and caloric value does not influence total consumption. During the

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session, the house light remained illuminated, and there were no scheduled light or tone stimuli presentations during food delivery. All inactive (right-side) lever presses were recorded but were without scheduled consequences. Because the subjects had a history of methamphetamine self-administration in which the right lever was active and upon infusion delivery both a stimulus light and tone were presented, the banana pellet protocol removed those stimuli and reversed the active lever position in order to avoid any confounding environmental conditioning. Following training, rats were required to meet stability criteria on a FR1 schedule for banana pellet delivery. Stability criteria were defined as the first and last session of three consecutive sessions had neither the highest nor lowest number of active lever responses, nor the number of responses during each session was more than ±20% from the mean. Additionally, rats did not always consume all of their earned banana pellets within the session so stability criteria were only met if <3 banana pellets remained following each of the three consecutive sessions.

Demand curve analyses

Following training, demand curve analyses were obtained for 0.1 and 0.03 mg/kg/inf methamphetamine and banana pellet self-administration. For each curve, the fixed ratio was increased daily in the following progression 1,3,6,9,13,19,26,35,47,62,82,108…using the formula (response ratio = [5e$^{(\text{injection number} \times j)}$] – 5, where j=0.26)) adapted from Richardson and Roberts (1996). Sessions continued until responding at a particular FR decreased to below that sufficient to earn a single reinforcer during the session. Rats were then returned to FR1, and were required to meet stability criteria before progressing to the alternate methamphetamine infusion dose or banana pellet administration.

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Methamphetamine-specific consumption effects analysis

BANANA group rats were maintained at 85% of their individual free feeding body weight and were required to meet stability criteria at FR1 for banana pellet administration. Once stability was reached, twice daily i.p. administration of ibudilast (10 mg/kg), AV1013 (10, 30 mg/kg) 1-h prior to testing or once daily i.p. administration of minocycline (10, 30, 60 mg/kg) 67-min prior to testing or their respective vehicles for three consecutive days under a FR1 schedule. Minocycline was administered 67-min prior to the test session to allow for similar pre-treatment, distribution, and absorption time to that of the self-administration tests. Between three-day sets of testing, rats were returned to FR1 training conditions and required to meet stability criteria.

Drugs

(±)-Methamphetamine hydrochloride (National Institute on Drug Abuse, Rockville, MD) was prepared in sterile 0.9% saline. Methamphetamine stock solutions were sterilized by filtration through 0.2 μm filtration disks. Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine) and AV1013 ((R)-2-amino-1-(2-isopropylpyrazolo[1,5-a]pyridin-3-yl)propan-1-one hydrochloride) were received as a gift from MediciNova, Inc. (San Diego, CA) and were dissolved in a 35% PEG400, 10% Cremophor® RH40 (BASF, Ludwigshafen, Germany) aqueous vehicle. Minocycline hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in saline and a few drops of 1 M hydrochloric acid. Immediately prior to administration, the minocycline solution was adjusted to pH 3-4 using a few drops of sodium hydroxide. Ibudilast, AV1013, and minocycline were all administered i.p. in 1 ml/kg body weight volume, except for 60 mg/kg minocycline that was given at 2 ml/kg body weight volume due to insolubility at the lower volume. Of note, two additional vehicles were tested during the minocycline banana pellet consumption assays. Veh30, a few drops of 1M hydrochloric acid added to sterile water to produce a vehicle with

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equivalent pH to 30 mg/kg minocycline, and Veh60 similar vehicle to Veh30 which was administered at 2 ml/kg injection volume to control for injection volume at 60 mg/kg minocycline administration, were tested. Neither vehicle produced significantly different total banana pellet consumption compared to sterile water vehicle, so data is not shown.

<u>Data Analysis</u>

Demand curves were generated using the formula $\log Q = \log Q_0 + k (e^{-\alpha P} - 1)$ from (Hursh & Silberberg, 2008). Non-linear regression best fit values for the alpha level parameter were compared between reinforcer types using an extra sum-of-squares F-test. When tested on a FR1 schedule, total consumption of METH (0.3 mg/kg/inf) or BANANA pellets was normalized by calculating the percentage of reinforcer consumption under test compound (ibudilast, AV1013 or minocycline) treatment compared to total consumption under vehicle conditions. Normalized consumption percentages were analyzed using a two-way repeated measures ANOVA (repeated measures on treatment dose and between comparisons on reinforcer type), and comparisons of ibudilast, AV1013, or minocycline treatment on consumption were assessed using Bonferroni Multiple Comparisons tests.

Results

The α levels (95% CI), the behavioral economic variable indicative of reinforcing strength of a particular commodity, for (0.03 and 0.1 mg/kg/inf) methamphetamine self-administration and banana pellet-maintained responding were calculated as 0.00012 (0.000075-0.00016), 0.000076 (0.000061-0.000090), 0.00013 (0.00011-0.00015) respectively. When the α levels of 0.3 and 0.1 mg/kg/inf methamphetamine were compared, the α level obtained under 0.1 mg/kg/inf methamphetamine conditions was significantly lower [$F_{(1, 101)} = 5.2$, p=0.0246] indicating that under these conditions, 0.1 mg/kg/inf methamphetamine had a stronger

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reinforcing strength. When 0.3 mg/kg/inf methamphetamine and banana pellets were compared, there was not a significant difference [F (1, 87) = 0.2, p=0.65] indicating that the baseline reinforcing strength of these two reinforcers, obtained under the specified conditions, was successfully matched (Fig. 7). Thus, all further methamphetamine demand analyses were performed using 0.03 mg/kg/inf methamphetamine.

There was a significant effect of ibudilast treatment on normalized consumption [F (1, 7) =30.26; p= 0.0009]. Bonferroni multiple comparison's indicated that 10 mg/kg ibudilast treatment significantly decreased normalized consumption of both 0.3 mg/kg methamphetamine self-administration (p<0.05) and banana pellets (p<0.05) compared to vehicle (Fig 8).

AV1013's treatment effect on normalized consumption of 0.03 mg/kg/inf methamphetamine self-administration and banana pellets was significant [F (2, 12) =12.63; p=0.0011]. Normalized consumption of 0.03 mg/kg/inf methamphetamine was significantly reduced by 10 and 30 mg/kg AV1013 compared to vehicle conditions (p<0.05). Banana pellet consumption, however, was only significantly decreased by 30 mg/kg AV1013 (p<0.05) (Fig. 9).

Figure 10 illustrates minocycline's effects on the normalized consumption of methamphetamine (0.03 mg/kg/inf) and banana pellet administration. There was a significant main effect of reinforcer type [F (1, 8) =7.741; p=0.0238] and minocycline treatment on consumption [F (3, 24) =12.76; p<0.0001]. Normalized consumption of 0.03 mg/kg/inf methamphetamine was significantly reduced by 60 mg/kg minocycline (p<0.05) compared to vehicle conditions. Bonferroni multiple comparison's also indicated that normalized consumption of banana pellets while the subjects were maintained at 85% of their free-feeding body weight was significantly decreased by 30 and 60 mg/kg minocycline (p<0.05).

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Summary

In order to support whether ibudilast, AV1013, and minocycline's effects on attenuating methamphetamine-induced behaviors are methamphetamine specific, the effects of the test compounds was measured on the total consumption of an alternative reinforcer, non-nutritive banana flavored cellulose pellets. Using behavioral economics, demand curves were generated for methamphetamine (0.03 mg/kg/inf) and banana flavored pellets when the subjects were maintained at 85% of their free-feeding body weight. Under those conditions, the reinforcing strength (α level) of the two different reinforcers was successfully matched. All three test compounds, ibudilast, AV1013, and minocycline, significantly attenuated total consumption of banana pellets at the same doses that attenuated methamphetamine self-administration with the exception of 10 mg/kg AV1013. This suggests that perhaps the intermediate dose of AV1013 (10 mg/kg) was specifically attenuating methamphetamine self-administration without affecting consumption of a non-nutritive food-like reinforcer.

Controlling for differential drug effects on differing reinforcing baselines

An assumption was made prior to initiating the previous studies involving the behavioral economic analysis of test drug effects on banana pellet and methamphetamine-maintained responding that baseline strength could be a determinant of whether a test compound affected responding or not. The assumption was that baselines maintained by very weak reinforcers would be affected before baselines maintained by stronger reinforcers. The following study attempted to provide a proof-of-concept to support this assumption. The present study examined food-maintained behavior while its baseline strength (α level) was manipulated by altering the subject's percentage of free feeding body weight. Test compounds were then

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evaluated to assess the extent to which baseline strength (i.e., efficacy of the food reinforcers) determined the magnitude of their effects.

Methods

Subjects

Adult male Long-Evans hooded rats (Harlan, Indianapolis, IN) with a history of methamphetamine self-administration were allowed to acclimate to the vivarium for at least one week before training began. When not in testing, rats were individually housed in standard plastic rodent cages in a temperature-controlled (22°C), AAALAC International-accredited facility in which they had *ad libitum* access to water. Before training, FOOD rats were allowed *ad libitum* rat chow for at least six days in order to determine each individual's free feeding body weight to be used throughout the study. FOOD rats were then maintained at a percentage of their own free feeding body weight for remainder of the study. All rats were maintained on a reversed, 12 h/12 h light-dark cycle (0600-1800 h lights off) for the duration of the experiment, and they were trained and tested during the dark phase of this cycle. All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996) and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Procedure

Food Pellet Training

Food pellet training was identical to banana pellet training as described above in which sessions occurred seven days a week for 15 min. Rats were trained to respond on a fixed ratio 10 (FR10) schedule in which 10 consecutive left-lever responses resulted in delivery of a 45 mg

rodent purified diet dustless precision pellet (BioServ, Frenchtown, NJ). During the session, the house light remained illuminated, and there were no scheduled light or tone stimuli presentations during food delivery. Because the subjects had a history of methamphetamine self-administration in which the right lever was active and upon infusion delivery both a stimulus light and tone were presented, the present protocol removed those stimuli and reversed the active lever position in order to avoid any confounding environmental conditioning. All inactive (right-side) lever presses were recorded but were without scheduled consequences. Following training, rats were required to meet stability criteria on a FR1 schedule for food pellet delivery. Stability criteria were defined as the first and last session of three consecutive sessions had neither the highest nor lowest number of active lever responses, nor the number of responses during each session was more than ±20% from the mean.

Demand Curve Analyses

The percent of *ad libitum* body weight was adjusted to vary the reinforcing efficacy (demand) of the food reinforcer. Rats were maintained at 85%, 90%, 100%, or 115% of their initial individual free feeding body weight by controlled feedings of rat chow following each daily session. Initial individual body weights were determined by averaging daily weights from six consecutive days of *ad libitum* feeding prior to initiation of weight maintenance and test sessions. Because Long-Evans hooded rats' growth and body weight continues to increase over time (Harlan Laboratories, 2006), maintaining the animals at 115% of their body weight was possible in later studies to introduce an even further reduction in the strength for food demand. Immediately following meeting criteria at FR1, the fixed ratio was increased daily in the following progression 1,3,6,9,13,19,26,35,47,62,82,108…using the formula (response ratio = $[5e^{(injection\ number\ x\ j)}] - 5$, where j=0.26)) adapted from Richardson and Roberts (1996). Sessions continued

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until responding at a particular FR decreased to below that sufficient to earn a single reinforcer during the session.

Drug Administration

Test compounds were administered acutely to determine their effects on food maintained responding under a FR10 schedule. A FR10 schedule was implemented in order to best maintain the rats' target body weight percentage because some animals were earning more nutritive food pellets under the FR1 schedule during the session than their daily food ration would allow. Administration of i.p. minocycline (0, 10, 30, 60 mg/kg) 67 minutes prior or i.p. buspirone (0, 1, 3, 10 mg/kg) was given 30 minutes prior to the test session. Minocycline's effects on differential reinforcing baselines of food-maintained behavior was examined given its effects on the previous self-administration studies as well as it's known reduction of other stimulant-induced behavioral outcomes (Zhang *et al.*, 2006; Chen *et al.*, 2009a; Fujita *et al.*, 2012). Buspirone, a partial 5-HT$_{1A}$ agonist with some dopaminergic antagonist activity, is an approved for treatment of anxiety. Buspirone was examined in the present study due to its ability to attenuate other psychostimulant-induced behaviors such as self-administration and re-instatement (Gold & Balster, 1992; Bergman *et al.*, 2013; Shelton *et al.*, 2013). Furthermore, the literature remains unclear as to whether buspirone significantly affects food-maintained behavior because significant rate decreasing were observed in a drug discrimination paradigm under buspirone treatment (Ator, 1991) but no significant decrease was observed in a self-administration procedure (Bergman *et al.*, 2013). Thus, buspirone provided a test compound which may or may not have significant effects on food-maintained behavior and significantly affected drug abuse-related behaviors. Between all test sessions with either minocycline or buspirone, rats were required to meet stability criteria on a FR10 schedule before moving to the next dose.

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Preliminary results suggested that while statistically significant, 85 and 100% BW conditions displayed a narrow window in demand between which to see differential effects of drug on food-maintained responding. Thus, widening the window between the initial demand conditions was hypothesized to allow for a better evaluation of differential drug effects. Thus, buspirone rats' percent body weight was increased to 115%, and they were re-tested under two intermediate doses of buspirone (1.3 and 1.8 mg/kg).

Drugs

Minocycline hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in saline and a few drops of 1 M hydrochloric acid. Immediately prior administration, the minocycline solution was adjusted to pH 3-4 using a few drops of sodium hydroxide. Buspirone was dissolved in saline. Minocycline and buspirone were both administered i.p. in 1 ml/kg body weight volume, except for 60 mg/kg minocycline that was given at 2 ml/kg body weight volume due to insolubility at the lower volume.

Data Analysis

Demand curves were generated using the formula $\log Q = \log Q_0 + k (e^{-\alpha P} - 1)$ from Hursh and Silberberg (2008). Non-linear regression best fit values for the alpha level parameter were compared between body weight conditions for each group (85%, 90%, 100%) or (85%, 100%, 115%) using an extra sum-of-squares F-test. When tested on a FR10 schedule, total consumption of food pellets was normalized by calculating the percentage of reinforcer consumption under test compound (minocycline or buspirone) treatment compared to total consumption under vehicle conditions. Normalized consumption percentages were analyzed using a two-way within subjects repeated measures ANOVA (repeated measures on treatment

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dose and body weight condition), and comparisons of minocycline and buspirone treatment on consumption were assessed using Sidak's Multiple Comparisons tests.

Results

The group α levels (95% CI) for each body weight condition (85%, 90%, 100%) were calculated as 0.000041 (0.000036-0.000045), 0.000058 (0.000052-0.000064), 0.000081 (0.000071-0.000092), respectively and were significantly different from one another [F (2, 178) = 35, p<0.0001] (Fig. 11). Responding by rats when maintained at 85% of their free-feeding body weight had a significantly smaller α level and a less elastic demand curve compared to animals maintained at 100% body weight [F(1,122)= 63, p<0.0001].

Figure 12 illustrates a significant main effect of minocycline on normalized consumption of the FOOD pellet reinforcer as compared to FOOD consumption under vehicle conditions [F(3,15)= 24.98, p<0.0001], however there was no main effect of body weight condition nor a significant interaction between the two variables. Multiple comparisons revealed that 30 and 60 mg/kg minocycline significantly attenuated FOOD consumption when it was normalized to its corresponding vehicle condition in both body weight conditions (p<0.05).

Similarly, there was a significant difference in α levels between body weights tested for the buspirone group [F (2,173) = 108, p<0.0001] (Fig. 13). The α levels for 85%, 100%, and 115% body weight were 0.000051 (0.000044-0.000057), 0.00025 (0.00019-0.00031), 0.00014 (0.00011-0.00017), respectively. Buspirone (1, 3, 10 mg/kg) produced a significant main effect on normalize FOOD consumption [(F (3, 36) = 26.20, p<0.0001], however there was no main effect of body weight condition nor a significant interaction. Buspirone (10 mg/kg) significantly

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reduced FOOD consumption when it was normalized to its corresponding vehicle and body weight conditions (p<0.05) (Fig. 14).

Buspirone at 1.3 and 1.8 mg/kg produced a significant main effect [$F_{(2, 8)}$ = 14.33 p= 0.0023] on normalized FOOD consumption, while there was no main effect of body weight condition when consumption was compared between animals maintained at 85% and 115% of their free-feeding body weight. Multiple comparisons analyses revealed that 1.8 mg/kg buspirone significantly attenuated FOOD consumption in both body weight conditions when normalized to its appropriate vehicle treated condition (p<0.05) (Fig. 15).

Summary

Given the results obtained from the methamphetamine self-administration studies, ibudilast, AV1013, and minocycline were all examined for their specificity of effect on methamphetamine-maintained relative to behavior maintained by a non-drug reinforcer, banana pellets. A behavioral economics analysis was applied to assess the baseline strengths maintained by methamphetamine and banana pellet delivery. This approach was based on the assumption that the most legitimate comparison of the effects of test drugs on methamphetamine-specific behavior would be obtained when baseline strengths of food and methamphetamine reinforcement were at similar levels. A follow-up study was conducted to demonstrate "proof of concept" that baseline strength can be a determinant of whether or not an effect by a challenge drug occurs. The baseline strengths utilizing the same reinforcer, food pellets, were altered by manipulating the percentage of the free feeding body weight of the subjects. Then, test compounds (minocycline and buspirone) were evaluated for their effects on food-maintained responding at differing levels of baseline strength. The results obtained indicated that there were not differential drug effects as a function of baseline strength under the

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conditions tested. However, there are several considerations of this study that limit the

conclusions that can be drawn from it that are discussed in the Discussion.

Chapter III: Figure Legends

Fig. 3. Effects of ibudilast or its vehicle on group mean infusions of methamphetamine (0.001, 0.03, and 0.1 mg/kg/inf) obtained during daily 2-h self-administration sessions. Ibudilast was administered at 1, 7.5, or 10 mg/kg i.p. b.i.d. for three consecutive days at each methamphetamine self-administered dose. Data points represent the group means of total infusions obtained during the third day of testing at each ibudilast dose. Bars through symbols indicate ±S.E.M. Data point above "S" on the abscissa indicates results when saline was self-administered when ibudilast's vehicle was given b.i.d. N=4 rats. *p<0.05 with respect to infusions obtained under ibudilast's vehicle condition.

Fig. 4. Response Rate Dependency analysis of effects of 10 mg/kg ibudilast on methamphetamine (0.03 mg/kg/inf or 0.1 mg/kg/inf) response rates when response rates were approximately matched under vehicle conditions. Under ibudilast's vehicle conditions the response rate maintained by 0.1 mg/kg/inf methamphetamine were altered by increasing the FR requirement (FR2-4). Ibudilast (10 mg/kg) was administered i.p. b.i.d. for three consecutive days. Data represent the group means (±S.E.M.) of response rate maintained by both methamphetamine infusion doses. N=4 rats. *p<0.05 with respect to response rate obtained under ibudilast's vehicle condition.

Fig. 5. Effects of AV1013 or its vehicle on group mean infusions of methamphetamine (0.001, 0.03, and 0.1 mg/kg/inf) obtained during daily 2-h self-administration sessions. AV1013 was administered at 1, 10, or 30 mg/kg i.p. b.i.d. for three consecutive days at each methamphetamine self-administered dose. Data points represent the group means of total

infusions obtained during the third day of testing at each AV1013 dose. Bars through symbols indicate ±S.E.M. Data point above "S" on the abscissa indicates results when saline was self-administered when AV1013s vehicle was given b.i.d. N=5 rats. *p<0.05 with respect to infusions obtained under AV1013s vehicle condition.

Fig. 6. Effects of minocycline or its vehicle on group mean infusions of methamphetamine (0.001, 0.03, and 0.1 mg/kg/inf) obtained during daily 2-h self-administration sessions. Minocycline was administered at 10, 30, or 60 mg/kg i.p once daily for three consecutive days at each methamphetamine self-administered dose. Data points represent the group means of total infusions obtained during the third day of testing at each minocycline dose. Bars through symbols indicate ±S.E.M. Data point above "S" on the abscissa indicates results when saline was self-administered when minocycline's vehicle (saline) was given daily. N=5 rats. *p<0.05 with respect to infusions obtained under minocycline's vehicle condition.

Fig. 7. Total demand for 0.1, 0.03 mg/kg/inf methamphetamine and banana pellets when rats were maintained at 85% of their free-feeding body weight. Methamphetamine demand was generated during daily 2-h self-administration session and banana pellet demand was generated during daily 15-min sessions. Data points represent the group means of total log of consumption of the reinforcing commodity (methamphetamine or banana pellets) as a function of unit price (FR value). Brackets through the symbols indicate ±S.E.M. Solid line curves represent the best non-linear fits for each reinforcer. N=5-6 rats.

Fig. 8. Effects of ibudilast (10mg/kg) on normalized consumption of 0.03 mg/kg/inf methamphetamine self-administration and banana pellets under a FR1 schedule. Data were

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collected during daily 2-h methamphetamine self-administration sessions or daily 15-min banana pellet sessions. Ibudilast (vehicle or 10 mg/kg) was administered i.p. b.i.d. for three consecutive days 1-h before testing. Bars represent group means of normalized consumption of either reinforcer obtained on the third day of testing ±S.E.M.   N=4-5 rats. *$p<0.05$ with respect to normalized consumption of either reinforcer under vehicle conditions.

Fig. 9. Effects of AV1013 (10, 30 mg/kg) on normalized consumption of 0.03 mg/kg/inf methamphetamine self-administration and banana pellets under a FR1 schedule. Data were collected during daily 2-h methamphetamine self-administration sessions or daily 15-min banana pellet sessions. AV1013 (vehicle, 10, 30 mg/kg) was administered i.p. b.i.d. for three consecutive days 1-h before testing. Bars represent group means of normalized consumption of either reinforcer obtained on the third day of testing. Brackets through bars represent ±S.E.M. N=3-5 rats. *$p<0.05$ with respect to normalized consumption of either reinforcer under vehicle conditions.

Fig. 10. Effects of minocycline (10, 30, 60 mg/kg) on normalized consumption of 0.03 mg/kg/inf methamphetamine self-administration and banana pellets under a FR1 schedule. Data were collected during daily 2-h methamphetamine self-administration sessions or daily 15-min banana pellet sessions. Minocycline (vehicle, 10, 30, 60 mg/kg) was administered i.p. once daily for three consecutive days 67-min before testing. Bars represent group means of normalized consumption of either reinforcer obtained on the third day of testing. Brackets through bars represent ±S.E.M. N=5 rats. *$p<0.05$ with respect to normalized consumption of either reinforcer under vehicle conditions.

Fig. 11. Total demand for 45 mg nutritive FOOD pellets when rats were maintained at 85%, 90%, and 100% of their individual free-feeding body weight. FOOD pellet demand was generated during daily 15-min sessions. As a within subject study, all animals were assessed under all three body weight conditions. Data points represent the group means of total log of consumption of the reinforcing commodity (FOOD) as a function of unit price (FR value). Brackets through the symbols indicate ±S.E.M. Solid line curves represent the best non-linear fits for each body weight condition. N=5 rats.

Fig. 12. Effects of minocycline (10, 30, 60 mg/kg) on normalized consumption of FOOD pellets under a FR10 schedule. Total consumption under minocycline treatment was normalized to total consumption of FOOD pellets following vehicle administration for each body weight condition. Data was collected during daily 15-min sessions. Data points represent the group mean of total normalized consumption as a function of minocycline dose in both body weight conditions. Brackets through symbols represent ±S.E.M.   N=6 rats. * and # indicate $p < 0.05$ with respect to normalized consumption of FOOD under vehicle conditions at 100% and 85% BW, respectively.

 Fig. 13. Total demand for 45 mg nutritive FOOD pellets when rats were maintained at 85%, 100%, and 115% of their individual free-feeding body weight. FOOD pellet demand was generated during daily 15-min sessions. As a within subject study, all animals were assessed under all three body weight conditions. Data points represent the group means of total log of consumption of the reinforcing commodity (FOOD) as a function of unit price (FR value). Brackets through the symbols indicate ±S.E.M. Solid line curves represent the best non-linear fits for each body weight condition. N=5 rats.

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Fig. 14. Effects of buspirone (1, 3, 10 mg/kg) on normalized consumption of FOOD pellets under a FR10 schedule. Total consumption under buspirone treatment was normalized to total consumption of FOOD pellets following vehicle administration for each body weight condition. Data were collected during daily 15-min sessions. Data points represent the group mean of total normalized consumption as a function of buspirone dose in two body weight conditions (85% and 100%). Brackets through symbols represent ±S.E.M.   N=13 rats. * and # indicate $p < 0.05$ with respect to normalized consumption of FOOD under vehicle conditions at 100% and 85% BW, respectively.

Fig. 15. Effects of buspirone (1.3, 1.8 mg/kg) on normalized consumption of FOOD pellets under a FR10 schedule. Total consumption under buspirone treatment was normalized to total consumption of FOOD pellets following vehicle administration for each body weight condition. Data was collected during daily 15-min sessions. Data points represent the group mean of total normalized consumption as a function of buspirone dose in two body weight conditions (85% and 115%). Brackets through symbols represent ±S.E.M.   N=5 rats. * and # indicate $p < 0.05$ with respect to normalized consumption of FOOD under vehicle conditions at 115% and 85% BW, respectively.
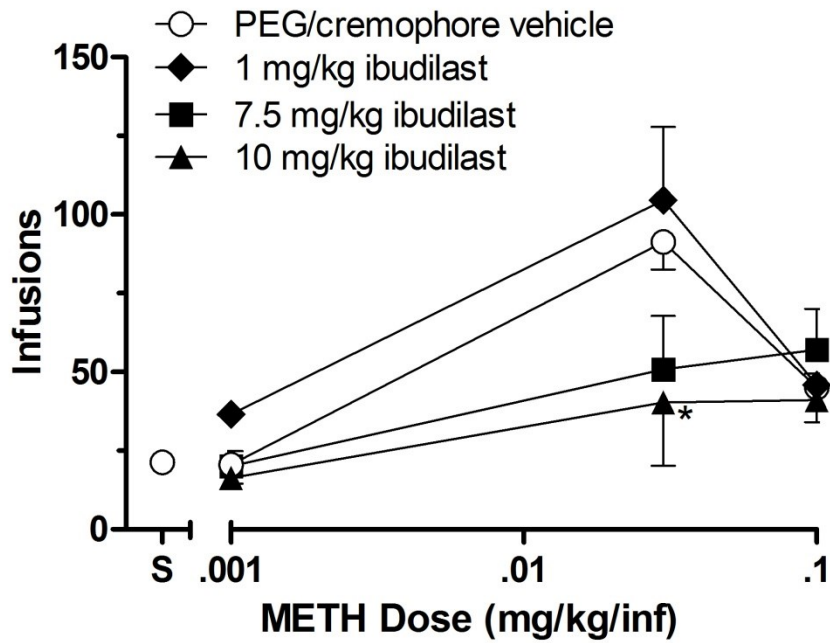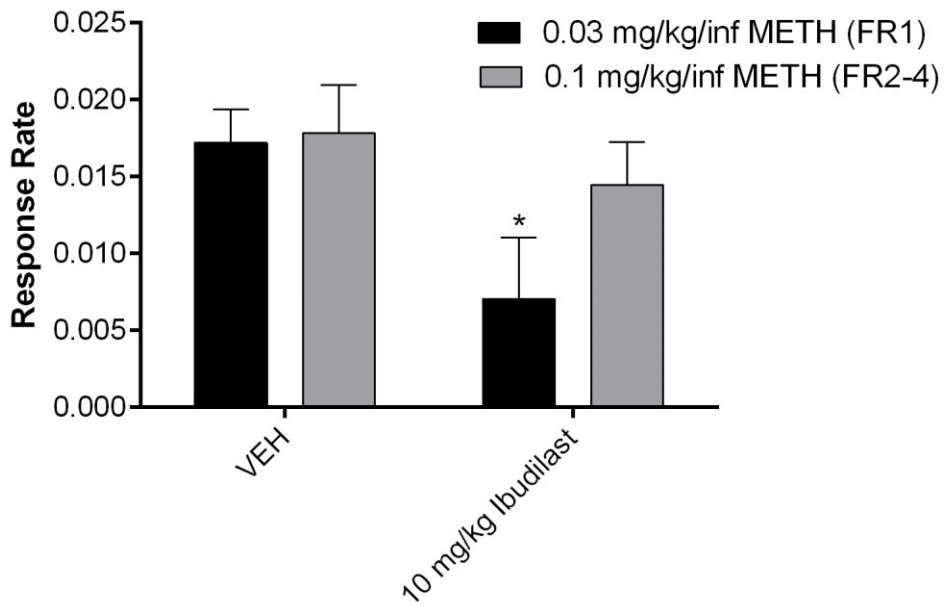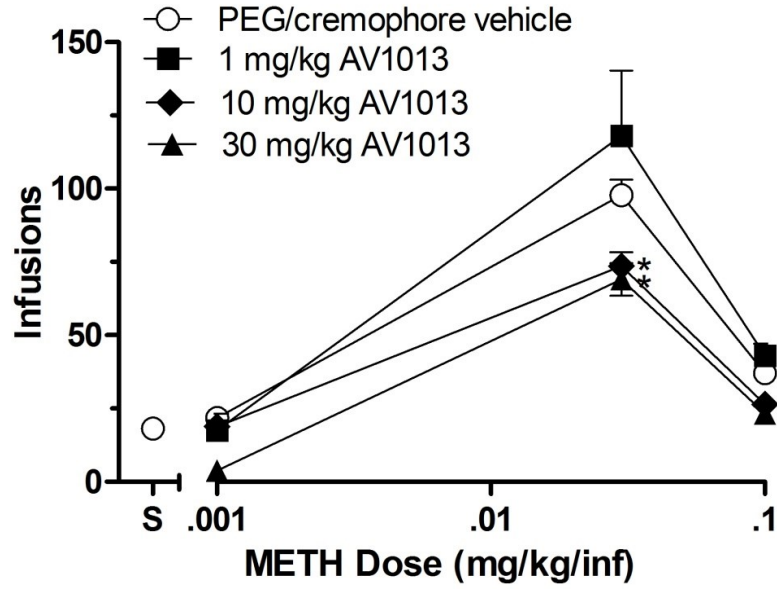
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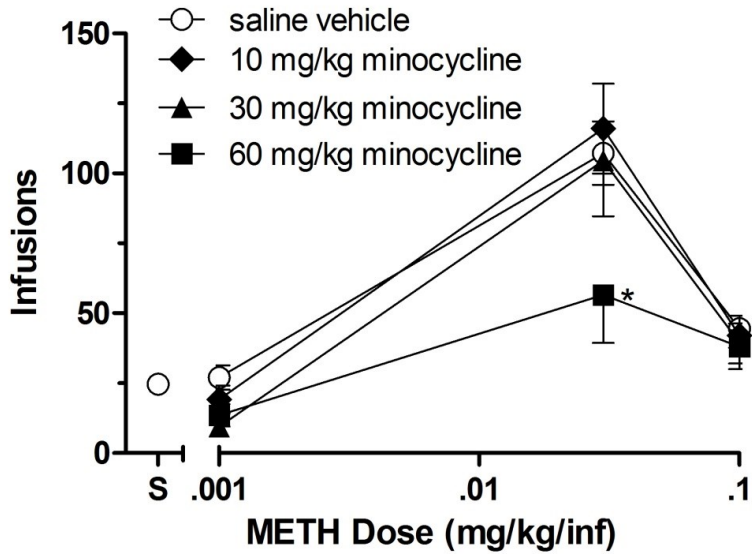
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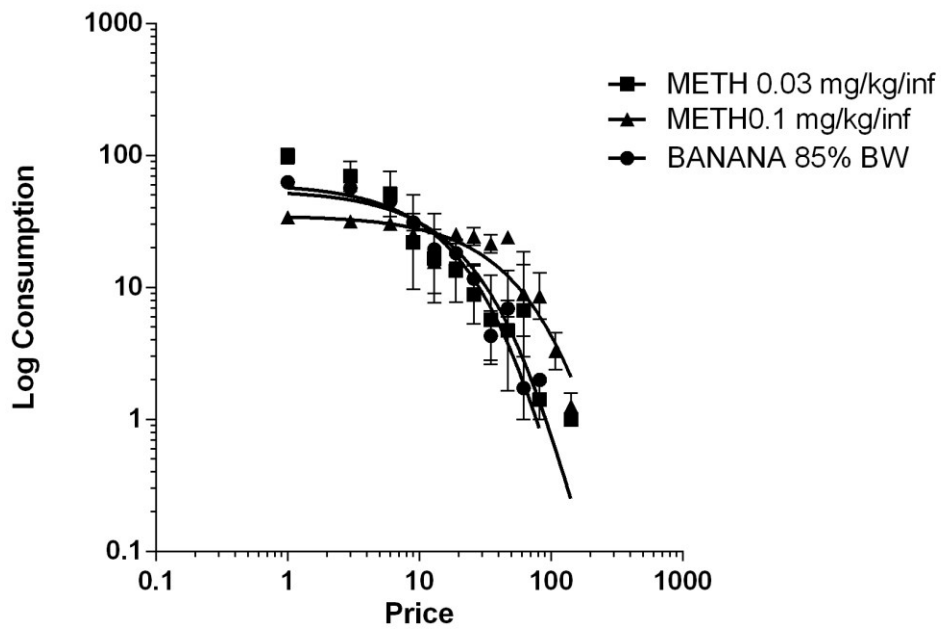
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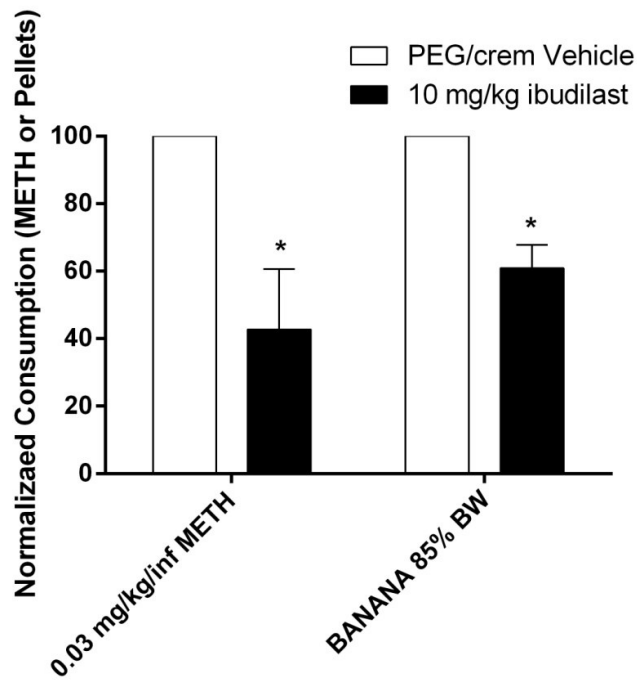
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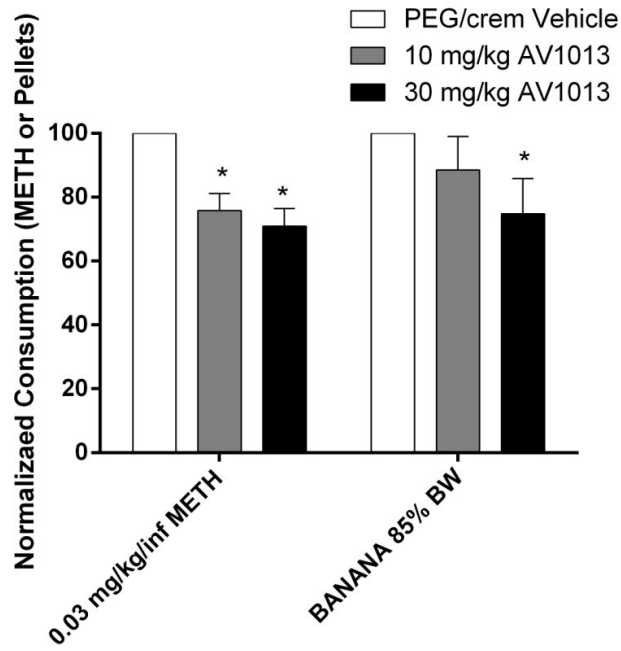
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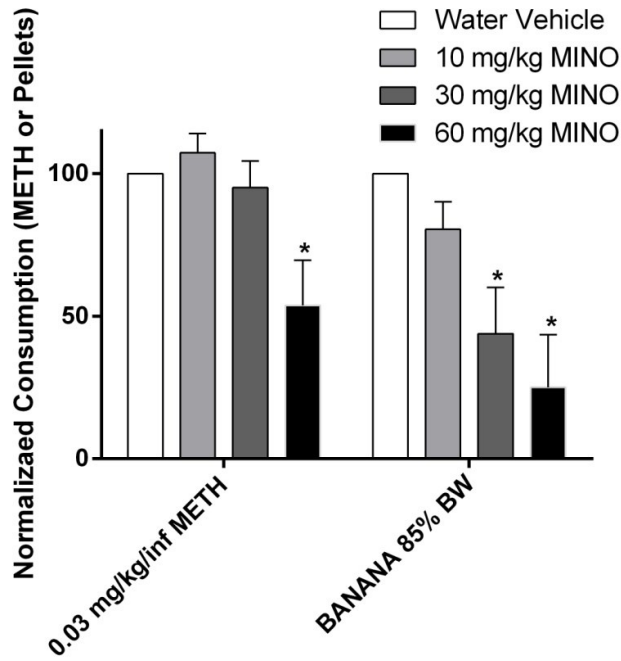
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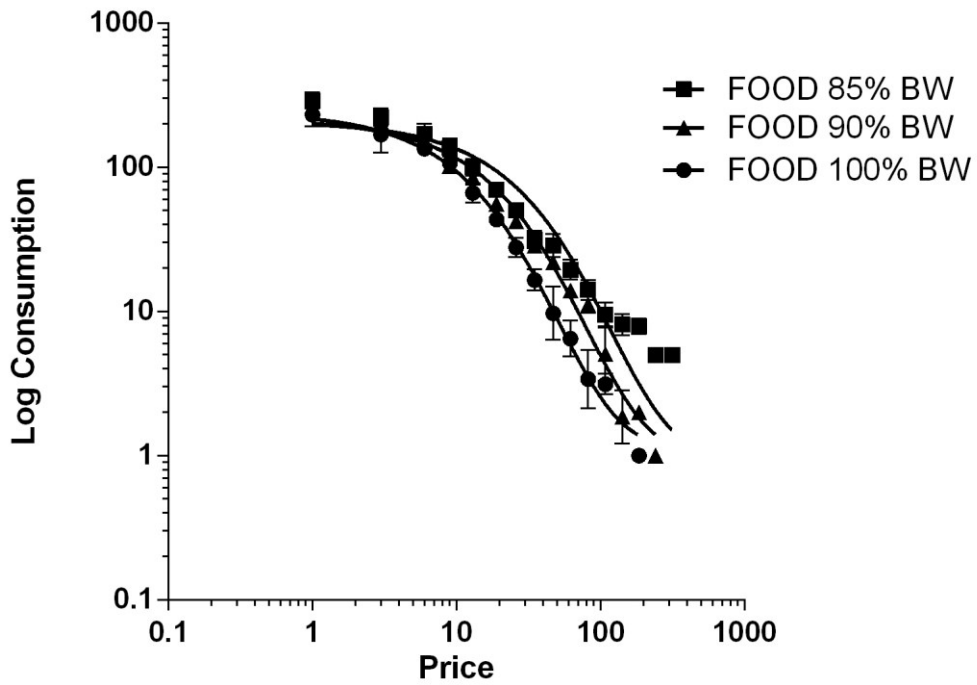
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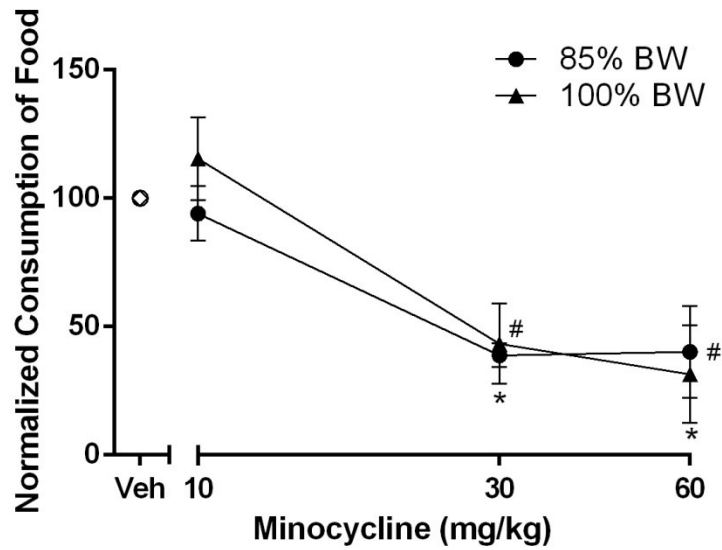
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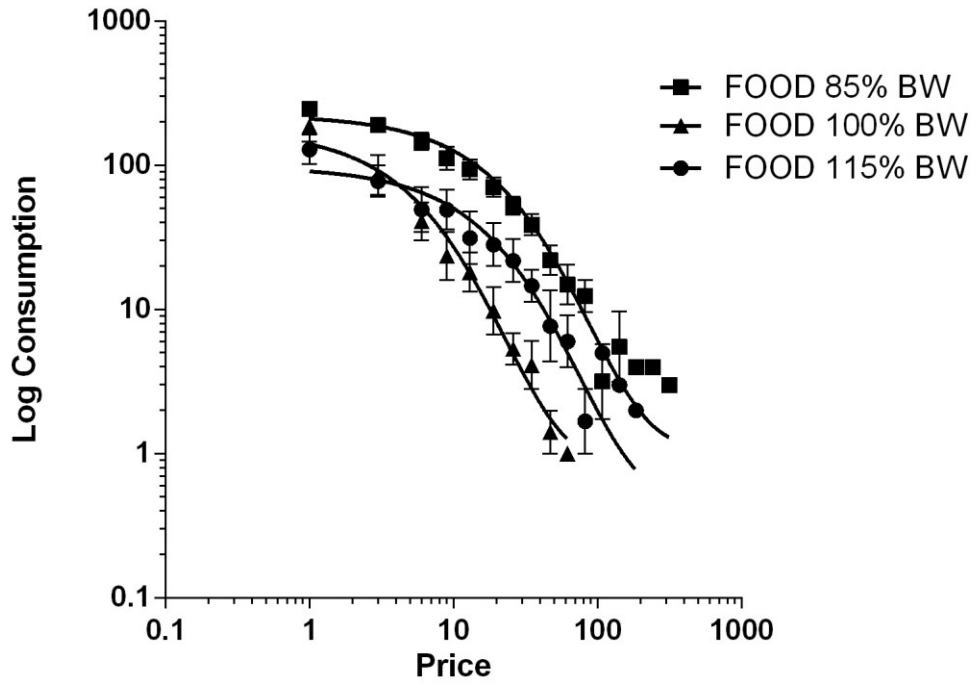
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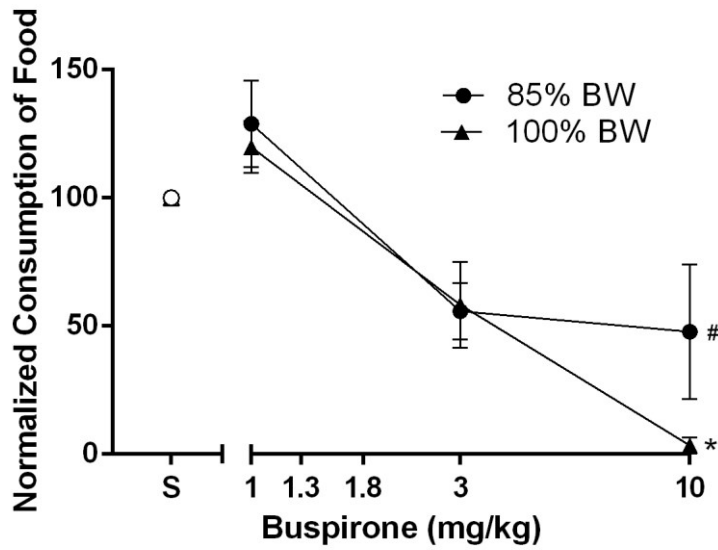
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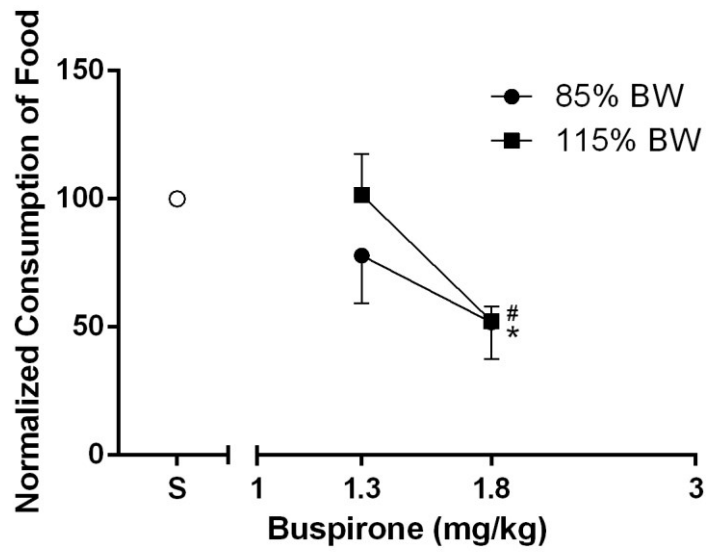
Chapter IV: Ibudilast's effects on methamphetamine drug discrimination in the rat

Introduction

The purpose of the final research aim of this dissertation was to determine whether the discriminative stimuli produced by methamphetamine administration are attenuated by ibudilast administration. Others have reported that the PDE inhibitors, rolipram and nefiracetam, significantly attenuate methamphetamine (0.2 and 0.5 mg/kg) drug discrimination in rats (Yan *et al.*, 2004; Yan *et al.*, 2006). Given that we have shown ibudilast, which exhibits PDE inhibitory activity, significantly attenuates methamphetamine-induced locomotor activity, sensitization, and self-administration (above), ibudilast was hypothesized to significantly attenuate methamphetamine's discriminative stimulus effects.

Methods

Subjects

Ten adult male Long-Evans hooded rats (Harlan, Indianapolis, IN) were allowed to acclimate to the vivarium for at least one week before training began. When not in testing, rats were individually housed in standard plastic rodent cages in a temperature-controlled (22°C), Association for the AAALAC International-accredited facility in which they had ad libitum access

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to water. Rats were allowed *ad libitum* rat chow (7012 Teklad LM-485 Mouse/Rat Sterilizable Diet, Harlan Laboratories, Inc., Indianapolis, IN) for at least ten days in order to calculate each individual's free feeding body weight. Individual body weights were determined by averaging daily weights from six consecutive days of *ad libitum* feeding prior to initiation of weight maintenance and test sessions Rats were then maintained at 85% of their free-feeding body weight by controlled feedings of rat chow after daily sessions and once daily over the weekend. All rats were maintained on a reversed, 12 h/12 h light-dark cycle (0600-1800 h lights off) for the duration of the experiment, and they were trained and tested during the dark segment of this cycle. All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996) and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Apparatus

Commercially-obtained test chambers equipped with two retractable levers, a 5-w houselight, a Sonalert tone generator, and a food pellet dispenser (MED Associates, Inc., St. Albans, VT) were used. Recording of lever presses and food dispenser activation was accomplished by a microcomputer, interface, and associated software (MED-PCs IV, MED Associates, Inc., St. Albans, VT).

Procedure

Discrimination Training

Rats were initially trained to lever press for 45 mg rodent purified diet dustless precision pellet (BioServ, Frenchtown, NJ) delivery according to a FR10 schedule during daily 15-min sessions (Mon-Fri). Five rats were trained to respond on the left lever and five rats on the right

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lever for food pellet delivery. This initially active lever eventually became the vehicle-designated lever. Rats were then trained to respond on the opposite lever for food pellet delivery under a FR10 schedule, which eventually became the drug-designated lever. During training sessions, either 1 mg/kg methamphetamine or saline i.p. injection was administered 15 min pre-session and the appropriately paired lever produced food pellets under a FR10 schedule. Training sessions occurred using the following sequence which renewed bi-monthly.

(1) D-V-V-D-V   (2) V-D-D-V-V
    V-D-D-V-D   D-V-D-V-D
    V-D-V-D-V   D-V-V-D-D
    D-V-D-V-D   V-D-V-D-V

Acute Tues/Fri Discrimination Testing

Testing commenced once the rats met training criteria in which the first fixed ratio (FFR) was completed on the appropriate lever during at least 8 out of 10 consecutive sessions, and 80% of total responses had been emitted on the appropriate lever during these 8 sessions. Test sessions subsequently occurred if the FFR was correct on both the most recent methamphetamine and saline training sessions, otherwise additional training sessions were given. During test sessions, which occurred on Tuesdays and Fridays, responding at either lever was reinforced according to a FR10 schedule. Initially, methamphetamine (0.1, 0.3, 0.56, 1, and 3 mg/kg) was tested to obtain a generalization curve for the training dose (1 mg/kg). Methamphetamine dose testing order was randomized across subjects.

Cumulative Dosing Training

An objective during testing was to rapidly obtain a complete dose-effect curve for methamphetamine at each ibudilast dose tested. To do so, training under cumulative dosing conditions was necessary before testing could begin to ensure that the procedure produced a

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generalization curve similar to that obtained under acute dosing conditions. After the acute

dose-effect curve for methamphetamine was obtained, cumulative dosing pilot training sessions

began. The pilot training sessions consisted of five 3-min reinforcement periods each preceded

by 10-min TO periods during which the test chambers darkened and response levers retracted.

Having five, 3-min food-reinforcement components preceded by 10-min TO periods ensured

that total food pellet availability remained constant at 15 min (similar to preceding training and

testing conditions) and that overall session duration did not exceed methamphetamine's

approximate elimination half-life of 70-min (Cho *et al.*, 2001). During pilot sessions, subjects

were administered saline 10-min prior to the first 3-min reinforcement period, methamphetamine

(1 mg/kg) at the commencement of the first TO period, and sham injections at the

commencement of the TO periods prior to the remaining reinforcement components. During

each reinforcement component, only the lever associated with the most recent injection

produced food reinforcement under a FR10 schedule. Pilot sessions were utilized to allow the

subjects to acclimate to the multi-component, multi-injection procedure, and to determine that

the pre-session injections controlled consistent lever selection for the 55 min session.

Cumulative dosing training was complete if subjects completed the FFR on the correct lever and

>80% of responding was emitted on the correct lever during all five food reinforcement periods.

Following pilot sessions, regular training with continuous 15-min training sessions resumed until

testing criteria were again met (i.e., correct FFR during the most recent drug and vehicle training

sessions).

Cumulative Dosing Testing

Once a subject met testing criteria, training was suspended for two full days (Sat-Sun)

during which ibudilast was administered b.i.d (see below) and the cumulative dosing testdays

occurred on the subsequent days (Mon-Tues). Beginning b.i.d. ibudilast dosing two days prior to

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the test sessions maintained consistency with the previously described behavioral protocols. Multiple treatments (i.e. b.i.d. dosing) prior to beginning and throughout testing are necessary to obtain steady-state drug levels of ibudilast in various tissue compartments and to enable minimally sufficient glial attenuation that relates to the onset of other effects (Ledeboer *et al.*, 2006; Hutchinson *et al.*, 2009a; Beardsley *et al.*, 2010). Furthermore, dosing over weekends avoided disruption of regularly scheduled training sessions (Mon-Fri). Cumulative dosing testdays consisted of five, 3-min food availability periods each but the first proceeded by 10-min TO periods. A methamphetamine (0, 0.1, 0.3, 0.56, 1, 3 mg/kg) dose effect curve was obtained by combining results from two testdays in which cumulative doses of 0 (saline), 0 (sham), 0.1, 0.56 and 1, and 0 (saline), 0 (sham), 0.3, 1, and 3 mg/kg were obtained (in which acute doses of 0 (saline), 0 (sham), .1, .46, and .44 mg/kg, and 0 (saline), 0 (sham), 0.3, 0.7, and 2 mg/kg were administered).

Ibudilast (0, 1, 7.5, 10 mg/kg) was administered b.i.d. beginning two days prior to the test days and 1-h prior to testing on both test days. Between Testdays, subjects returned to daily acute dosing 15-min session training and were required to meet test criteria before advancing. Tested ibudilast doses were randomized between individual rats.

<u>Testing and Training Lower Methamphetamine Doses (1 mg/kg vs. 0.56 or 0.3 mg/kg)</u>

The literature suggests that the training dose utilized during a drug discrimination procedure can be a pivotal component of the assay. For example, progressively lowering a PCP training dose produces marked decreases in the ED50 for stimulus generalization and parallel leftward shifts of the dose response curves indicative of greater stimulus generalization at lower training doses (Beardsley *et al.*, 1987). Further, an antagonist can more readily disrupt discrimination of a particular drug when trained at a low training dose (Picker *et al.*, 1993). Yan et al (2006) first trained animals to discriminate 0.5 mg/kg methamphetamine and tested PDE

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inhibitors' effects under 0.2 mg/kg methamphetamine conditions. Thus, in order to better compare ibudilast's effects as a PDE inhibitor and glial cell modulator to rolipram and nefiracetam's effects on methamphetamine drug discrimination, the subjects were re-trained at a lower methamphetamine training doses (0.56 and 0.3 mg/kg).

Animals were re-trained to discriminate 0.56 mg/kg methamphetamine using the procedures described above that involved only a single injection prior to each 15-min session until training criteria were again met. As a probe to determine if even the high dose of ibudilast (10 mg/kg) would now have effects different from those following discrimination training at the 1 mg/kg dose, the effects of 10 mg/kg ibudilast were tested on discrimination performance. To do so, training was suspended for two full days during which (10 mg/kg) ibudilast was administered b.i.d and then ibudilast was administered 1-h prior to a 15-min 0.56 mg/kg methamphetamine testing session on the third day. The results from the probe were compared to the %DLR for 0.56 mg/kg methamphetamine obtained during a non ibudilast pre-treated 15-min control test session.

Following this initial probe, the group was divided in two. Five of the 10 animals remained at the 0.56 mg/kg methamphetamine training dose and underwent the cumulative dosing procedure in order to obtain a full dose response curve for the 0.56 mg/kg training dose and determine whether there was a leftward shift of the curve and reduction of the ED50 for methamphetamine generalization. Further, the cumulative dosing procedure allowed for the potential to examine ibudilast's effects on methamphetamine discrimination at all doses of methamphetamine, not just the training dose (0.56 mg/kg methamphetamine). Second, to better approximate the training and testing conditions previously reported to result in antagonism of the methamphetamine discriminative stimulus by other PDE inhibitors (Yan *et al.*, 2004; Yan *et al.*, 2006), the methamphetamine training dose was further decreased to 0.3 mg/kg for the

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remaining five animals. Subjects were re-trained, as before, and required to meet training and testing criteria at the 0.3 mg/kg methamphetamine training dose. A methamphetamine dose response curve (0, 0.1, 0.3, 0.56, 1, 3 mg/kg) was obtained using the acute Tues/Fri testing procedure where dose order was randomized between subjects. The acute procedure was utilized here because initial acquisition to the lower training dose took much longer and stability of discrimination performance was unstable.

Drugs

(±)-Methamphetamine hydrochloride (National Institute on Drug Abuse, Rockville, MD) was prepared in sterile 0.9% saline. Methamphetamine stock solutions were sterilized by filtration through 0.2 mm filtration disks. Ibudilast (3-isobutyryl-2-isopropylpyrazolo [1, 5-a] pyridine) was received as a gift from MediciNova, Inc. (San Diego, CA) and was dissolved in a 35% PEG400, 10%Cremophor® RH40 (BASF, Ludwigshafen, Germany) aqueous vehicle. Ibudilast was administered i.p.in 1 ml/kg body weight volume.

Data Analysis

The percentage of methamphetamine-lever presses (%DLR) was calculated for each subject by dividing the number of lever presses emitted upon the methamphetamine-designated lever by the total number of presses emitted, and multiplying this quotient by 100. Individual values of %DLR were then averaged (±SEM). Complete generalization to the methamphetamine discriminative stimulus was inferred when %DLR ≥80%. If a rat failed to make at least 10 lever presses during a test session, its data were excluded from calculations of %DLR but were included for mean response rate determinations. This exclusion was made to prevent near-zero rates of responding from disproportionately influencing estimates of %DLR. ED50 values and their confidence intervals (CI) were calculated for %DLR and for reducing

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response rates after a log-dose transformation using a variable slope, nonlinear regression analysis. Methamphetamine slopes with and without co-administration of the test drug were then determined parallel or not using the F test, and if parallel, intercepts were determined equal or not as a measure of potency. Average ED50s from each condition were analyzed using an un-paired t-test (in comparing acute vs. cumulative dosing procedures) or a one-way ANOVA with Dunnett's multiple comparisons post hoc tests (in ibudilast treatment conditions). Response rates were calculated for each drug condition by dividing the total number of lever presses emitted during the session by the number of seconds in the acute 15-min session (900-sec) or for each individual 3-min test bin during the cumulative testing procedure (180-sec). Calculated response rates under acute Tues/Fri methamphetamine conditions were compared using a one-way ANOVA with Dunnett's multiple comparison's post hoc tests. Calculated response rates for acute vs. cumulative dosing procedures and ibudilast treated conditions were analyzed with a two-way ANOVA (repeated measures on methamphetamine dose and between dosing procedure or ibudilast condition). Differences in response rates from saline vehicle conditions and/or compared to PEG/crem veh response rates were assessed using Bonferroni post hoc analyses. All statistical tests were conducted using commercial computer software (Prism5d for Macintosh, GraphPad Software, Inc., San Diego, CA), and all types of comparisons were considered statistically significant if $p < 0.05$.

Results

The upper frame of Figure 15 shows the percentage of methamphetamine lever presses obtained during acute Tues/Fri tests when the rats were trained to discriminate 1 mg/kg methamphetamine from saline. Complete generalization (>80% DLR) occurred at 1 and 3 mg/kg

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methamphetamine, while saline produced near-zero drug lever presses. The ED50 (CI) obtained was 0.53 (0.46-0.58) mg/kg methamphetamine for occasioning the 1 mg/kg methamphetamine stimulus. There were no statistically significant effects of methamphetamine on response rates at any dose compared to vehicle conditions (Fig. 15; lower frame).

Results from the cumulative dosing discrimination procedure produced similar results in that both 1 and 3 mg/kg produced complete generalization to the 1 mg/kg methamphetamine training stimulus, while saline and sham injections produced near-zero %DLR (Fig. 16; upper frame). The ED50 for methamphetamine to occasion its 1 mg/kg discriminative stimulus was 0.58 (0.51-0.65) mg/kg. There was a significant main effect of methamphetamine dose on response rates [$F_{(5, 35)} = 5.326$, $p=0.001$] and interaction between methamphetamine dose and the dosing procedure used [$F_{(5, 35)} = 3.982$, $p=0.0058$]. Bonferroni post hoc analyses indicated that in the cumulative dosing procedure there was a significant decrease in response rates at 3 mg/kg methamphetamine compared to the saline condition ($p<0.05$). There were no significant differences in the ED50s for %DLR or for suppressing response rates (Fig. 16; lower frame) between the acute Tues/Fri and the cumulative dosing procedures.

During cumulative doing testing, complete generalization occurred at 1 and 3 mg/kg methamphetamine with near-zero %DLR following saline and sham injections under PEG/crem vehicle, 7.5, and 10 mg/kg ibudilast pre-treatment conditions (Fig. 17; upper frame). Complete generalization occurred at 3 mg/kg of methamphetamine; however 1 mg/kg methamphetamine fell just short of full generalization with an average of 79 %DLR for methamphetamine under 1 mg/kg ibudilast pre-treatment conditions. The calculated ED50s (CI) were 0.49 (0.42-0.56), 0.711 (0.62-0.82), 0.49 (0.42-0.57), and 0.50 (0.37-0.67) mg/kg for occasioning the 1 mg/kg methamphetamine training dose under PEG/crem vehicle, 1, 7.5, and 10 mg/kg ibudilast pre-treatment conditions, respectively. Further, there were no significant differences in ED50s for

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occasioning the 1 mg/kg methamphetamine stimulus following any ibudilast pretreatment condition compared to pretreatment with PEG/crem vehicle. Methamphetamine [F (6, 54) = 16.18, p<0.0001] and ibudilast [F (3, 27) =14.71, p<0.0001] both had a significant main effects on response rates in the cumulative dosing procedure (Fig. 17; lower frame). The high dose of methamphetamine (3 mg/kg) significantly depressed response rates compared to saline and sham conditions regardless of ibudilast pre-treatment dose (p<0.05). Ibudilast (7.5 mg/kg) pre-treatment significantly decreased response rates at all methamphetamine doses compared to PEG/crem vehicle and 10 mg/kg ibudilast pre-treatment decreased response rates at all the methamphetamine doses with the exception of the high dose of methamphetamine (3 mg/kg) (p<0.05).

Figure 18 illustrates the %DLR for methamphetamine obtained when the training dose was lowered to 0.56 mg/kg. Unlike results when trained at 1 mg/kg methamphetamine, lowering the training dose to 0.56 mg/kg methamphetamine now resulted with 0.56 mg/kg methamphetamine completely occasioning methamphetamine lever pressing. A probe (n=7) to determine the effects of 10 mg/kg ibudilast pretreatment on methamphetamine discrimination at the new training dose (0.56 mg/kg) indicated no significant attenuation in %DLR for methamphetamine compared to non-treated control test sessions.

When the rats were sub-divided into groups of five, subjects whose training dose remained at 0.56 mg/kg methamphetamine were tested under cumulative dosing conditions in order to characterize a complete dose-effect curve. Under ibudilast's vehicle (PEG/crem) pre-treatment conditions, methamphetamine produced increasingly more methamphetamine appropriate responding with increasing cumulative dose, with complete generalization at 0.56, 1 and 3 mg/kg methamphetamine. Although ibudilast was planned to be tested under these conditions, proceeding forward with these tests seemed unwarranted and were not conducted

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considering that 0.3 mg/kg methamphetamine elicited near zero methamphetamine appropriate responding and the probe analysis indicated that 10 mg/kg ibudilast does not affect % DLR even at 0.56 mg/kg methamphetamine.

The remaining five animals were re-trained to discriminate 0.3 mg/kg methamphetamine from saline. Although the subjects reached both training and testing criteria, their discrimination behavior was not consistently stable from week to week thus promoting the use of the acute Tues/Fri testing procedure. For example, it took an average of 16 training days to complete the methamphetamine dose response curve when animals were trained at 1 mg/kg. Conversely, the same five animals required an average of 28.8 training days to complete the entire dose response curve when trained at 0.3 mg/kg methamphetamine. Further, while the training dose (0.3 mg/kg) produced full generalization, both 0.56 and 1 mg/kg methamphetamine failed to produce greater than 80% of methamphetamine appropriate lever responding. Thus, these inconsistencies in discrimination at 0.3 mg/kg precluded further testing with ibudilast or its vehicle.

Summary

The results from drug discrimination tests indicated that when trained to discriminate 1 mg/kg methamphetamine, increasingly greater proportions of % DLR occurred. There was not a significant difference in the ED50s for %DLR or response rates when tested under the acute or in a two-day cumulative dosing procedure. When ibudilast was administered as a pretreatment following drug discrimination training at 1 mg/kg methamphetamine, there were no significant differences in ED50 for methamphetamine at any ibudilast dose tested. However, 7.5 and 10 mg/kg ibudilast significantly attenuated session response rates compared to vehicle

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conditions. These results suggest that ibudilast was ineffective at attenuating methamphetamine's discriminative stimulus effects following training at 1 mg/kg methamphetamine.

When animals were re-trained to discriminate methamphetamine at lower doses, a training dose of 0.56 mg/kg methamphetamine was still not affected by 10 mg/kg ibudilast. Finally, training at the 0.3 mg/kg methamphetamine training dose resulted in inconsistent discriminative performance, which was considered too unreliable to permit subsequent tests with ibudilast.

Table 4. Number of rats included in each condition based on session response criteria

Tues/Fri Acute Vs. Cumulative Dosing Procedure

Meth Dose	Procedure			
	Tues/Fri Acute	Response Rate	Cumulative Dosing	Response Rate
0.1 mg/kg	*n=10*	*n=10*	*n=8*	*n=8*
0.3 mg/kg	*n=10*	*n=10*	*n=8*	*n=8*
0.56 mg/kg	*n=10*	*n=10*	*n=8*	*n=8*
1 mg/kg	*n=10*	*n=10*	*n=8*	*n=8*
3 mg/kg	*n=9*	*n=10*	*n=7*	*n=8*

2-Day Cumulative Dosing Procedure

Meth Dose	Ibudilast Dose (b.i.d.)				Response Rate
	PEG/crem veh	1 mg/kg	7.5 mg/kg	10 mg/kg	
0.1 mg/kg	*n=10*	*n=10*	*n=10*	*n=9*	*n=10*
0.3 mg/kg	*n=10*	*n=9*	*n=10*	*n=10*	*n=10*
0.56 mg/kg	*n=10*	*n=10*	*n=8*	*n=9*	*n=10*
1 mg/kg	*n=10*	*n=10*	*n=9*	*n=10*	*n=10*
3 mg/kg	*n=7*	*n=8*	*n=9*	*n=7*	*n=10*

Methamphetamine Probe Analysis (0.56 mg/kg Training Dose)

Meth Dose	Treatment Condition			
	No Pre-treatment	Response Rate	10 mg/kg ibudilast	Response Rate
0.56 mg/kg	*n=8*	*n=8*	*n=7*	*n=8*

1 vs 0.56 mg/kg Training Dose Comparison (Cumulative Dosing)

Meth Dose	PEG/crem veh	
	1 mg/kg Training	0.56 mg/kg Training
0.1 mg/kg	*n=10*	*n=3*
0.3 mg/kg	*n=10*	*n=3*
0.56 mg/kg	*n=10*	*n=3*
1 mg/kg	*n=10*	*n=3*
3 mg/kg	*n=7*	*n=2*

1 vs 0.3 mg/kg Training Dose Comparison (Tues/Fri Acute Dosing)

Meth Dose	PEG/crem veh	
	1 mg/kg Training	0.3 mg/kg Training
0.1 mg/kg	*n=10*	*n=5*
0.3 mg/kg	*n=10*	*n=5*
0.56 mg/kg	*n=10*	*n=5*
1 mg/kg	*n=10*	*n=5*
3 mg/kg	*n=9*	*n=5*

Chapter V: Figure Legends

Fig. 15. <u>Upper Frame</u>: Methamphetamine %DLR during acute Tues/Fri tests. Discrimination sessions were 15-min in duration. Data points represent the group means of %DLR ± S.E.M. for each dosing procedure. "S" indicates results with saline vehicle. "Sh" indicates results following sham injections. See Table 4 for groups sizes for each methamphetamine dose. <u>Lower Frame</u>: Response rates during acute dosing tests. Data points represent the group means of response rates ± S.E.M. for each dosing procedure. Other details identical to those described in the upper frame. N=10 rats.

Fig. 16. <u>Upper Frame</u>: Comparison of %DLR of methamphetamine between acute Tues/Fri dosing and cumulative dosing tests. Acute Tues/Fri dosing sessions were 15-min in duration. Cumulative dosing data are a compilation of two consecutive days of testing in which five 3-min active reinforcement periods were separated by 10-min TO periods to allow for injections and drug absorption. Data points represent the group means of %DLR ± S.E.M. for each dosing procedure. "S" indicates discrimination of saline vehicle. See Table 4 for group sizes for each methamphetamine dose. <u>Lower Frame</u>: Response rates between acute Tues/Fri dosing and cumulative dosing tests. Data points represent the group means of response rates ± S.E.M. for each dosing procedure. Other details identical to those described in the upper frame. *p<0.05 with respect to saline response rates. N=10 rats.

Fig. 17. <u>Upper Frame:</u> Ibudilast's effects on %DLR of methamphetamine during cumulative dosing tests. Cumulative dosing data are a compilation of two consecutive days of testing in which five 3-min active reinforcement periods were separated by 10-min TO periods to allow for injections and drug absorption. Data points represent the group means of %DLR ± S.E.M. for

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each dosing procedure. "S" indicates results with saline vehicle. "Sh" indicates results following sham injections. See Table 4 for groups sizes for each ibudilast dose. <u>Lower Frame</u>: Ibudilast's effects on responses rate during cumulative dosing tests. Data points represent the group means of response rates ± S.E.M. for each dosing procedure. Other details identical to those described in the upper frame. *p<0.05 with respect to PEG/crem vehicle response rates. #p<0.05 with respect to saline and sham condition response rates. N=10 rats.

Fig. 18. Ibudilast's (10 mg/kg) effects on %DLR for methamphetamine training dose (0.56 mg/kg) tests. Filled circle points represent %DLR ± S.E.M. from Tues/Fri acute dosing methamphetamine dosing procedure when the animals were trained to discriminate 1 mg/kg methamphetamine from saline. The filled square represents %DLR ± S.E.M. of an acute dose of methamphetamine (0.56 mg/kg) training dose. The filled triangle represents ibudilast (10 mg/kg) treatment effects on 0.56 mg/kg methamphetamine %DLR ± S.E.M. "S" indicates results with saline vehicle. See Table 4 for group sizes for each condition.

Fig. 19. Comparison of 1 vs. 0.56 mg/kg methamphetamine training dose effects on %DLR of methamphetamine during cumulative dose tests. Both curves generated following ibudilast's vehicle (PEG/crem) pre-treatment. Cumulative dosing data is a compilation of two consecutive days of testing in which five 3-min active reinforcement periods were separated by 10-min TO periods to allow for dosing and absorption. Data points represent the group means of %DLR ± S.E.M. for each dosing procedure. "S" indicates results with saline vehicle. "Sh" indicates results following sham injections. See Table 4 for groups sizes for each methamphetamine dose.

Fig. 20. Comparison of 1 vs. 0.3 mg/kg methamphetamine training dose effects on %DLR of methamphetamine during acute Tues/Fri test sessions. Acute Tues/Fri sessions were 15-min in duration. Data points represent the group means of %DLR ± S.E.M. for each dosing procedure. "S" indicates results with saline vehicle. See Table 4 for group sizes for each methamphetamine dose.

Figure 15.
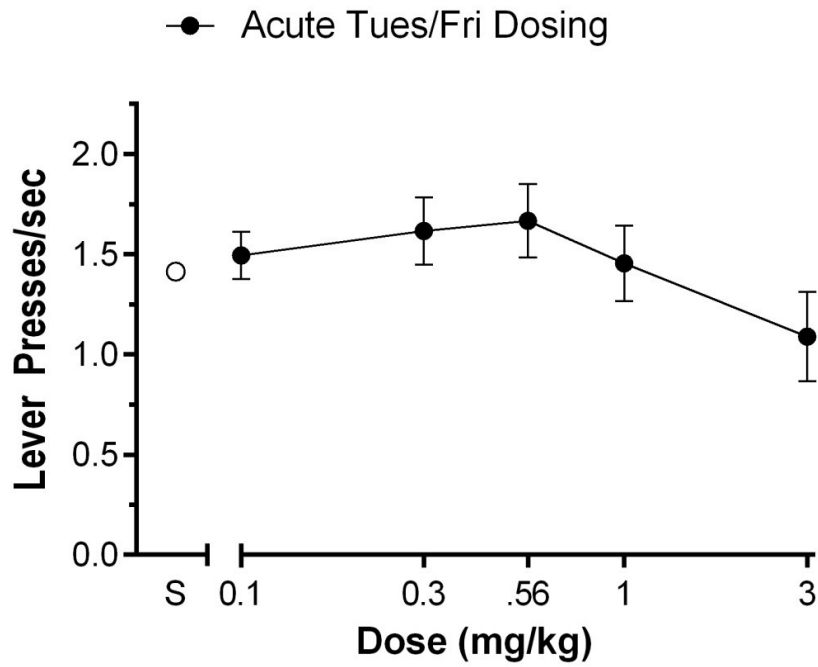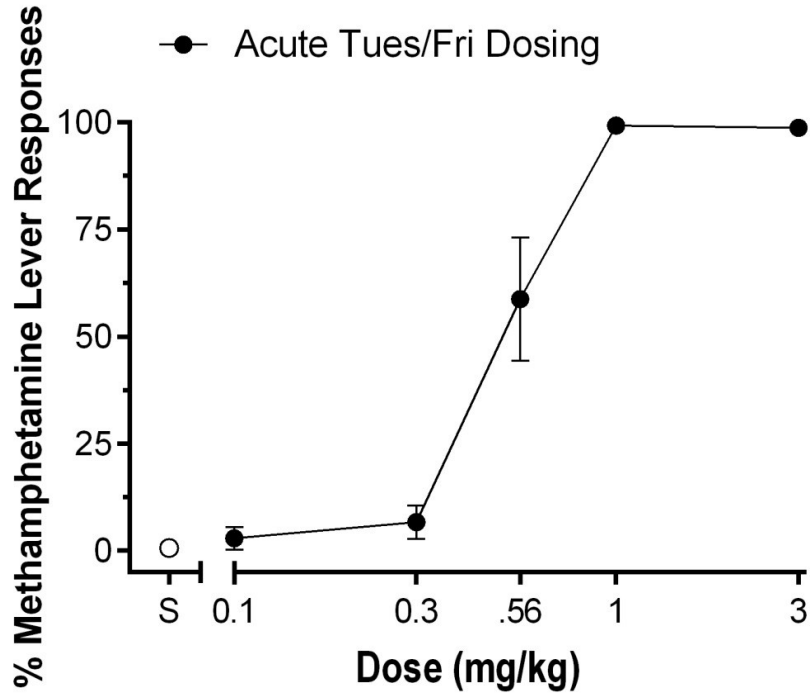
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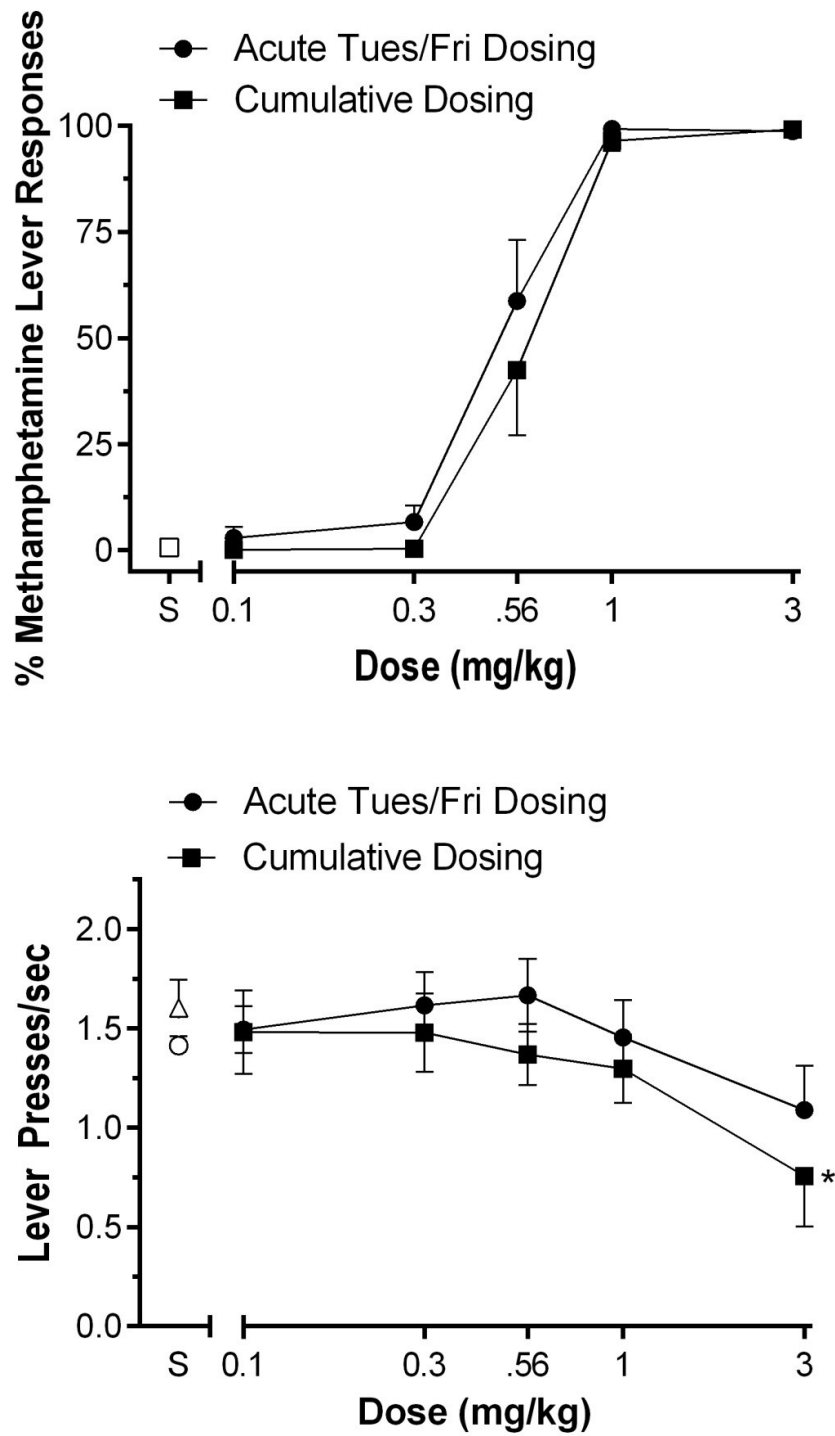
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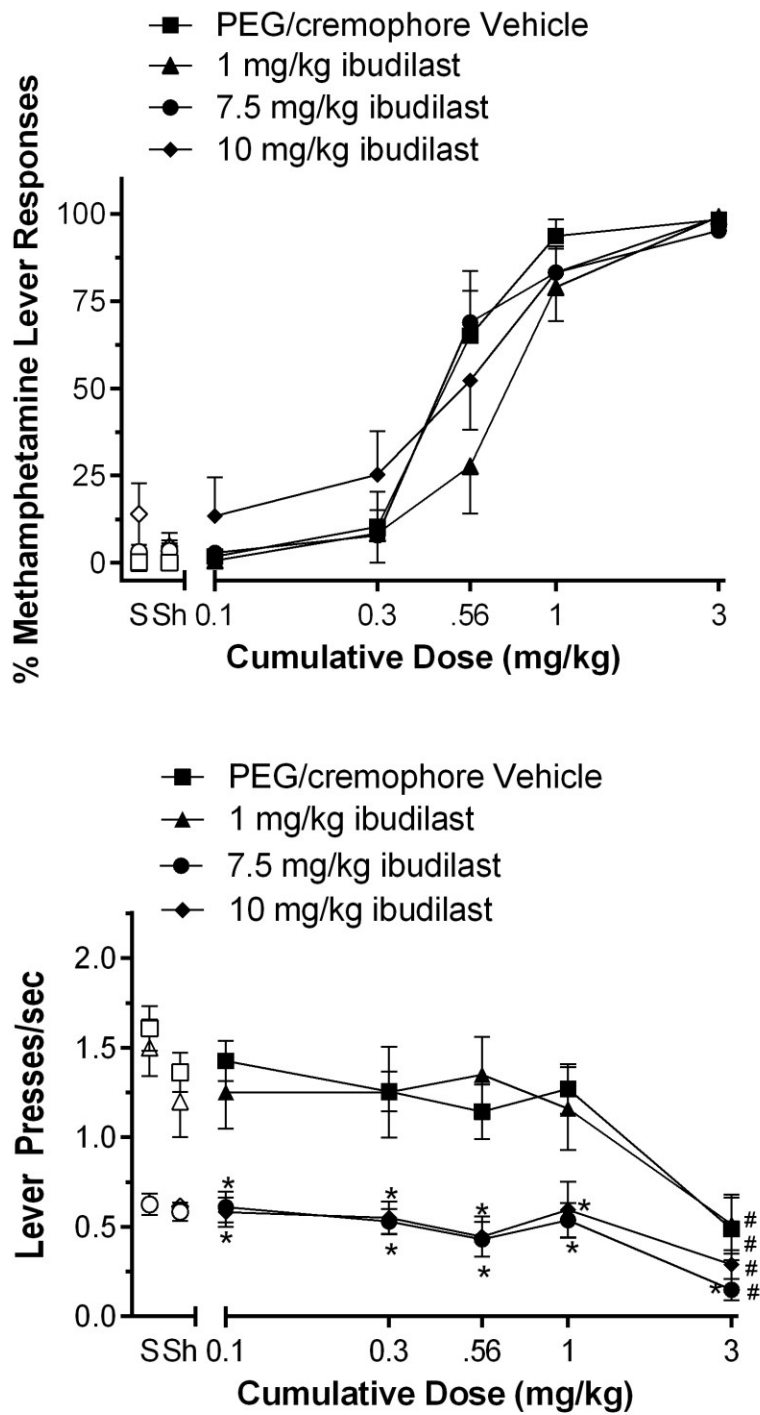
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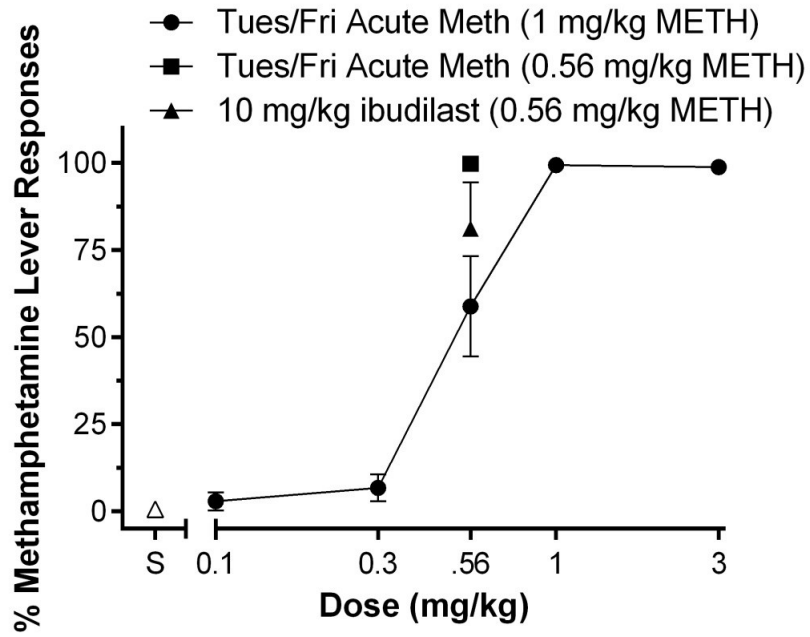
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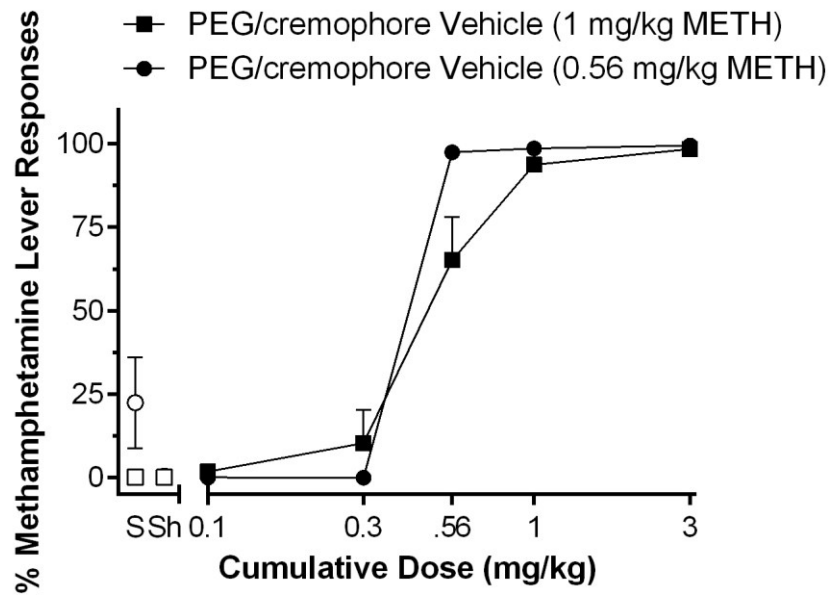
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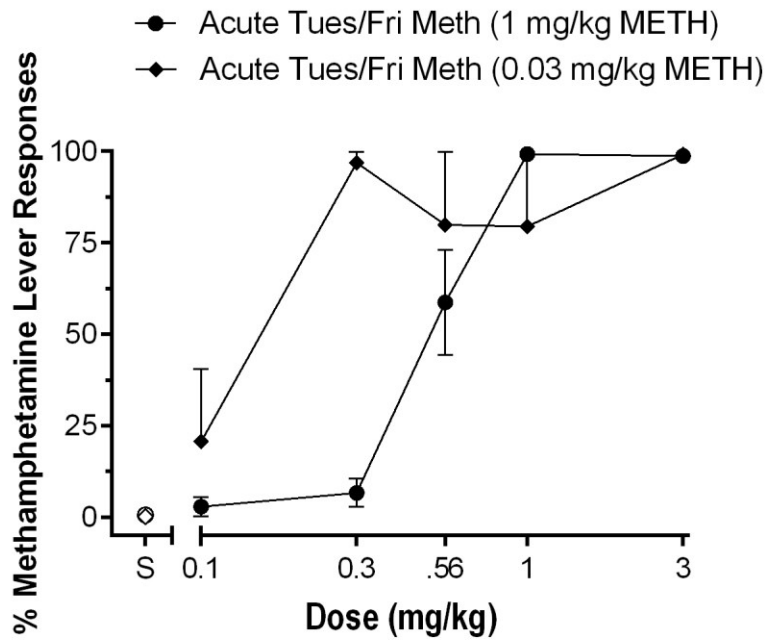
Chapter V: DISCUSSION

Introduction

The evidence provided above suggests that modulating glial cell activity, and consequently reducing glial cell associated neuroinflammation, and/or inhibiting PDE activity, may modulate behaviors in animal models presumably predictive of clinical behavior associated with drug abuse disorders. More specifically, the evidence suggests that attenuating microglial and astrocyte activation and its subsequent inflammatory events elicited by methamphetamine administration can reduce methamphetamine locomotor activity and sensitization, self-administration, but perhaps not drug discrimination. Furthermore, given the control procedures performed as a result of the self-administration study results, the data suggests that the glial modulating compounds are not acting in a rate dependent manner, but may not be specific for only reducing methamphetamine-specific behavior. Further studies are required in order to determine whether the initial reinforcing efficacy of alternative reinforcers is important in examining non-specific effects of potential pharmacotherapies.

Chapter II

Results from Chapter II indicated that ibudilast dose-dependently reduced the chronic and acute effects of methamphetamine on locomotor activity in the mouse. Chronic treatment with methamphetamine provided evidence of sensitization as subsequent administrations elicited greater increases in distance traveled. The highest dose of ibudilast (13 mg/kg) tested

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significantly attenuated these methamphetamine-induced sensitization effects. Ibudilast's analog, AV1013, which lacks its potency for inhibiting PDE, but retains its ability to suppress activated glial activity, similarly dose-dependently attenuated methamphetamine's chronic and acute locomotor activity effects, although it was ~ 6–9 fold less potent in doing so. These later observations suggest that the ability to modulate glial activity is sufficient to attenuate methamphetamine's locomotor activity effects, although PDE inhibition likely can additionally contribute if present.

Ibudilast is a non-selective PDE inhibitor (Kishi *et al.*, 2001; Gibson *et al.*, 2006), glial cell modulator and anti-inflammatory agent (Suzumura *et al.*, 1999; Mizuno *et al.*, 2004), and an inhibitor of macrophage migration inhibitory factor (Cho *et al.*, 2010). As such, its effects could be a result of any or a combination of all these mechanisms. Some of these effects have already been reported to reduce methamphetamine activity (see below). It is unlikely ibudilast's effects on methamphetamine are a result of directly affecting conventional mechanisms, for it doesn't have effective activity at ~100 other radioligand binding and enzyme targets (Ledeboer *et al.*, 2006).

PDE inhibition, by itself, significantly reduces some methamphetamine behaviors. PDE inhibitors, such as rolipram and nefiracetam, attenuate methamphetamine-induced locomotor activity, sensitization, and the discriminative stimulus effects of methamphetamine (Iyo *et al.*, 1995; Iyo *et al.*, 1996a; Iyo *et al.*, 1996b; Mori *et al.*, 2000; Yan *et al.*, 2004; Yan *et al.*, 2006).

Methamphetamine increases levels of cytokines and inflammatory factors, such as tumor necrosis factor (TNFα), interleukin 6 (IL-6), interleukin 1β (IL-1β) mRNA levels, monocyte chemo-attractant protein 1 (MCP-1), and intercellular adhesion molecule (ICAM-1) (Yamaguchi *et al.*, 1991; Nakajima *et al.*, 2004b; Goncalves *et al.*, 2008). Attenuation of glial cell activation and pro-inflammatory signaling, and up-regulation of neuroprotective factors, activities of both

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ibudilast and AV1013, have also been reported to attenuate some of methamphetamine's behavioral effects, including locomotor sensitization (Zhang *et al.*, 2006; Niwa *et al.*, 2007c). Conversely, reducing GDNF levels potentiates methamphetamine self-administration and reinstatement vulnerability (Yan *et al.*, 2007). GDNF is a neurotrophic factor best known for its role in cell survival and re-growth, especially in dopamine neurons, that has been recently implicated negatively regulating drug abuse behaviors (Carnicella & Ron, 2009). Ibudilast's anti-inflammatory action reduces glial activation by suppressing TNFα, IL-6, IL-1β, MCP-1, and nitric oxide (NO), while also increasing production of GDNF (Suzumura *et al.*, 1999; Mizuno *et al.*, 2004). Although AV1013 lacks the efficacy of PDE inhibition of ibudilast, it has similar glial cell modulatory activity (Cho *et al.*, 2010). Both ibudilast and AV1013 reduce methamphetamine-induced locomotor behavior, suggesting that AV1013's modulation of glial cell activation is sufficient to attenuate methamphetamine effects.

Lilius (2009) reported that ibudilast could induce decreases in spontaneous locomotor activity in rats following its acute administration. Although the Lilius study used rats, and the present study used mice, the possibility of direct locomotor decreasing effects needs to be considered in interpreting ibudilast's and AV1013's modulation of methamphetamine's locomotor activity effects. It is unlikely that these potential locomotor decreasing effects could explain the magnitude of their effects on methamphetamine's activity. Importantly, none of the dosage regimens of ibudilast or AV1013 produced statistically significant reductions in locomotor activity during either baseline test. Perhaps if there had been important locomotor decreasing effects of these drugs initially, tolerance developed to them, for the drugs were given b.i.d beginning two days prior to the initiation of testing, whereas in the Lilius study ibudilast was given acutely. Additionally, acute tolerance may have occurred as well, for in the Lilius study, ibudilast was administered 15 min before testing, whereas in the present study it was given one hour prior to

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locomotor tests, and sedative-like effects appear to wane within 30 min of its administration (Ledeboer *et al.*, 2006).

Both ibudilast and AV1013 attenuated methamphetamine-induced locomotor activity when administered concurrently with methamphetamine. These results suggest that these drugs potentially could blunt methamphetamine's stimulatory effects or "value" to chronic users potentially facilitating the effectiveness of other interventions such as psychotherapy. Additionally, ibudilast and AV1013 significantly attenuated the hyperactivity effects following acute methamphetamine challenge. Considering that limited re-exposure to an abused drug can precipitate a longer-termed relapse in an abstinent abuser (Bigelow *et al.*, 1977; Chornock *et al.*, 1992; de Wit, 1996), ibudilast and AV1013's attenuation of an acute methamphetamine challenge suggests usefulness as a relapse prevention treatment in abstinent abusers, which is consistent with a previous report that ibudilast reduces reinstatement precipitated by methamphetamine primes in rats previously reinforced with methamphetamine (Beardsley *et al.*, 2010). The possibility of clinically useful relapse prevention now extends to AV1013 as well.

The AD50 values for reducing the effects of the 3 mg/kg methamphetamine challenge dose did not differ within ibudilast and AV1013 groups between the acutely and chronically treated mice, suggesting that peak ability to blunt methamphetamine's effects was reached by two days of b.i.d. administration. This speculation requires the qualification that only a single methamphetamine dose (3 mg/kg) was tested, and administration of these drugs was not given for longer than seven days. Similarity between these AD50 values also suggests that tolerance to their effectiveness did not develop, a desirable feature in a potential pharmacotherapeutic.

Several additional observations strengthen the interest in these drugs. In the present study, repeated administration of methamphetamine-induced sensitization to its locomotor activity effects was significantly attenuated by 13 mg/kg ibudilast. It has been suggested that

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sensitization plays a key role in drug addiction in humans (Sax & Strakowski, 2001; Chen *et al.*, 2009b). For example, three doses of d-amphetamine given to healthy human volunteers produces significant increases in eye-blink and locomotor scores, as well as in reported mood and subjective drug effects (i.e. euphoria) suggestive of sensitization (Strakowski & Sax, 1998). In conjunction with the behavioral effects, the adaptations of specific brain regions implicated in the process of sensitization have been associated with reward pathways linked to drug-seeking and addiction (Robinson & Berridge, 1993). Thus, a compound that blunts sensitization may have additional merit for consideration as a pharmacotherapy for drug abuse. Furthermore, because the neurocircuitry, neurotransmitter, and neuronal receptor systems activated in reinstatement models of drug abuse are similar to those systems involved in the process of sensitization (Steketee & Kalivas, 2011), the potential usefulness of these drugs in treating methamphetamine relapse is even further enhanced.

Summary

The present study identified that both ibudilast and its analog, AV1013, are able to attenuate methamphetamine-induced locomotor activity in mice. Given AV1013's impotency to affect PDE activity, these results suggest that glial cell modulation alone may be sufficient for attenuating these methamphetamine effects. Treatments for stimulant abuse targeting conventional mechanisms have generally proven unsuccessful. The present results are consistent with others suggesting that modulating glial cell activity with drugs could provide a novel, and perhaps fruitful target for treating methamphetamine abuse.

Chapter III

Results from Chapter III reported that methamphetamine was established as a positive reinforcer and was self-administered under vehicle pretreatment conditions characterized by an inverted U-shaped curve relating infusion numbers to dose, with significantly more methamphetamine infusions being obtained at the intermediate dose (0.03 mg/kg/inf) during testing of all drugs, and at the highest dose (0.1 mg/kg/inf) during testing of ibudilast and AV1013, relative to those obtained of saline. Ibudilast (10 mg/kg), AV1013 (10 and 30 mg/kg) and minocycline (60 mg/kg) significantly reduced total 0.03 mg/kg/inf methamphetamine infusions compared to vehicle pretreatment conditions. These results suggest that modulating glial cell activity, and consequent neuroinflammatory processes, can modulate abuse-related effects of methamphetamine.

All three compounds, ibudilast, AV1013, and minocycline; reduced infusion rates for 0.03 mg/kg methamphetamine self-administration. None of the test drugs increased infusion rates of 0.001 mg/kg/inf, the lowest tested dose of methamphetamine and dose that was not self-administered under vehicle conditions. These observations suggest that the infusion-rate reducing effects of these drugs at the 0.03 mg/kg/inf dose of methamphetamine was not attributable to the test drugs "enhancing" the effects 0.03 mg/kg/inf methamphetamine to be functionally experienced as a higher dose (and thus, advancing it along the descending limb of the dose-effect curve). Instead, the data suggest that the effects promoting methamphetamine self-administration were diminished at 0.03 mg/kg/inf by the test compounds. None of the compounds, however, affected infusions maintained by the highest self-administered methamphetamine dose (0.1 mg/kg/inf). There are several possible levels of explanation for the lack of effect on 0.1 mg/kg/inf methamphetamine-maintained behavior, the first being at the neurochemical level. Methamphetamine's effects on glial cell activation and induction of pro-

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inflammatory signals have been well established (Yamaguchi *et al.*, 1991; Nakajima *et al.*, 2004b; Goncalves *et al.*, 2008). Additionally, methamphetamine's glial cell activation is associated with changes in behavior (Miguel-Hidalgo, 2009). In the present study, rats self-administered methamphetamine at an average of 3.7-4.5 mg/kg/2-h session when given access to the 0.1 mg/kg/inf dose, and an average of 2.74-3.2 mg/kg/2-h session methamphetamine at the 0.03 mg/kg/inf dose. Self-administration of both doses are likely high enough to produce pro-inflammatory conditions, as it has been shown that a single dose of 1 mg/kg methamphetamine administered subcutaneously produces a significant enhancement of cytokine and chemokine induction in mice (Loftis *et al.*, 2011). However, perhaps 0.1 mg/kg/inf methamphetamine produces a glial response "insurmountable" by the tested doses of ibudilast, AV1013, or minocycline. For instance, as the dose of methamphetamine increases, it may activate glial cells faster and promote more cytokine transcription to induce its neuroinflammatory effects. Thomas et al. (2004a) noted that there was a dose-dependent effect of methamphetamine on microglial activation in the mouse striatum. Thus, the test compounds may not be effective against those processes recruited at higher doses.

Relative potency analysis revealed that ibudilast was ~6 times more potent than AV1013, and ~13 times more potent than minocycline in reducing self-administration of methamphetamine. These data are consistent with my previous report that ibudilast is ~4-7 more potent in reducing methamphetamine-induced hyperlocomotion in mice than AV1013 (Snider *et al.*, 2012). These findings also support that while glial cell modulation is sufficient to attenuate some methamphetamine-induced behaviors, ibudilast's PDE inhibition activity may have acted in combination with glial suppression.

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Summary

The present study established methamphetamine self-administration at levels likely great enough to induce glial activation and pro-inflammatory signaling. Ibudilast, AV1013, and minocycline, three compounds that attenuate glial activity, all reduced self-administration of 0.03 mg/kg/inf methamphetamine. While the mechanism of these effects has yet to be definitively identified, the previously published data and the present report, strengthen the linkage between glial cell activation, neuroinflammation, and the behavioral effects of abused drugs.

Clarifying Ibudilast's Behavioral Mechanism of Action and Defining the Limits of its Effectiveness

Ibudilast attenuated rates of self-administration at the self-administered dose of methamphetamine that maintained the highest baseline response rate (0.03 mg/kg/infusion), and did not significantly affect self-administration of another self-administered dose that maintained a lower baseline rate (0.1 mg/kg/infusion). This raises the possibility that there was a response-rate determinant of ibudilast's effectiveness. Also, although ibudilast was effective in attenuating the locomotor activity and reinforcing effects of methamphetamine, it could not be assumed that it would attenuate all effects of methamphetamine. Because of these two observations, tests were conducted to determine if ibudilast's effects on methamphetamine self-administration were attributable to response rate determinants, and other tests were conducted to determine if its range of effectiveness and specificity in attenuating methamphetamine's effects.

A possible behavioral mechanism for the effectiveness of ibudilast for reducing 0.03 mg/kg/inf but not 0.1 mg/kg/inf methamphetamine self-administration may be attributable to a rate dependent effect. Dews (1955) reported that a dose of pentobarbital that increases low

reinforced rates of responding can also decrease high rates of responding. Thus, a drug's effect on behavior can be a function of the baseline response rate. In the present study, total responses (and infusions) emitted during the sessions in which responding was maintained by 0.03 mg/kg/inf methamphetamine were greater than that maintained by 0.1 mg/kg/inf methamphetamine. Thus, it may be argued that the pre-treatment compounds differentially affected responding because baseline responding (i.e. total responses and infusions obtained under vehicle-treatment conditions) differed between methamphetamine doses to start. However, this explanation is unlikely because when response rates were specifically matched in rats maintained by 0.03 and 0.1 mg/kg/inf methamphetamine during Rate Dependency Analysis tests, 10 mg/kg ibudilast still did not reduce response rates for 0.1 mg/kg/inf methamphetamine. Furthermore, the response rate reductions at 0.03 mg/kg/inf methamphetamine were not likely a result of non-specific behavioral suppression because total infusions (and consequently, response rate) following pretreatment with ibudilast did not differ from baseline rates at either the lowest or highest tested methamphetamine doses in the present study (and they would have been if there was non-specific behavioral suppression) nor did ibudilast or AV1013 significantly suppress locomotor activity in mice when tested alone under similar dosage regimens (Snider *et al.*, 2012). Ibudilast has been reported, however, to produce transient sedation and decreased reactivity to touch in the Irwin test in Wistar (Han) rats (Ledeboer *et al.*, 2006).

In observing the effectiveness of ibudilast reducing methamphetamine self-administration, the question emerges regarding the degree to which ibudilast's effectiveness is restricted to methamphetamine-maintained responding. Considering that ibudilast is a PDE inhibitor with likely broad-ranging effects, and that its modulation of glial activity could also have broad-ranging down-stream effects, ibudilast is unlikely to be *solely* specific in affecting methamphetamine-maintained as opposed to other reinforcer-maintained responding. It is

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important, if only from a drug developmental view, to begin to characterize the range of reinforced behavior ibudilast can affect. Given these considerations, the effects of ibudilast on banana pellet maintained-behavior were examined. It was hypothesized that the baseline strength of behavior (i.e., the efficacy of the reinforcer) could determine whether or not ibudilast reduced responding. This was somewhat suggested by the observation that ibudilast reduced responding maintained by 0.03 mg/kg/inf methamphetamine, and not at the higher dose of 0.1 mg/kg/inf, and often the higher the dose of a self-administered drug, the greater the reinforcing efficacy (Griffiths *et al.*, 1979). To ensure similar baseline strengths of banana- and methamphetamine-maintained responding, a behavioral economic approach was used to help equate them. The baseline reinforcing strength (α level) of both non-nutritive banana flavored cellulose pellets (banana pellets) and methamphetamine self-administration were successfully equated. The baseline reinforcing strengths of these two commodities were generated while both reinforcers were controlled within closed economies.

When tested on a FR1 schedule, all compounds that significantly attenuated 0.03 mg/kg/inf methamphetamine-maintained responding also attenuated banana pellet consumption, with the exception of 10 mg/kg AV1013. These findings possibly suggest that an intermediate dose of AV1013 may be the best potential pharmacotherapeutic as it is the most selective for methamphetamine-induced behaviors without affecting non-drug-maintained behavior. The initial hypothesis asserted that none of the test compounds would alter food-maintained behavior because banana pellet consumption does not inherently induce glial cell activation and neuroinflammatory activity. There are at least two opposing conclusions that could be drawn from the gathered results. First, all three test compounds affected both methamphetamine and food maintained behavior, thus glial cell attenuation could reduce behavior maintained by many reinforcer types through a similar mechanism. Mild chronic food

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restriction (85% body weight restriction), however, does not cause glial cell activation; in fact, chronic food restriction attenuates age-related astrocyte and microglial activity (Morgan *et al.*, 1999). Hence, it is likely that the test compounds are not attenuating a banana pellet-induced inflammatory action to reduce reinforcer-maintained behavior. Furthermore, while the animals used for this study had a history of methamphetamine self-administration and abstinent human methamphetamine abusers show protracted microglial activation (Sekine *et al.*, 2008), the activation is negatively correlated with time of abstinence (Sekine *et al.*, 2008) and can return to baseline levels in rodent subjects in as little as seven days (Thomas *et al.*, 2004c).

A second conclusion that could be drawn from the behavioral economic study results is that all three of the test compounds are exhibiting non-specific effects in which all behavior is suppressed. As discussed above, while there was no significant effect on locomotor activity in mice by ibudilast or AV1013, ibudilast (7.5 and 10 mg/kg) did significantly suppress response rates for food pellet delivery in the drug discrimination studies. Minocycline (56 mg/kg) causes locomotor activity suppression at 100 mg/kg i.p. (Kofman *et al.*, 1990) and suppresses response rates for food pellet reinforcer delivery in a drug discrimination paradigm but response rates are only moderately affected by 32 mg/kg minocycline (Munzar *et al.*, 2002). Symptoms such as light-headedness, headaches and nausea have all also been reported as side effects of high doses of minocycline in humans (Gump *et al.*, 1977). However, disentangling the relationship between methamphetamine's, or any drug's, specific effects on drug maintained behavior and those of "non-specificity" is complex at best. The importance of determining this specificity should be based on the usefulness of the drug's outcome as a potential pharmacotherapeutic. If a particular compound reduces drug self-administration by anesthetizing the animal, disentangling that relationship is a vital component to determine for the progression of that compound as a therapeutic tool. However, there are commonly prescribed

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pharmacotherapeutics that engender non-specific effects (i.e. side effects). For example, methadone is a widely utilized pharmacotherapeutic agent that has been used to help treat opiate dependence for almost 50 years (Dole & Nyswander, 1965). Methadone maintenance treatment also significantly suppresses food intake in rhesus monkeys, in fact with greater specificity than it suppresses heroin self-administration (Mello *et al.*, 1983). Thus, it may be argued that suppression of food intake does not necessarily eliminate a compound from consideration as a potential therapy, but rather adds important information to its pharmacological profile to be considered in future studies.

Can Baseline Strength Determine Sensitivity to a Drug's Effect

To evaluate the assumption that a drug would differentially affect reinforced behavior when differential baseline strengths were involved, 45 mg food pellets were used as the reinforcer when subjects were maintained at three different body weight conditions (85, 90 and 100% BW). Initial food pellet reinforcer demand curves indicated that the α level for food when maintained at 85% BW produced significantly more demand than when animals were maintained at 90% and 100% BW, indicating that the baseline strength (reinforcing efficacy of food pellets) at 85% was significantly stronger. When minocycline (30 & 60 mg/kg) was administered, there was a significant decrease in total consumption of food compared to vehicle, however there was no significant effect of body weight condition. Likewise, 10 mg/kg buspirone produced similar results in significantly decreasing total consumption of food independent of body weight condition.

The differences between the α levels obtained at 85, 90%, and 100% BW conditions during the initial reinforcing strength assessment, while statistically significant, displayed a

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narrow window in demand between which to see differential effects of drug on food-maintained responding. Thus, widening the window between the initial demand conditions was hypothesized to allow for a better evaluation of differential drug effects. When animals' percent body weight was increased to 115%, they were re-tested under two intermediate doses of buspirone. Again, while the demand for food between the 85% and 115% body weight conditions was statistically different, there was no main effect of body weight condition under buspirone treatment. The possibility of being below threshold for detecting differences under these conditions remains a plausible possibility, however. Any attempt at widening the range between α values by manipulation of body weight was precluded by concerns of maintaining the animals' health. A more thorough evaluation of the possibility that baseline strength can be a determinant of the sensitivity to a drug's effect (while having a broad range between α values) should involve reinforcers not directly essential for health, although this would likely necessitate specialized reinforcer delivery equipment that was not available during the present tests.

General Conclusions

While seemingly intuitive to assess the effects of test compounds on responding maintained by a non-drug reinforcer (i.e. non-nutritive banana pellets) in this manner, the limitations of using banana pellets as a reinforcer may preclude further analyses using this approach. For example, animals were observed to respond for but not necessarily eat all earned banana pellets during a given session, even while maintained at 85% of their free-feeding body weight. Thus, it could be argued that the animals' responding was maintained by conditioned reinforcers other than the banana pellet delivery. However, the purpose of generating α levels for methamphetamine and banana pellet maintained responding was to approximate the baseline strength of responding for two distinct reinforcers. Whether those

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reinforcers were the primary reinforcers themselves (i.e. methamphetamine or banana pellets) or conditioned stimuli does not matter. Baseline strengths for methamphetamine and banana pellet maintained responding were also matched under incongruent experimental stimuli (i.e. active lever, lights, tones), to reduce the probability conditioned stimuli from a methamphetamine self-administration history would confound response strengths. Actual consumption of the banana pellets was inconsequential to the question posed. The test compounds were still assessed against two different reinforcer types while they were maintained under similar reinforcing strength regardless of what unconditioned or conditioned stimuli the animals were responding to or as a result of.

Secondly, while differences between α levels for pellet delivery at different body weights was significantly different in tests with nutritive food-pellets as the reinforcer, the range of α levels was relatively tight perhaps limiting detection of differential drug effects. Widening the differences in initial baseline strength would require further food restriction and/or a pre-feeding procedure that might compromise the health of the animals or add confounding variables.

An assessment of any test compounds' effects on food maintained behavior is still a valuable addition to basic information surrounding a potential pharmacotherapy. Thus, as an alternative approach, food (or food-like) reinforcers may be examined concurrently with methamphetamine in a choice procedure. Test compounds could then be assessed for their ability to re-allocate methamphetamine maintained responding to food maintained responding. For example, when cocaine and food are concurrently available, administration of monoamine releaser compounds produce right-ward shifts in the cocaine choice dose response curve (Negus, 2003; Banks *et al.*, 2011) indicating a reduction in cocaine maintained behavior when food is also available. This approach could be developed by first determining whether the two reinforcers in question interact with one another before posing the choice. Those interactions, in

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behavioral economic terms could include two commodities acting as complements, substitutes or independents.

Behavioral economics provides ways for describing interactions between two reinforcers such as drug and food in choice procedures (Hursh, 1978; Elsmore *et al.*, 1980; Bickel *et al.*, 1995). Two reinforcers available concurrently may act as substitutes, complements, or independent reinforcers (Hursh, 1980; Hursh *et al.*, 2005). The two reinforcers are considered substitutes if consumption of commodity B increases with an increase in price of commodity A (Hursh, 1980). Reinforcers are complements when consumption of commodity B decreases with increases in the price of commodity A (Hursh, 1980; Hursh & Roma, 2013) indicating that one reinforcer affects the reinforcing strength of the second reinforcer. Finally, the price of each independent reinforcer has no effect on the consumption of the other (Hursh, 1980; Hursh & Roma, 2013). In order to ascertain the specificity of a test compound on attenuating only methamphetamine's reinforcing strength, and not food, the two reinforcers would first need to be matched in initial baseline strength and assessed as substitutes, complements, or independent reinforcers in a behavioral economic paradigm. This would theoretically allow for an initial baseline in which reinforcing strength is equated and interactions between the two commodities are noted and controlled for. Then, administration of the test compounds during concurrent choice of both reinforcers might elucidate whether the test compounds can re-allocate responding to and from drug to food-maintained behavior and interactions of substitutes or complement reinforcers does not confound a shift in choice.

Rats trained to discriminate 1 mg/kg methamphetamine from saline emitted progressively more methamphetamine-lever responses as methamphetamine dose increased until at the training dose and above, nearly 100% of lever presses were emitted on the drug-associated lever. These results are consistent with previous reports of methamphetamine drug discrimination in rats. Methamphetamine drug discrimination is well established, and methamphetamine is known to substitute for other stimulants such as ephedrine, cocaine, methylphenidate, d-amphetamine (Schechter, 1997b; Bondareva *et al.*, 2002; Sevak *et al.*, 2009). Further, SKF77434 (a partial D1 agonist), SCH39166 (selective D1 antagonist), varenicline (nicotinic agonist), pentobarbital, aripiprazole, and clomipramine (serotonin re-uptake inhibitor) all attenuate methamphetamine or amphetamine drug discrimination (Tidey & Bergman, 1998; Gatch *et al.*, 2005; Lile *et al.*, 2005; Desai & Bergman, 2010). Furthermore, and most pertinent for the present studies, nefiracetam and rolipram, two PDE inhibitors, reduce methamphetamine drug discrimination in rats (Yan *et al.*, 2004; Yan *et al.*, 2006). Given that ibudilast also engenders PDE inhibitory activity, ibudilast was hypothesized to attenuate methamphetamine's discriminative stimulus properties.

While drug discrimination procedures conventionally utilize a single test drug administration during test sessions (Brady & Balster, 1980), cumulative dosing procedures, in which multiple administrations of test drug occur between periods of reinforcer availability within a single test session, are also used (Walker & Young, 1993). Direct comparison of these two types of procedures produce similar dose response relationships and ED50 values for drug-paired lever responding (Schechter, 1997a). Cumulative dosing testing procedures thus allows for rapid data collection and flexibility between procedure types. Similarly, in the present study, there were no significant differences between ED50s of methamphetamine generalization

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between the acute and cumulative dosing procedures. Thus, the cumulative dosing procedure was utilized to assess ibudilast's effects on methamphetamine drug discrimination.

Ibudilast did not significantly alter methamphetamine drug discrimination when the animals were trained with 1 mg/kg methamphetamine. There are several possibilities for why this might be the case including both neurochemical and behavioral explanations.

First, drug discrimination is proposed as a pre-clinical model for the subjective effects of a drug (Schuster, 1976; Brady & Balster, 1981). The present study hypothesized that methamphetamine's glial and related neuroinflammatory effects are involved with methamphetamine's discriminative stimulus effects, and these stimulus effects could be reduced by attenuating the associated glial effects with the chosen test compounds. Given the present results, it remains possible that while methamphetamine-induced neuroinflammation may be involved with some aspects of abuse-related behaviors, such as locomotor sensitization and self-administration, it may not be involved with methamphetamine's discriminative stimulus effects. The drug discrimination procedure can be used to determine the mechanism by which drugs of abuse produce their interoceptive effects (Balster, 1990). Thus, perhaps the proposed indirect neurochemical mechanism through which glial cell modulation affects methamphetamine-induced behavior (see below) is separate from affecting interoceptive cues. In addition to its glial cell modulatory and anti-inflammatory activity, ibudilast is also a non-selective PDE inhibitor for PDEs 3, 4, 10, and 11 (Kishi *et al.*, 2001; Gibson *et al.*, 2006). Rolipram and nefiracetam, two selective PDE4 inhibitors, have been reported to attenuate methamphetamine drug discrimination (Yan *et al.*, 2006). While ibudilast is not more selective for PDE4 over the others, it has relatively similar binding affinity to that of rolipram at all PDE4 isotypes (Gibson *et al.*, 2006). Thus, ibudilast's selectivity is likely not the reason it did not affect methamphetamine discrimination while rolipram and nefiracetam were reported to do so.

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Behaviorally, previous literature has shown that it is plausible to have a disconnection in effects between self-administration and drug discrimination involving the same compound because the two procedures are measuring different pharmacological effects (Woolverton & Balster, 1982). Experimenter-administered test compounds, such as used in drug discrimination procedures, compared to actively self-administered drug, such as in typical self-administration procedures, can elicit different behavioral, neurochemical, and even proteomic differences (Jacobs *et al.*, 2003). Thus, these potential differences could explain the dissociation between ibudilast significantly affecting self-administration yet not drug discrimination in the present study. Finally, drug discrimination training and tests with ibudilast occurred using a 1 mg/kg methamphetamine training dose in the present study. However, the training dose utilized during a drug discrimination procedure can be a pivotal component of the assay. For example, progressively lowering a PCP training dose produces marked decreases in the ED50 for stimulus generalization and parallel leftward shifts of the dose response curves indicative of greater stimulus generalization at lower training doses (Beardsley *et al.*, 1987). Further, an antagonist can more readily disrupt discrimination of a particular drug when trained at a low training dose (Picker *et al.*, 1993). Yan et al (2006) trained animals to discriminate 0.5 mg/kg methamphetamine and tested under 0.2 mg/kg methamphetamine conditions. Thus, in order to better compare ibudilast's to rolipram and nefiracetam's effects on methamphetamine drug discrimination, the subjects were re-trained in the present study at lower methamphetamine training doses (0.56 and 0.3 mg/kg).

Rats re-trained at 0.56 mg/kg methamphetamine were probed with 10 mg/kg ibudilast pretreatment at the 0.56 training dose and there were no significant effects on discrimination. Further analysis with the cumulative dosing procedure revealed that under ibudilast's vehicle conditions, the training dose was the lowest methamphetamine dose to completely occasion the

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0.56 mg/kg discriminative stimulus. Generating the entire dose response curve allowed for the possibility that 0.3 mg/kg methamphetamine might produce partial generalization to the training dose, in which case ibudilast would have been tested to determine if it could reduce generalization. However, 0.3 mg/kg produced near zero methamphetamine appropriate lever responding eliminating the opportunity to continue testing ibudilast under those conditions.

Finally, the remaining animals were retrained at 0.3 mg/kg methamphetamine. While the animals eventually acquired the discrimination, performance during their training sessions were not as stable or consistent as compared to when animals were trained at 1 mg/kg methamphetamine. These findings are consistent with previous reports in that progressively lower doses of a training dose increase the number of training sessions required to acquire the discrimination and the number of errors occurring on training days following acquisition (Beardsley *et al.*, 1987). Generalization test results indicated that 0.56 and 1 mg/kg methamphetamine, doses higher than the 0.3 mg/kg training dose, failed to produce full generalization to the 0.3 mg/kg training stimulus, and this uncharacteristic result of well-trained discriminative performance suspended enthusiasm to proceed with ibudilast testing.

In sum, ibudilast did not significantly affect methamphetamine generalization when involving cumulative dosing procedures when rats were trained at 1 mg/kg methamphetamine, or when tested with an acute dose of 0.56 mg/kg methamphetamine when animals were retrained at 0.56 mg/kg. Stimulus control was not stable enough to warrant further testing with animals trained at either 0.56 or 0.3 mg/kg.

Potential mechanisms connecting methamphetamine, PDE, inflammation, and behavior

How methamphetamine promotes neuroinflammation precisely is not yet known. Reactive oxygen species, substance P, and dopamine quinones contribute to

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methamphetamine-induced cellular damage and apoptosis (Fleckenstein *et al.*, 1997; Zhu *et al.*, 2006). As a result of the damaged cells and neurotoxicity, astrocytes and microglia become activated and elicit an immune response and increase pro-inflammatory cytokine production (Kita *et al.*, 2008; Clark *et al.*, 2012). While cell damage and death is a common catalyst for inflammation induction, methamphetamine's effects on inflammatory pathways can also temporally occur before dopamine cell terminal pathology (LaVoie *et al.*, 2004). Furthermore, there is growing evidence that psychostimulants can activate both astrocytes and microglial cells directly through a variety of mechanisms (Beardsley & Hauser, 2014). Thus, methamphetamine-induced inflammation can occur at non-neurotoxic levels and independently of cell damage. One mechanism that has been proposed for methamphetamine-induced inflammation via the nuclear transcription factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Shah *et al.*, 2012). Methamphetamine's release of excitatory neurotransmitters activates the metabotropic glutamate receptor, mGluR5. mGluR5 is described to activate the intracellular signaling pathway, AKT/PI3K, that downstream induces the release of NF-κB, which, in turn, translocates to the nucleus to promote transcription of inflammatory cytokine proteins such as TNFα, IL-6 and IL-8 (Shah *et al.*, 2012).

Bacterial lipopolysaccharide (LPS) is a gram-negative endotoxin that stimulates inflammation via toll-like receptor-4 (TLR-4) (Chow *et al.*, 1999). Methamphetamine and LPS both induce inflammation through the AKT/PI3K pathways and induce NF-κB to translocate to the nucleus and promote transcription of inflammatory cytokines (Ojaniemi *et al.*, 2003; Shah *et al.*, 2012). Methamphetamine exacerbates LPS's inflammatory signal (Liu *et al.*, 2012a). These effects are likely attributable to both compounds acting via NF-κB, MAPK, and AKT/PI3K pathways (Liu *et al.*, 2012a). Ibudilast and AV1013 antagonize macrophage migration inhibitory factor (MIF) (Cho *et al.*, 2010), a pro-inflammatory factor essential for TLR-4 function and

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inflammatory response (Roger *et al.*, 2001). If LPS and methamphetamine's inflammatory signals are similar, ibudilast and AV1013's antagonism of the TLR-4 receptor via modulation of MIF may be one mechanism in which these compounds are reducing cytokine production and inflammation. Furthermore, ibudilast reduces nuclear translocation of p65, a subunit of NF-κB, potentially suggesting a mechanism for ibudilast's reduction of MIF (El-Hage *et al.*, 2014). AV1013's minimal PDE inhibitory effects could contribute to its lower efficacy relative to ibudilast in attenuating methamphetamine induced locomotor activity in mice and on-going self-administration in rats. AV1013's lower efficacy in these assays could be due to reduced potency at the drugs' glial targets. AV1013 is a less potent inhibitor of MIF with a Ki = 74.9 (± 8.5) μM than is ibudilast, which has a Ki of 30.9 (± 2.8) μM (Cho *et al.*, 2010). Given this, AV1013 may be less effective in reducing methamphetamine activity due to a combination of decreased potency at both PDE and glial targets. Interestingly, morphine's inflammatory response occurs when the glycoprotein, MD-2, forms a complex with TLR-4 and induces inflammation similar to LPS (Wang *et al.*, 2012a), thus providing evidence for ibudilast's mechanism of action in reducing opioid-induced inflammation and behavior as well.

Minocycline also inhibits pro-inflammatory action of microglia without affecting the anti-inflammatory functionality of the cells (Kobayashi *et al.*, 2013). Minocycline's proposed mechanism also includes interaction with LPS and the NF-κB pathway. Minocycline prevents LPS induced degradation of IκBα, an inhibitory factor, which ultimately prevents NF-κB translocation to the nucleus and induction of inflammatory cytokine production (Nikodemova *et al.*, 2006). Minocycline also decreases binding of NF-κB to DNA which disrupts transcription (Bernardino *et al.*, 2009). Thus, all three test compounds are hypothesized to inhibit inflammation and methamphetamine-induced behaviors via a similar neurochemical pathway.

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How attenuation of glial cell activation and neuroinflammatory activity translates into modulating methamphetamine's behavioral effects is also unknown. However, stimulant-activated glial cells can respond, as a result, to influence the behavioral effects of the drug (Beardsley & Hauser, 2014). One proposed mechanism involves the ability of glial cells to regulate neurotransmission and synaptic strength by affecting the cell surface delivery and retention of glutamatergic NMDA and AMPA receptors (Eroglu & Barres, 2010). Interestingly, an up-regulation of TNFα, elicited from activated astrocytes, increases AMPA receptor expression on the cell surface and increases NMDA and AMPA receptor-mediated synaptic currents (Beattie *et al.*, 2002; Stellwagen & Malenka, 2006) that improves synaptic efficacy. Conversely, blockade of TNFα has the opposite effect (Beattie *et al.*, 2002). Therefore, methamphetamine-induced increases in TNFα could indirectly increase the concentration of AMPA receptors and their activation. In contrast, ibudilast and AV1013's attenuation of TNFα levels would inhibit delivery of these receptors preventing signaling and synaptic change. Excessive activation of both AMPA and metabotropic glutamate receptors may play a role in behavioral sensitization and in the rewarding properties of stimulants such as cocaine and methamphetamine (Wolf, 1998). Thus, the blockade of these processes may be a link to suppressing the effects of stimulant drugs.

What about PDE inhibition?

Increases in cAMP, by a PDE inhibitor like rolipram for example, reduce microglial activation as well (Atkins *et al.*, 2007). TNFα levels may also be affected via cAMP production, which reduces further TNFα synthesis (Kast, 2000; Shames *et al.*, 2001). Thus, under ibudilast treatment, increasing cAMP and inhibiting TNFα synthesis both work to reduce further glial activation. Consistently, elevations in inflammatory cytokines such as TNFα and IL1-β reduce

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the level of cAMP in microglia that can be reversed by PDE inhibition (Ghosh *et al.*, 2012) suggesting that PDE inhibition can be a potential therapeutic target even after the initial inflammatory response has occurred.

Amphetamine derivatives, including methamphetamine and MDMA, have been identified as agonists for an orphaned GPCR, the rat trace amine receptor (TAAR1), and work to up-regulate cAMP release (Bunzow *et al.*, 2001). TAAR1 is widely distributed and co-expresses with both DAT and D2 receptors on DA neurons (Xie & Miller, 2007; Espinoza *et al.*, 2011). Cells incubated in 8-Bromo-cAMP mediated a PKA-dependent up-regulation in DAT uptake of extracellular substrates (Batchelor & Schenk, 1998; Page *et al.*, 2004) indicating cAMP's importance in DAT functionality. Further, MDMA was suggested to auto-inhibit dopaminergic transmission via recruitment of TAAR1 (Di Cara *et al.*, 2011) suggesting that TAAR1 is a negative modulator of DAT (Xie & Miller, 2007). Interestingly, TAAR1 knockout mice exhibited significantly more sensitization to amphetamine than wild type mice and TAAR1 decreased the firing rate of DA neurons in the VTA suggesting that TAAR1 is also a negative modulator of the behavioral effects of amphetamines (Lindemann *et al.*, 2008). With ibudilast administration it remains a possibility that an increase in cAMP, given its PDE inhibitory activity, might act to recruit TAAR1 receptors to negatively modulate methamphetamine's neurochemical and behavioral effects. In sum, perhaps ibudilast's PDE inhibition and glial modulatory effects are working in conjunction to produce the observed results.

Future Directions

Ibudilast and AV1013 are suggested to inhibit the p65 subunit of NF-κB which subsequently inhibits its translocation to the nucleus and subsequent pro-inflammatory release

(El-Hage *et al.*, 2014). However, is there a molecular correlation between inflammatory activity expression and behavior? The literature has suggested that a single bolus dose of 1 mg/kg methamphetamine administered subcutaneously produces a significant enhancement of cytokine and chemokine induction in mice (Loftis *et al.*, 2011). If separated into individual treatment groups, an important future direction would be to examine the ability of daily methamphetamine self-administration to produce cytokine and chemokine induction, as well as the ability of the test compounds ibudilast, AV1013, and minocycline to reduce that induction in vivo. Further analysis of these hypotheses would include an examination of the molecular time-course of methamphetamine's induction of glial cell activation, the test compounds' ability to reduce it, and whether a tolerance to the immune reduction occurred following chronic treatment.

In order to further clarify the range of behavior, beyond that modified by methamphetamine, that these glial cell modulators attenuate it would be important to expand upon the banana pellet and food-maintained behavior studies. The banana pellet studies suggested that the test compounds do not just solely reduce methamphetamine-affected behavior. The second control procedure attempted to examine drug effects on differential reinforcer demand, however there were health-related and potentially behavioral limitations using food reinforcement. An expansion of this line of study might include testing alternative reinforcers when baseline reinforcing efficacy is matched to that of self-administration. For example, would ibudilast attenuate a reinforcer that is not consumable such as intracranial self-stimulation (ICSS)? Another advantage to using ICSS would be the ability to differentially control a wider range of baseline strengths of behavior by altering the frequency of stimulation.

Finally, in addition to the development of a potential pharmacotherapeutics for treating drug-abuse behavior, there may also be some validity in exploring the possibility of alternative

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interventions such as healthy diet regimens to reduce inflammatory activity in the CNS. Thus, would altering an abuser's diet and nutrition be enough to reduce drug abuse behavior? The literature suggests that dibenzocyclooctadiene lignans from the fruit of *Schisandra wilsoniana,* a plant grown in Asia, attenuates agonist-induced action of the TLR2/4 receptor on microglia by inhibiting MAPK and NF-κB pathways (Park *et al.*, 2013). More readily recognizable foods available in the US, including pomegranate (Rojanathammanee *et al.*, 2013) onion, oregano, and red sweet potato, also show the ability to reduce microglial activation even following standardized "cooking" preparation (Gunawardena *et al.*, 2014). Cinnamon and fresh ginger also exhibit anti-inflammatory activity and attenuate microglial activation via the NF-κB pathway (Ho *et al.*, 2013a; Ho *et al.*, 2013b). Thus, while an alteration in an addict's diet may likely not produce full abstinence, perhaps different types of nutrition programming used in conjunction with behavioral and/or pharmacological therapies could be beneficial in sustaining abstinence and reducing the risk of relapse.

Finally, the growing literature regarding the linkage between glial cell modulators affecting stimulant abuse behavior has initiated the study of both ibudilast and minocycline in clinical trials. Minocycline significantly reduced the "feel good drug effects" and "I feel high" subjective ratings of d-amphetamine in an outpatient procedure using non-dependent healthy volunteers (Sofuoglu *et al.*, 2011). Furthermore, ibudilast is now in Phase IIb clinical trials to assess its safety and efficacy in treatment seeking methamphetamine dependent volunteers half of whom are also HIV positive. This trial will also be sufficiently powered to ascertain whether ibudilast can significantly improve methamphetamine abstinence over the two-week treatment period (Johnson & Iwaki, 2014).

Conclusions

Methamphetamine's induction of glial cell activation and neuroinflammatory activity causes effects in the periphery, CNS, and on behavior. The present dissertation supports evidence that there is a linkage between glial cell modulation and abuse-related behavior. The glial cell modulators ibudilast and AV1013 were observed to significantly attenuate methamphetamine-induced locomotor activity and sensitization in mice. In addition, ibudilast, AV1013, and minocycline significantly attenuated methamphetamine self-administration in rats. The range of abuse-related behavior that ibudilast could affect, however, had limits in that it did not alter methamphetamine's discriminative stimulus effects.

Each of these three assays illustrates a different component of drug abuse and thus all three were important to assess as a collection. The results suggest that glial cell modulation affects drug abuse-related behaviors associated with methamphetamine-induced hyperactivity, sensitization and drug seeking, however they do not suggest a linkage with the subjective cues of methamphetamine intoxication. Furthermore, studies utilizing a behavioral economics approach indicated that these glial cell modulators may also affect behavior maintained by alternative reinforcers, such as non-nutritive banana pellets, in which case further studies may be warranted to assess the extent to which these compounds affect behavior maintained by other reinforcers. In summary, compounds that modulate glial cell activation and neuroinflammatory activity appear to be associated with some methamphetamine abuse-like behaviors, and while there is more to understand regarding these mechanisms, compounds such as those tested may provide novel targets for potential drug abuse pharmacotherapeutics.

References

Agrawal, R.G., Hewetson, A., George, C.M., Syapin, P.J. & Bergeson, S.E. (2011) Minocycline reduces ethanol drinking. *Brain Behav Immun*, **25 Suppl 1**, S165-169.

Ang, E., Chen, J., Zagouras, P., Magna, H., Holland, J., Schaeffer, E. & Nestler, E.J. (2001) Induction of nuclear factor-kappaB in nucleus accumbens by chronic cocaine administration. *J Neurochem*, **79**, 221-224.

Araque, A., Parpura, V., Sanzgiri, R.P. & Haydon, P.G. (1999) Tripartite synapses: glia, the unacknowledged partner. *Trends in neurosciences*, **22**, 208-215.

Atkins, C.M., Oliva, A.A., Jr., Alonso, O.F., Pearse, D.D., Bramlett, H.M. & Dietrich, W.D. (2007) Modulation of the cAMP signaling pathway after traumatic brain injury. *Experimental neurology*, **208**, 145-158.

Ator, N.A. (1991) Discriminative stimulus effects of the novel anxiolytic buspirone. *Behavioural pharmacology*, **2**, 3-14.

Balster, R.L. (1990) Perception of Drug Effects. In Berkley, M.A.a.S., W. C. (ed) *Comparative Perception Vol 1. Basics Mechanisms*. John Wiley & Sons, Inc, New York, pp. 127-154.

Banks, M.L., Blough, B.E. & Negus, S.S. (2011) Effects of monoamine releasers with varying selectivity for releasing dopamine/norepinephrine versus serotonin on choice between cocaine and food in rhesus monkeys. *Behavioural pharmacology*, **22**, 824-836.

Barbour, B., Brew, H. & Attwell, D. (1988) Electrogenic glutamate uptake in glial cells is activated by intracellular potassium. *Nature*, **335**, 433-435.

Batchelor, M. & Schenk, J.O. (1998) Protein kinase A activity may kinetically upregulate the striatal transporter for dopamine. *J Neurosci*, **18**, 10304-10309.

Beardsley, P., Shelton, K., Hendrick, E. & Johnson, K. (2010) The glial cell modulator and phosphodiesterase inhibitor, AV411 (ibudilast), attenuates prime- and stress-induced methamphetamine relapse. *Eur J Pharmacol*, **637**, 102-108.

Beardsley, P.M., Balster, R.L. & Salay, J.M. (1987) Separation of the Response Rate and Discriminative Stimulus Effects of Phencyclidine - Training Dose as a Factor in Phencyclidine-Saline Discrimination. *Journal of Pharmacology and Experimental Therapeutics*, **241**, 159-165.

Beardsley, P.M. & Hauser, K.F. (2014) Glial modulators as potential treatments of psychostimulant abuse. *Advances in pharmacology*, **69**, 1-69.

Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Von Zastrow, M., Beattie, M.S. & Malenka, R.C. (2002) Control of synaptic strength by glial TNFalpha. *Science*, **295**, 2282-2285.

Beavo, J.A. (1995) Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. *Physiological reviews*, **75**, 725-748.

Benveniste, E.N. (1992) Inflammatory cytokines within the central nervous system: sources, function, and mechanism of action. *The American journal of physiology*, **263**, C1-16.

Bergman, J., Roof, R.A., Furman, C.A., Conroy, J.L., Mello, N.K., Sibley, D.R. & Skolnick, P. (2013) Modification of cocaine self-administration by buspirone (buspar(R)): potential involvement of D3 and D4 dopamine receptors. *Int J Neuropsychopharmacol*, **16**, 445-458.

Berkley, M.A. & Stebbins, W.C. (1990) *Comparative perception*. Wiley, New York.

Bernardino, A.L., Kaushal, D. & Philipp, M.T. (2009) The antibiotics doxycycline and minocycline inhibit the inflammatory responses to the Lyme disease spirochete Borrelia burgdorferi. *J Infect Dis*, **199**, 1379-1388.

Berta, T., Liu, T., Liu, Y.C., Xu, Z.Z. & Ji, R.R. (2012) Acute morphine activates satellite glial cells and up-regulates IL-1beta in dorsal root ganglia in mice via matrix metalloprotease-9. *Molecular pain*, **8**, 18.

Bickel, W.K., Degrandpre, R.J. & Higgins, S.T. (1995) The Behavioral Economics of Concurrent Drug Reinforcers - a Review and Reanalysis of Drug Self-Administration Research. *Psychopharmacology*, **118**, 250-259.

Bickel, W.K., Marsch, L.A. & Carroll, M.E. (2000) Deconstructing relative reinforcing efficacy and situating the measures of pharmacological reinforcement with behavioral economics: a theoretical proposal. *Psychopharmacology (Berl)*, **153**, 44-56.

Bigelow, G.E., Griffiths, R.R. & Liebson, I.A. (1977) Pharmacological influences upon human ethanol self-administration. *Advances in experimental medicine and biology*, **85B**, 523-538.

Blackstone, K., Iudicello, J.E., Morgan, E.E., Weber, E., Moore, D.J., Franklin, D.R., Ellis, R.J., Grant, I., Woods, S.P. & the Translational Methamphetamine, A.R.C.G. (2013) Human Immunodeficiency Virus Infection Heightens Concurrent Risk of Functional Dependence in Persons With Long-Term Methamphetamine Use. *Journal of addiction medicine*.

Blanco, A.M. & Guerri, C. (2007) Ethanol intake enhances inflammatory mediators in brain: role of glial cells and TLR4/IL-1RI receptors. *Frontiers in bioscience : a journal and virtual library*, **12**, 2616-2630.

Blanco, A.M., Pascual, M., Valles, S.L. & Guerri, C. (2004) Ethanol-induced iNOS and COX-2 expression in cultured astrocytes via NF-kappa B. *Neuroreport*, **15**, 681-685.

Blanco, A.M., Perez-Arago, A., Fernandez-Lizarbe, S. & Guerri, C. (2008) Ethanol mimics ligand-mediated activation and endocytosis of IL-1RI/TLR4 receptors via lipid rafts caveolae in astroglial cells. *J Neurochem*, **106**, 625-639.

Bland, S., Hutchinson, M., Maier, S., Watkins, L. & Johnson, K. (2009) The glial activation inhibitor AV411 reduces morphine-induced nucleus accumbens dopamine release. *Brain Behav Immun*, **23**, 492-497.

Blednov, Y.A., Benavidez, J.M., Geil, C., Perra, S., Morikawa, H. & Harris, R.A. (2011) Activation of inflammatory signaling by lipopolysaccharide produces a prolonged increase of voluntary alcohol intake in mice. *Brain Behav Immun*, **25 Suppl 1**, S92-S105.

Bondareva, T.S., Young, R. & Glennon, R.A. (2002) Central stimulants as discriminative stimuli. Asymmetric generalization between (-)ephedrine and S(+)methamphetamine. *Pharmacol Biochem Behav*, **74**, 157-162.

Borgkvist, A. & Fisone, G. (2007) Psychoactive drugs and regulation of the cAMP/PKA/DARPP-32 cascade in striatal medium spiny neurons. *Neuroscience and biobehavioral reviews*, **31**, 79-88.

Bowers, M.S. & Kalivas, P.W. (2003) Forebrain astroglial plasticity is induced following withdrawal from repeated cocaine administration. *The European journal of neuroscience*, **17**, 1273-1278.

Brackins, T., Brahm, N.C. & Kissack, J.C. (2011) Treatments for methamphetamine abuse: a literature review for the clinician. *J Pharm Pract*, **24**, 541-550.

Brady, K.T. & Balster, R.L. (1980) Phencyclidine Discrimination in Squirrel-Monkeys - Generalization to Structural Analogs and Structurally Dissimilar Compounds. *Federation proceedings*, **39**, 303-303.

Brady, K.T. & Balster, R.L. (1981) Discriminative stimulus properties of phencyclidine and five analogues in the squirrel monkey. *Pharmacol Biochem Behav*, **14**, 213-218.

Brothers, H.M., Marchalant, Y. & Wenk, G.L. (2010) Caffeine attenuates lipopolysaccharide-induced neuroinflammation. *Neurosci Lett*, **480**, 97-100.

Brown, J.M., Hanson, G.R. & Fleckenstein, A.E. (2001) Regulation of the vesicular monoamine transporter-2: a novel mechanism for cocaine and other psychostimulants. *J Pharmacol Exp Ther*, **296**, 762-767.

Bruce-Keller, A.J., Turchan-Cholewo, J., Smart, E.J., Geurin, T., Chauhan, A., Reid, R., Xu, R., Nath, A., Knapp, P.E. & Hauser, K.F. (2008) Morphine causes rapid increases in glial activation and neuronal injury in the striatum of inducible HIV-1 Tat transgenic mice. *Glia*, **56**, 1414-1427.

Buccafusco, J.J. (2001) *Methods of behavior analysis in neuroscience*. CRC Press, Boca Raton.

Buening, M.K. & Gibb, J.W. (1974) Influence of methamphetamine and neuroleptic drugs on tyrosine hydroxylase activity. *Eur J Pharmacol*, **26**, 30-34.

Bunzow, J.R., Sonders, M.S., Arttamangkul, S., Harrison, L.M., Zhang, G., Quigley, D.I., Darland, T., Suchland, K.L., Pasumamula, S., Kennedy, J.L., Olson, S.B., Magenis, R.E., Amara, S.G. & Grandy, D.K. (2001) Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Mol Pharmacol*, **60**, 1181-1188.

Cadet, J.L. & Brannock, C. (1998) Free radicals and the pathobiology of brain dopamine systems. *Neurochemistry international*, **32**, 117-131.

Calabresi, P., Maj, R., Mercuri, N.B. & Bernardi, G. (1992) Coactivation of D1 and D2 dopamine receptors is required for long-term synaptic depression in the striatum. *Neurosci Lett*, **142**, 95-99.

Carey, C.L., Woods, S.P., Rippeth, J.D., Gonzalez, R., Heaton, R.K. & Grant, I. (2006) Additive deleterious effects of methamphetamine dependence and immunosuppression on neuropsychological functioning in HIV infection. *AIDS and behavior*, **10**, 185-190.

Carlezon, W.A., Jr., Duman, R.S. & Nestler, E.J. (2005) The many faces of CREB. *Trends in neurosciences*, **28**, 436-445.

Carnicella, S. & Ron, D. (2009) GDNF--a potential target to treat addiction. *Pharmacology & therapeutics*, **122**, 9-18.

Cearley, C.N., Blindheim, K., Sorg, B.A., Krueger, J.M. & Churchill, L. (2011) Acute cocaine increases interleukin-1beta mRNA and immunoreactive cells in the cortex and nucleus accumbens. *Neurochem Res*, **36**, 686-692.

Chen, H., Uz, T. & Manev, H. (2009a) Minocycline affects cocaine sensitization in mice. *Neurosci Lett*, **452**, 258-261.

Chen, J.C., Chen, P.C. & Chiang, Y.C. (2009b) Molecular mechanisms of psychostimulant addiction. *Chang Gung Med J*, **32**, 148-154.

Chen, M.K. & Guilarte, T.R. (2008) Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair. *Pharmacology & therapeutics*, **118**, 1-17.

Chen, P.C. & Chen, J.C. (2005) Enhanced Cdk5 activity and p35 translocation in the ventral striatum of acute and chronic methamphetamine-treated rats. *Neuropsychopharmacology*, **30**, 538-549.

Chen, S.L., Tao, P.L., Chu, C.H., Chen, S.H., Wu, H.E., Tseng, L.F., Hong, J.S. & Lu, R.B. (2012) Low-dose memantine attenuated morphine addictive behavior through its anti-inflammation and neurotrophic effects in rats. *J Neuroimmune Pharmacol*, **7**, 444-453.

Chiamulera, C., Epping-Jordan, M.P., Zocchi, A., Marcon, C., Cottiny, C., Tacconi, S., Corsi, M., Orzi, F. & Conquet, F. (2001) Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nature neuroscience*, **4**, 873-874.

Cho, A.K., Melega, W.P., Kuczenski, R. & Segal, D.S. (2001) Relevance of pharmacokinetic parameters in animal models of methamphetamine abuse. *Synapse*, **39**, 161-166.

Cho, A.K. & Segal, D.S. (1994) *Amphetamine and its analogs : psychopharmacology, toxicology, and abuse*. Academic Press, San Diego.

Cho, Y., Crichlow, G., Vermeire, J., Leng, L., Du, X., Hodsdon, M., Bucala, R., Cappello, M., Gross, M., Gaeta, F., Johnson, K. & Lolis, E. (2010) Allosteric inhibition of macrophage migration inhibitory factor revealed by ibudilast. *Proc Natl Acad Sci U S A*, **107**, 11313-11318.

Choi, J., Ifuku, M., Noda, M. & Guilarte, T.R. (2011) Translocator protein (18 kDa)/peripheral benzodiazepine receptor specific ligands induce microglia functions consistent with an activated state. *Glia*, **59**, 219-230.

Chornock, W.M., Stitzer, M.L., Gross, J. & Leischow, S. (1992) Experimental model of smoking re-exposure: effects on relapse. *Psychopharmacology (Berl)*, **108**, 495-500.

Chow, J.C., Young, D.W., Golenbock, D.T., Christ, W.J. & Gusovsky, F. (1999) Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem*, **274**, 10689-10692.

Chuck, R.S., Williams, J.M., Goldberg, M.A. & Lubniewski, A.J. (1996) Recurrent corneal ulcerations associated with smokeable methamphetamine abuse. *American journal of ophthalmology*, **121**, 571-572.

Chung, Y.C., Bok, E., Huh, S.H., Park, J.Y., Yoon, S.H., Kim, S.R., Kim, Y.S., Maeng, S., Park, S.H. & Jin, B.K. (2011) Cannabinoid receptor type 1 protects nigrostriatal dopaminergic neurons against MPTP neurotoxicity by inhibiting microglial activation. *J Immunol*, **187**, 6508-6517.

Clark, K.H., Wiley, C.A. & Bradberry, C.W. (2012) Psychostimulant Abuse and Neuroinflammation: Emerging Evidence of Their Interconnection. *Neurotox Res*.

Coller, J.K. & Hutchinson, M.R. (2012) Implications of central immune signaling caused by drugs of abuse: mechanisms, mediators and new therapeutic approaches for prediction and treatment of drug dependence. *Pharmacology & therapeutics*, **134**, 219-245.

Control, O.o.D. (2012) Title 21 United States Code (USC) Controlled Substances Act. In Justice, U.S.D.o. (ed).

Cooper, J.R., Bloom, F.E. & Roth, R.H. (2003) *The biochemical basis of neuropharmacology*. Oxford University Press, Oxford ; New York.

Creese, I. (1983) *Stimulants, neurochemical, behavioral, and clinical perspectives*. Raven Press, New York.

Crews, F.T., Zou, J. & Qin, L. (2011) Induction of innate immune genes in brain create the neurobiology of addiction. *Brain Behav Immun*, **25 Suppl 1**, S4-S12.

Crime, U.N.O.o.D.a. (2012) World Drug Report 2012, New York.

Cui, Y., Liao, X.X., Liu, W., Guo, R.X., Wu, Z.Z., Zhao, C.M., Chen, P.X. & Feng, J.Q. (2008) A novel role of minocycline: attenuating morphine antinociceptive tolerance by inhibition of p38 MAPK in the activated spinal microglia. *Brain Behav Immun*, **22**, 114-123.

D'Ascenzo, M., Fellin, T., Terunuma, M., Revilla-Sanchez, R., Meaney, D.F., Auberson, Y.P., Moss, S.J. & Haydon, P.G. (2007) mGluR5 stimulates gliotransmission in the nucleus accumbens. *Proc Natl Acad Sci U S A*, **104**, 1995-2000.

Davis, L.E., Kalousek, G. & Rubenstein, E. (1970) Hepatitis associated with illicit use of intravenous methamphetamine. *Public health reports*, **85**, 809-813.

Davis, R.L. & Syapin, P.J. (2004) Ethanol increases nuclear factor-kappa B activity in human astroglial cells. *Neurosci Lett*, **371**, 128-132.

De Simone, R., Ajmone-Cat, M.A., Carnevale, D. & Minghetti, L. (2005) Activation of alpha7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures. *Journal of neuroinflammation*, **2**, 4.

de Wit, H. (1996) Priming effects with drugs and other reinforcers. *Experimental Clincal Psychopharmacology*, **4**, 5-10.

DEA, D.E.A. (2012) Total of All Meth Clandestine Laboratory Incidents Including Labs, Dumpsites, Chem/Glass/Equipment. In Justice, U.D.o. (ed).

DeGrandpre, R.J., Bickel, W.K., Hughes, J.R., Layng, M.P. & Badger, G. (1993) Unit price as a useful metric in analyzing effects of reinforcer magnitude. *Journal of the experimental analysis of behavior*, **60**, 641-666.

Desai, R.I. & Bergman, J. (2010) Drug Discrimination in Methamphetamine-Trained Rats: Effects of Cholinergic Nicotinic Compounds. *Journal of Pharmacology and Experimental Therapeutics*, **335**, 807-816.

Dews, P.B. (1955) Studies on behavior. I. Differential sensitivity to pentobarbital of pecking performance in pigeons depending on the schedule of reward. *J Pharmacol Exp Ther*, **113**, 393-401.

Di Cara, B., Maggio, R., Aloisi, G., Rivet, J.M., Lundius, E.G., Yoshitake, T., Svenningsson, P., Brocco, M., Gobert, A., De Groote, L., Cistarelli, L., Veiga, S., De Montrion, C., Rodriguez, M., Galizzi, J.P., Lockhart, B.P., Coge, F., Boutin, J.A., Vayer, P., Verdouw, P.M., Groenink, L. & Millan, M.J. (2011) Genetic deletion of trace amine 1 receptors reveals their role in auto-inhibiting the actions of ecstasy (MDMA). *J Neurosci*, **31**, 16928-16940.

Dole, V.P. & Nyswander, M. (1965) A Medical Treatment for Diacetylmorphine (Heroin) Addiction. A Clinical Trial with Methadone Hydrochloride. *JAMA : the journal of the American Medical Association*, **193**, 646-650.

Dong, Y., Green, T., Saal, D., Marie, H., Neve, R., Nestler, E.J. & Malenka, R.C. (2006) CREB modulates excitability of nucleus accumbens neurons. *Nature neuroscience*, **9**, 475-477.

El-Hage, N., Rodriguez, M., Podhaizer, E., Zou, S., Dever, S., Snider, S., Knapp, P., Beardsley, P. & Hauser, K. (2014) Ibudilast (AV411), and its AV1013 analogue, reduce HIV-1 replication and neuronal death induced by HIV-1 and morphine *AIDS*, **in press**.

Elsmore, T.F., Fletcher, G.V., Conrad, D.G. & Sodetz, F.J. (1980) Reduction of Heroin Intake in Baboons by an Economic Constraint. *Pharmacol Biochem Be*, **13**, 729-731.

Eriksson, C., Zou, L.P., Ahlenius, S., Winblad, B. & Schultzberg, M. (2000) Inhibition of kainic acid induced expression of interleukin-1 beta and interleukin-1 receptor antagonist mRNA in the rat brain by NMDA receptor antagonists. *Brain research. Molecular brain research*, **85**, 103-113.

Eroglu, C. & Barres, B.A. (2010) Regulation of synaptic connectivity by glia. *Nature*, **468**, 223-231.

Espinoza, S., Salahpour, A., Masri, B., Sotnikova, T.D., Messa, M., Barak, L.S., Caron, M.G. & Gainetdinov, R.R. (2011) Functional interaction between trace amine-associated receptor 1 and dopamine D2 receptor. *Mol Pharmacol*, **80**, 416-425.

Everitt, B.J. & Robbins, T.W. (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature neuroscience*, **8**, 1481-1489.

Fattorini, G., Melone, M., Bragina, L., Candiracci, C., Cozzi, A., Pellegrini Giampietro, D.E., Torres-Ramos, M., Perez-Samartin, A., Matute, C. & Conti, F. (2008) GLT-1 expression and Glu uptake in rat cerebral cortex are increased by phencyclidine. *Glia*, **56**, 1320-1327.

143

FDA, U.F.a.D.A. (2010) Minocin (minocycline hydrochloride) pellet-filled capsules Prescribing Information September 2010, Silver Spring, MD, pp. 3-23.

Fellin, T., D'Ascenzo, M. & Haydon, P.G. (2007) Astrocytes control neuronal excitability in the nucleus accumbens. *TheScientificWorldJournal*, **7**, 89-97.

Fernandez-Lizarbe, S., Montesinos, J. & Guerri, C. (2013) Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells. *J Neurochem*, **126**, 261-273.

Fiala, M., Gan, X.H., Zhang, L., House, S.D., Newton, T., Graves, M.C., Shapshak, P., Stins, M., Kim, K.S., Witte, M. & Chang, S.L. (1998) Cocaine enhances monocyte migration across the blood-brain barrier. Cocaine's connection to AIDS dementia and vasculitis? *Advances in experimental medicine and biology*, **437**, 199-205.

Fleckenstein, A.E., Gibb, J.W. & Hanson, G.R. (2000) Differential effects of stimulants on monoaminergic transporters: pharmacological consequences and implications for neurotoxicity. *Eur J Pharmacol*, **406**, 1-13.

Fleckenstein, A.E., Haughey, H.M., Metzger, R.R., Kokoshka, J.M., Riddle, E.L., Hanson, J.E., Gibb, J.W. & Hanson, G.R. (1999) Differential effects of psychostimulants and related agents on dopaminergic and serotonergic transporter function. *Eur J Pharmacol*, **382**, 45-49.

Fleckenstein, A.E., Metzger, R.R., Beyeler, M.L., Gibb, J.W. & Hanson, G.R. (1997) Oxygen radicals diminish dopamine transporter function in rat striatum. *Eur J Pharmacol*, **334**, 111-114.

Fox, H.C., D'Sa, C., Kimmerling, A., Siedlarz, K.M., Tuit, K.L., Stowe, R. & Sinha, R. (2012) Immune system inflammation in cocaine dependent individuals: implications for medications development. *Human psychopharmacology*, **27**, 156-166.

Frau, L., Simola, N., Plumitallo, A. & Morelli, M. (2013) Microglial and astroglial activation by 3,4-methylenedioxymethamphetamine (MDMA) in mice depends on S(+) enantiomer and is associated with an increase in body temperature and motility. *J Neurochem*, **124**, 69-78.

Fujita, Y., Kunitachi, S., Iyo, M. & Hashimoto, K. (2012) The antibiotic minocycline prevents methamphetamine-induced rewarding effects in mice. *Pharmacol Biochem Behav*, **101**, 303-306.

Gan, X., Zhang, L., Berger, O., Stins, M.F., Way, D., Taub, D.D., Chang, S.L., Kim, K.S., House, S.D., Weinand, M., Witte, M., Graves, M.C. & Fiala, M. (1999) Cocaine enhances brain endothelial adhesion molecules and leukocyte migration. *Clin Immunol*, **91**, 68-76.

Garrido-Mesa, N., Zarzuelo, A. & Galvez, J. (2013) Minocycline: far beyond an antibiotic. *British journal of pharmacology*, **169**, 337-352.

Gass, J.T., Osborne, M.P., Watson, N.L., Brown, J.L. & Olive, M.F. (2009) mGluR5 antagonism attenuates methamphetamine reinforcement and prevents reinstatement of methamphetamine-seeking behavior in rats. *Neuropsychopharmacology*, **34**, 820-833.

Gatch, M.B., Selvig, M. & Forster, M.J. (2005) GABAergic modulation of the discriminative stimulus effects of methamphetamine. *Behavioural pharmacology*, **16**, 261-266.

Gekker, G., Hu, S., Sheng, W.S., Rock, R.B., Lokensgard, J.R. & Peterson, P.K. (2006) Cocaine-induced HIV-1 expression in microglia involves sigma-1 receptors and transforming growth factor-beta1. *International immunopharmacology*, **6**, 1029-1033.

Ghosh, M., Garcia-Castillo, D., Aguirre, V., Golshani, R., Atkins, C.M., Bramlett, H.M., Dietrich, W.D. & Pearse, D.D. (2012) Proinflammatory cytokine regulation of cyclic AMP-phosphodiesterase 4 signaling in microglia in vitro and following CNS injury. *Glia*, **60**, 1839-1859.

Gibson, L.C., Hastings, S.F., McPhee, I., Clayton, R.A., Darroch, C.E., Mackenzie, A., Mackenzie, F.L., Nagasawa, M., Stevens, P.A. & Mackenzie, S.J. (2006) The inhibitory profile of Ibudilast against the human phosphodiesterase enzyme family. *Eur J Pharmacol*, **538**, 39-42.

Giordano, L.A., Bickel, W.K., Shahan, T.A. & Badger, G.J. (2001) Behavioral economics of human drug self-administration: progressive ratio versus random sequences of response requirements. *Behavioural pharmacology*, **12**, 343-347.

Gold, L.H. & Balster, R.L. (1992) Effects of buspirone and gepirone on i.v. cocaine self-administration in rhesus monkeys. *Psychopharmacology (Berl)*, **108**, 289-294.

Goncalves, J., Martins, T., Ferreira, R., Milhazes, N., Borges, F., Ribeiro, C.F., Malva, J.O., Macedo, T.R. & Silva, A.P. (2008) Methamphetamine-induced early increase of IL-6 and TNF-alpha mRNA expression in the mouse brain. *Ann N Y Acad Sci*, **1139**, 103-111.

Granja, A.G., Carrillo-Salinas, F., Pagani, A., Gomez-Canas, M., Negri, R., Navarrete, C., Mecha, M., Mestre, L., Fiebich, B.L., Cantarero, I., Calzado, M.A., Bellido, M.L., Fernandez-Ruiz, J., Appendino, G., Guaza, C. & Munoz, E. (2012) A cannabigerol quinone alleviates neuroinflammation in a chronic model of multiple sclerosis. *J Neuroimmune Pharmacol*, **7**, 1002-1016.

Green-Sadan, T., Kinor, N., Roth-Deri, I., Geffen-Aricha, R., Schindler, C.J. & Yadid, G. (2003) Transplantation of glial cell line-derived neurotrophic factor-expressing cells into the striatum and nucleus accumbens attenuates acquisition of cocaine self-administration in rats. *The European journal of neuroscience*, **18**, 2093-2098.

Green-Sadan, T., Kuttner, Y., Lublin-Tennenbaum, T., Kinor, N., Boguslavsky, Y., Margel, S. & Yadid, G. (2005) Glial cell line-derived neurotrophic factor-conjugated nanoparticles suppress acquisition of cocaine self-administration in rats. *Experimental neurology*, **194**, 97-105.

145

Green, A.L. & El Hait, M.A. (1980) A new approach to the assessment of the potency of reversible monoamine oxidase inhibitors in vivo, and its application to (+)-amphetamine, p-methoxyamphetamine and harmaline. *Biochemical pharmacology*, **29**, 2781-2789.

Griffiths, R.R., Bradford, L.D. & Brady, J.V. (1979) Progressive ratio and fixed ratio schedules of cocaine-maintained responding in baboons. *Psychopharmacology (Berl)*, **65**, 125-136.

Gump, D.W., Ashikaga, T., Fink, T.J. & Radin, A.M. (1977) Side effects of minocycline: different dosage regimens. *Antimicrobial agents and chemotherapy*, **12**, 642-646.

Gunawardena, D., Shanmugam, K., Low, M., Bennett, L., Govindaraghavan, S., Head, R., Ooi, L. & Munch, G. (2014) Determination of anti-inflammatory activities of standardised preparations of plant- and mushroom-based foods. *European journal of nutrition*, **53**, 335-343.

Hall, C. (1934) Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Psychology*, **18**, 385-403.

Hamamoto, D.T. & Rhodus, N.L. (2009) Methamphetamine abuse and dentistry. *Oral Dis*, **15**, 27-37.

Hardingham, G.E., Fukunaga, Y. & Bading, H. (2002) Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature neuroscience*, **5**, 405-414.

Harlan Laboratories, I.U. (2006) Long Evans (Blue Spruce) Growth Chart, New York.

Harms, R., Morsey, B., Boyer, C.W., Fox, H.S. & Sarvetnick, N. (2012) Methamphetamine administration targets multiple immune subsets and induces phenotypic alterations suggestive of immunosuppression. *PLoS One*, **7**, e49897.

Hasbi, A., O'Dowd, B.F. & George, S.R. (2010) Heteromerization of dopamine D2 receptors with dopamine D1 or D5 receptors generates intracellular calcium signaling by different mechanisms. *Current opinion in pharmacology*, **10**, 93-99.

Haughey, H.M., Brown, J.M., Fleckenstein, A.E. & Hanson, G.R. (1999) Oxygen radicals differentially affect Na+/Cl(-)-dependent transporters. *Eur J Pharmacol*, **379**, 107-110.

Haughey, H.M., Brown, J.M., Wilkins, D.G., Hanson, G.R. & Fleckenstein, A.E. (2000) Differential effects of methamphetamine on Na(+)/Cl(-)-dependent transporters. *Brain Res*, **863**, 59-65.

Haydon, P.G., Blendy, J., Moss, S.J. & Rob Jackson, F. (2009) Astrocytic control of synaptic transmission and plasticity: a target for drugs of abuse? *Neuropharmacology*, **56 Suppl 1**, 83-90.

Haydon, P.G. & Carmignoto, G. (2006) Astrocyte control of synaptic transmission and neurovascular coupling. *Physiological reviews*, **86**, 1009-1031.

He, J. & Crews, F.T. (2008) Increased MCP-1 and microglia in various regions of the human alcoholic brain. *Experimental neurology*, **210**, 349-358.

He, S.Y., Matoba, R., Fujitani, N., Sodesaki, K. & Onishi, S. (1996) Cardiac muscle lesions associated with chronic administration of methamphetamine in rats. *The American journal of forensic medicine and pathology*, **17**, 155-162.

Heberlein, A., Muschler, M., Wilhelm, J., Frieling, H., Lenz, B., Groschl, M., Kornhuber, J., Bleich, S. & Hillemacher, T. (2010) BDNF and GDNF serum levels in alcohol-dependent patients during withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry*, **34**, 1060-1064.

Hemmings, H.C., Jr., Greengard, P., Tung, H.Y. & Cohen, P. (1984) DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. *Nature*, **310**, 503-505.

Hernandez-Lopez, S., Tkatch, T., Perez-Garci, E., Galarraga, E., Bargas, J., Hamm, H. & Surmeier, D.J. (2000) D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. *J Neurosci*, **20**, 8987-8995.

Hirabayasi, M., Saito, T. & Tadokoro, S. (1991) Differential sensitization to ambulation-increasing effect of methamphetamine after repeated administration to mice in activity cages of different sizes. *Jpn J Pharmacol*, **57**, 91-97.

Ho, S.C., Chang, K.S. & Chang, P.W. (2013a) Inhibition of neuroinflammation by cinnamon and its main components. *Food chemistry*, **138**, 2275-2282.

Ho, S.C., Chang, K.S. & Lin, C.C. (2013b) Anti-neuroinflammatory capacity of fresh ginger is attributed mainly to 10-gingerol. *Food chemistry*, **141**, 3183-3191.

Hotchkiss, A.J., Morgan, M.E. & Gibb, J.W. (1979) The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxylase, tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase activities. *Life sciences*, **25**, 1373-1378.

House, R.V., Thomas, P.T. & Bhargava, H.N. (1994) Immunological consequences of in vitro exposure to lysergic acid diethylamide (LSD). *Immunopharmacology and immunotoxicology*, **16**, 23-40.

Hsiang, C.Y., Wu, S.L., Cheng, S.E. & Ho, T.Y. (2005) Acetaldehyde-induced interleukin-1beta and tumor necrosis factor-alpha production is inhibited by berberine through nuclear factor-kappaB signaling pathway in HepG2 cells. *Journal of biomedical science*, **12**, 791-801.

Hursh, S.R. (1978) Economics of Daily Consumption Controlling Food-Reinforced and Water-Reinforced Responding. *Journal of the experimental analysis of behavior*, **29**, 475-491.

147

Hursh, S.R. (1980) Economic Concepts for the Analysis of Behavior. *Journal of the experimental analysis of behavior*, **34**, 219-238.

Hursh, S.R. (1984) Behavioral economics. *Journal of the experimental analysis of behavior*, **42**, 435-452.

Hursh, S.R., Galuska, C.M., Winger, G. & Woods, J.H. (2005) The economics of drug abuse: a quantitative assessment of drug demand. *Molecular interventions*, **5**, 20-28.

Hursh, S.R. & Roma, P.G. (2013) Behavioral Economics and Empirical Public Policy. *Journal of the experimental analysis of behavior*, **99**, 98-124.

Hursh, S.R. & Silberberg, A. (2008) Economic demand and essential value. *Psychological review*, **115**, 186-198.

Hursh, S.R. & Winger, G. (1995) Normalized demand for drugs and other reinforcers. *Journal of the experimental analysis of behavior*, **64**, 373-384.

Hutchinson, M., Lewis, S., Coats, B., Skyba, D., Crysdale, N., Berkelhammer, D., Brzeski, A., Northcutt, A., Vietz, C., Judd, C., Maier, S., Watkins, L. & Johnson, K. (2009a) Reduction of opioid withdrawal and potentiation of acute opioid analgesia by systemic AV411 (ibudilast). *Brain Behav Immun*, **23**, 240-250.

Hutchinson, M.R., Northcutt, A.L., Chao, L.W., Kearney, J.J., Zhang, Y., Berkelhammer, D.L., Loram, L.C., Rozeske, R.R., Bland, S.T., Maier, S.F., Gleeson, T.T. & Watkins, L.R. (2008) Minocycline suppresses morphine-induced respiratory depression, suppresses morphine-induced reward, and enhances systemic morphine-induced analgesia. *Brain Behav Immun*, **22**, 1248-1256.

Hutchinson, M.R., Ramos, K.M., Loram, L.C., Wieseler, J., Sholar, P.W., Kearney, J.J., Lewis, M.T., Crysdale, N.Y., Zhang, Y., Harrison, J.A., Maier, S.F., Rice, K.C. & Watkins, L.R. (2009b) Evidence for a role of heat shock protein-90 in toll like receptor 4 mediated pain enhancement in rats. *Neuroscience*, **164**, 1821-1832.

Iyo, M., Bi, Y., Hashimoto, K., Inada, T. & Fukui, S. (1996a) Prevention of methamphetamine-induced behavioral sensitization in rats by a cyclic AMP phosphodiesterase inhibitor, rolipram. *Eur J Pharmacol*, **312**, 163-170.

Iyo, M., Bi, Y., Hashimoto, K., Tomitaka, S.I., Inada, T. & Fukui, S. (1996b) Does an increase of cyclic AMP prevent methamphetamine-induced behavioral sensitization in rats? *Ann N Y Acad Sci*, **801**, 377-383.

Iyo, M., Maeda, Y., Inada, T., Kitao, Y., Sasaki, H. & Fukui, S. (1995) The effects of a selective cAMP phosphodiesterase inhibitor, rolipram, on methamphetamine-induced behavior. *Neuropsychopharmacology*, **13**, 33-39.

Jacobs, E.H., Smit, A.B., de Vries, T.J. & Schoffelmeer, A.N.M. (2003) Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends Pharmacol Sci*, **24**, 566-573.

Johnson, K.M., K & Iwaki, Y. (2014) Ibudilast for the treatment of drug addiction and other neurological conditions. *Clinical Investigations*, **4**, 1-11.

Johnston, L., O'Malley, PM, Bachman, JG, Schulenberg, JE (2011) Monitoring the Future: National Results on Adolescent Drug Use. Institute for Social Research, University of Michigan, Ann Arbor MI.

Karch, S.B., Stephens, B.G. & Ho, C.H. (1999) Methamphetamine-related deaths in San Francisco: demographic, pathologic, and toxicologic profiles. *Journal of forensic sciences*, **44**, 359-368.

Karila, L., Weinstein, A., Aubin, H.J., Benyamina, A., Reynaud, M. & Batki, S.L. (2010) Pharmacological approaches to methamphetamine dependence: a focused review. *Br J Clin Pharmacol*, **69**, 578-592.

Kast, R.E. (2000) Tumor necrosis factor has positive and negative self regulatory feed back cycles centered around cAMP. *International journal of immunopharmacology*, **22**, 1001-1006.

Kay-Lambkin, F.J. (2008) Technology and innovation in the psychosocial treatment of methamphetamine use, risk and dependence. *Drug and alcohol review*, **27**, 318-325.

Kebabian, J.W., Beaulieu, M. & Itoh, Y. (1984) Pharmacological and biochemical evidence for the existence of two categories of dopamine receptor. *The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques*, **11**, 114-117.

Kelly, M.A., Low, M.J., Rubinstein, M. & Phillips, T.J. (2008) Role of dopamine D1-like receptors in methamphetamine locomotor responses of D2 receptor knockout mice. *Genes Brain Behav*, **7**, 568-577.

Kermanian, F., Soleimani, M., Ebrahimzadeh, A., Haghir, H. & Mehdizadeh, M. (2013) Effects of adenosine A2a receptor agonist and antagonist on hippocampal nuclear factor-kB expression preceded by MDMA toxicity. *Metabolic brain disease*, **28**, 45-52.

Khairnar, A., Plumitallo, A., Frau, L., Schintu, N. & Morelli, M. (2010) Caffeine enhances astroglia and microglia reactivity induced by 3,4-methylenedioxymethamphetamine ('ecstasy') in mouse brain. *Neurotoxicity research*, **17**, 435-439.

Khoshbouei, H., Wang, H., Lechleiter, J.D., Javitch, J.A. & Galli, A. (2003) Amphetamine-induced dopamine efflux. A voltage-sensitive and intracellular Na+-dependent mechanism. *The Journal of biological chemistry*, **278**, 12070-12077.

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Kishi, Y., Ohta, S., Kasuya, N., Sakita, S., Ashikaga, T. & Isobe, M. (2001) Ibudilast: a non-selective PDE inhibitor with multiple actions on blood cells and the vascular wall. *Cardiovasc Drug Rev*, **19**, 215-225.

Kita, T., Miyazaki, I., Asanuma, M., Takeshima, M. & Wagner, G.C. (2009) Dopamine-induced behavioral changes and oxidative stress in methamphetamine-induced neurotoxicity. *International review of neurobiology*, **88**, 43-64.

Kita, T., Takeshima, M., Wagner, G.C., Hozumi, H., Miyazaki, I. & Asanuma, M. (2008) [New perspectives on the mechanism of methamphetamine-induced neurotoxicity]. *Nihon Shinkei Seishin Yakurigaku Zasshi*, **28**, 49-61.

Kiyatkin, E.A. & Sharma, H.S. (2012) Environmental conditions modulate neurotoxic effects of psychomotor stimulant drugs of abuse. *International review of neurobiology*, **102**, 147-171.

Kobayashi, K., Imagama, S., Ohgomori, T., Hirano, K., Uchimura, K., Sakamoto, K., Hirakawa, A., Takeuchi, H., Suzumura, A., Ishiguro, N. & Kadomatsu, K. (2013) Minocycline selectively inhibits M1 polarization of microglia. *Cell death & disease*, **4**, e525.

Kofman, O., Klein, E., Newman, M., Hamburger, R., Kimchi, O., Nir, T., Shimon, H. & Belmaker, R.H. (1990) Inhibition by antibiotic tetracyclines of rat cortical noradrenergic adenylate cyclase and amphetamine-induced hyperactivity. *Pharmacol Biochem Behav*, **37**, 417-424.

Kogan, F.J., Nichols, W.K. & Gibb, J.W. (1976) Influence of methamphetamine on nigral and striatal tyrosine hydroxylase activity and on striatal dopamine levels. *Eur J Pharmacol*, **36**, 363-371.

Kousik, S.M., Napier, T.C. & Carvey, P.M. (2012) The effects of psychostimulant drugs on blood brain barrier function and neuroinflammation. *Frontiers in pharmacology*, **3**, 121.

Kozela, E., Pietr, M., Juknat, A., Rimmerman, N., Levy, R. & Vogel, Z. (2010) Cannabinoids Delta(9)-tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF-kappaB and interferon-beta/STAT proinflammatory pathways in BV-2 microglial cells. *The Journal of biological chemistry*, **285**, 1616-1626.

Kuribara, H. (1997) Effects of postmethamphetamine treatment with restraint on ambulatory sensitization to methamphetamine in mice. *Brain research bulletin*, **43**, 97-100.

Laso, F.J., Vaquero, J.M., Almeida, J., Marcos, M. & Orfao, A. (2007) Chronic alcohol consumption is associated with changes in the distribution, immunophenotype, and the inflammatory cytokine secretion profile of circulating dendritic cells. *Alcoholism, clinical and experimental research*, **31**, 846-854.

LaVoie, M.J., Card, J.P. & Hastings, T.G. (2004) Microglial activation precedes dopamine terminal pathology in methamphetamine-induced neurotoxicity. *Exp Neurol*, **187**, 47-57.

Lawrence, D.M., Thomas, D.A. & Wu, D.Y. (2007) Glial cells and the neurobiology of addiction. *TheScientificWorldJournal*, **7**, 86-88.

Ledeboer, A., Hutchinson, M.R., Watkins, L.R. & Johnson, K.W. (2007) Ibudilast (AV-411). A new class therapeutic candidate for neuropathic pain and opioid withdrawal syndromes. *Expert Opin Investig Drugs*, **16**, 935-950.

Ledeboer, A., Liu, T., Shumilla, J.A., Mahoney, J.H., Vijay, S., Gross, M.I., Vargas, J.A., Sultzbaugh, L., Claypool, M.D., Sanftner, L.M., Watkins, L.R. & Johnson, K.W. (2006) The glial modulatory drug AV411 attenuates mechanical allodynia in rat models of neuropathic pain. *Neuron Glia Biol*, **2**, 279-291.

Lee, K.W., Kim, Y., Kim, A.M., Helmin, K., Nairn, A.C. & Greengard, P. (2006) Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc Natl Acad Sci U S A*, **103**, 3399-3404.

Lewis, S.S., Hutchinson, M.R., Rezvani, N., Loram, L.C., Zhang, Y., Maier, S.F., Rice, K.C. & Watkins, L.R. (2010) Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement via toll-like receptor 4/MD-2 and interleukin-1beta. *Neuroscience*, **165**, 569-583.

Liang, N.Y. & Rutledge, C.O. (1982) Comparison of the release of [3H]dopamine from isolated corpus striatum by amphetamine, fenfluramine and unlabelled dopamine. *Biochemical pharmacology*, **31**, 983-992.

Lile, J.A., Stoops, W.W., Vansickel, A.R., Glaser, P.E.A., Hays, L.R. & Rush, C.R. (2005) Aripiprazole attenuates the discriminative-stimulus and subject-rated effects of D-amphetamine in humans. *Neuropsychopharmacology*, **30**, 2103-2114.

Lilius, T.O., Rauhala, P.V., Kambur, O. & Kalso, E.A. (2009) Modulation of morphine-induced antinociception in acute and chronic opioid treatment by ibudilast. *Anesthesiology*, **111**, 1356-1364.

Lin, K.Y., Cherng, C.G., Yang, F.R., Lin, L.C., Lu, R.B. & Yu, L. (2011) Memantine abolishes the formation of cocaine-induced conditioned place preference possibly via its IL-6-modulating effect in medial prefrontal cortex. *Behav Brain Res*, **220**, 126-131.

Lindemann, L., Meyer, C.A., Jeanneau, K., Bradaia, A., Ozmen, L., Bluethmann, H., Bettler, B., Wettstein, J.G., Borroni, E., Moreau, J.L. & Hoener, M.C. (2008) Trace amine-associated receptor 1 modulates dopaminergic activity. *J Pharmacol Exp Ther*, **324**, 948-956.

Liu, Q., Zhang, J., Zhu, H., Qin, C., Chen, Q. & Zhao, B. (2007) Dissecting the signaling pathway of nicotine-mediated neuroprotection in a mouse Alzheimer disease model. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, **21**, 61-73.

Liu, X., Silverstein, P.S., Singh, V., Shah, A., Qureshi, N. & Kumar, A. (2012a) Methamphetamine increases LPS-mediated expression of IL-8, TNF-alpha and IL-1beta in human macrophages through common signaling pathways. *PLoS One*, **7**, e33822.

Liu, Y., Hu, J., Wu, J., Zhu, C., Hui, Y., Han, Y., Huang, Z., Ellsworth, K. & Fan, W. (2012b) alpha7 nicotinic acetylcholine receptor-mediated neuroprotection against dopaminergic neuron loss in an MPTP mouse model via inhibition of astrocyte activation. *Journal of neuroinflammation*, **9**, 98.

Loftis, J.M., Choi, D., Hoffman, W. & Huckans, M.S. (2011) Methamphetamine causes persistent immune dysregulation: a cross-species, translational report. *Neurotox Res*, **20**, 59-68.

Loop, T., Humar, M., Pischke, S., Hoetzel, A., Schmidt, R., Pahl, H.L., Geiger, K.K. & Pannen, B.H. (2003) Thiopental inhibits tumor necrosis factor alpha-induced activation of nuclear factor kappaB through suppression of kappaB kinase activity. *Anesthesiology*, **99**, 360-367.

Loweth, J.A., Baker, L.K., Guptaa, T., Guillory, A.M. & Vezina, P. (2008) Inhibition of CaMKII in the nucleus accumbens shell decreases enhanced amphetamine intake in sensitized rats. *Neurosci Lett*, **444**, 157-160.

Lynch, J. & House, M.A. (1992) Cardiovascular effects of methamphetamine. *The Journal of cardiovascular nursing*, **6**, 12-18.

MacKenzie, R.G. & Heischober, B. (1997) Methamphetamine. *Pediatrics in review / American Academy of Pediatrics*, **18**, 305-309.

Mahajan, S.D., Aalinkeel, R., Reynolds, J.L., Nair, B.B., Sykes, D.E., Hu, Z., Bonoiu, A., Ding, H., Prasad, P.N. & Schwartz, S.A. (2009) Therapeutic targeting of "DARPP-32": a key signaling molecule in the dopiminergic pathway for the treatment of opiate addiction. *International review of neurobiology*, **88**, 199-222.

Mandell, A.J. & Morgan, M. (1970) Amphetamine induced increase in tyrosine hydroxylase activity. *Nature*, **227**, 75-76.

Mello, N.K., Bree, M.P. & Mendelson, J.H. (1983) Comparison of buprenorphine and methadone effects on opiate self-administration in primates. *J Pharmacol Exp Ther*, **225**, 378-386.

Mello, N.K. & Negus, S.S. (1996) Preclinical evaluation of pharmacotherapies for treatment of cocaine and opioid abuse using drug self-administration procedures. *Neuropsychopharmacology*, **14**, 375-424.

Merighi, S., Gessi, S., Varani, K., Fazzi, D., Stefanelli, A. & Borea, P.A. (2013) Morphine mediates a proinflammatory phenotype via mu-opioid receptor-PKCvarepsilon-Akt-ERK1/2 signaling pathway in activated microglial cells. *Biochemical pharmacology*, **86**, 487-496.

Miguel-Hidalgo, J.J. (2005) Lower packing density of glial fibrillary acidic protein-immunoreactive astrocytes in the prelimbic cortex of alcohol-naive and alcohol-drinking alcohol-preferring rats as compared with alcohol-nonpreferring and Wistar rats. *Alcoholism, clinical and experimental research*, **29**, 766-772.

Miguel-Hidalgo, J.J. (2006) Withdrawal from free-choice ethanol consumption results in increased packing density of glutamine synthetase-immunoreactive astrocytes in the prelimbic cortex of alcohol-preferring rats. *Alcohol and alcoholism*, **41**, 379-385.

Miguel-Hidalgo, J.J. (2009) The Role of Glial Cells in Drug Abuse. *Curr Drug Abuse Rev*, **2**, 76-82.

Miller, S., Kesslak, J.P., Romano, C. & Cotman, C.W. (1995) Roles of metabotropic glutamate receptors in brain plasticity and pathology. *Ann N Y Acad Sci*, **757**, 460-474.

Mizuno, T., Kurotani, T., Komatsu, Y., Kawanokuchi, J., Kato, H., Mitsuma, N. & Suzumura, A. (2004) Neuroprotective role of phosphodiesterase inhibitor ibudilast on neuronal cell death induced by activated microglia. *Neuropharmacology*, **46**, 404-411.

Morgan, T.E., Xie, Z., Goldsmith, S., Yoshida, T., Lanzrein, A.S., Stone, D., Rozovsky, I., Perry, G., Smith, M.A. & Finch, C.E. (1999) The mosaic of brain glial hyperactivity during normal ageing and its attenuation by food restriction. *Neuroscience*, **89**, 687-699.

Mori, T., Baba, J., Ichimaru, Y. & Suzuki, T. (2000) Effects of rolipram, a selective inhibitor of phosphodiesterase 4, on hyperlocomotion induced by several abused drugs in mice. *Jpn J Pharmacol*, **83**, 113-118.

Munzar, P., Li, H., Nicholson, K.L., Wiley, J.L. & Balster, R.L. (2002) Enhancement of the discriminative stimulus effects of phencyclidine by the tetracycline antibiotics doxycycline and minocycline in rats. *Psychopharmacology (Berl)*, **160**, 331-336.

Nakajima, A., Yamada, K., Nagai, T., Uchiyama, T., Miyamoto, Y., Mamiya, T., He, J., Nitta, A., Mizuno, M., Tran, M.H., Seto, A., Yoshimura, M., Kitaichi, K., Hasegawa, T., Saito, K., Yamada, Y., Seishima, M., Sekikawa, K., Kim, H.C. & Nabeshima, T. (2004a) Role of tumor necrosis factor-alpha in methamphetamine-induced drug dependence and neurotoxicity. *J Neurosci*, **24**, 2212-2225.

Nakajima, A., Yamada, K., Nagai, T., Uchiyama, T., Miyamoto, Y., Mamiya, T., He, J., Nitta, A., Mizuno, M., Tran, M.H., Seto, A., Yoshimura, M., Kitaichi, K., Hasegawa, T., Saito, K., Yamada, Y., Seishima, M., Sekikawa, K., Kim, H.C. & Nabeshima, T. (2004b) Role of tumor necrosis factor-alpha in methamphetamine-induced drug dependence and neurotoxicity. *J Neurosci*, **24**, 2212-2225.

Nakki, R., Koistinaho, J., Sharp, F.R. & Sagar, S.M. (1995) Cerebellar toxicity of phencyclidine. *J Neurosci*, **15**, 2097-2108.

Nakki, R., Nickolenko, J., Chang, J., Sagar, S.M. & Sharp, F.R. (1996a) Haloperidol prevents ketamine- and phencyclidine-induced HSP70 protein expression but not microglial activation. *Experimental neurology*, **137**, 234-241.

Nakki, R., Sharp, F.R. & Sagar, S.M. (1996b) FOS expression in the brainstem and cerebellum following phencyclidine and MK801. *J Neurosci Res*, **43**, 203-212.

Nakki, R., Sharp, F.R., Sagar, S.M. & Honkaniemi, J. (1996c) Effects of phencyclidine on immediate early gene expression in the brain. *J Neurosci Res*, **45**, 13-27.

Narita, M., Miyatake, M., Shibasaki, M., Shindo, K., Nakamura, A., Kuzumaki, N., Nagumo, Y. & Suzuki, T. (2006) Direct evidence of astrocytic modulation in the development of rewarding effects induced by drugs of abuse. *Neuropsychopharmacology*, **31**, 2476-2488.

Negus, S.S. (2003) Rapid assessment of choice between cocaine and food in rhesus monkeys: effects of environmental manipulations and treatment with d-amphetamine and flupenthixol. *Neuropsychopharmacology*, **28**, 919-931.

Neugebauer, N.M., Harrod, S.B., Stairs, D.J., Crooks, P.A., Dwoskin, L.P. & Bardo, M.T. (2007) Lobelane decreases methamphetamine self-administration in rats. *Eur J Pharmacol*, **571**, 33-38.

Nevin, J.A. (1992) An integrative model for the study of behavioral momentum. *Journal of the experimental analysis of behavior*, **57**, 301-316.

Nicosia, N., Laccardo Pacula, R. & Kilmer, B.L., R
Chiesa, J (2009) The Economic Cost of Methamphetamine Use in the United States, 2005. RAND Corporation, Santa Monica, CA, pp. 169.

Nikodemova, M., Duncan, I.D. & Watters, J.J. (2006) Minocycline exerts inhibitory effects on multiple mitogen-activated protein kinases and IkappaBalpha degradation in a stimulus-specific manner in microglia. *J Neurochem*, **96**, 314-323.

Nishikawa, T., Mataga, N., Takashima, M. & Toru, M. (1983) Behavioral sensitization and relative hyperresponsiveness of striatal and limbic dopaminergic neurons after repeated methamphetamine treatment. *Eur J Pharmacol*, **88**, 195-203.

Niwa, M., Nitta, A., Shen, L., Noda, Y. & Nabeshima, T. (2007a) Involvement of glial cell line-derived neurotrophic factor in inhibitory effects of a hydrophobic dipeptide Leu-Ile on morphine-induced sensitization and rewarding effects. *Behav Brain Res*, **179**, 167-171.

Niwa, M., Nitta, A., Yamada, Y., Nakajima, A., Saito, K., Seishima, M., Noda, Y. & Nabeshima, T. (2007b) Tumor necrosis factor-alpha and its inducer inhibit morphine-induced rewarding effects and sensitization. *Biological psychiatry*, **62**, 658-668.

Niwa, M., Nitta, A., Yamada, Y., Nakajima, A., Saito, K., Seishima, M., Shen, L., Noda, Y., Furukawa, S. & Nabeshima, T. (2007c) An inducer for glial cell line-derived neurotrophic

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factor and tumor necrosis factor-alpha protects against methamphetamine-induced rewarding effects and sensitization. *Biological psychiatry*, **61**, 890-901.

Obert, J.L., McCann, M.J., Marinelli-Casey, P., Weiner, A., Minsky, S., Brethen, P. & Rawson, R. (2000) The matrix model of outpatient stimulant abuse treatment: history and description. *J Psychoactive Drugs*, **32**, 157-164.

Ohnishi, M., Katsuki, H., Takagi, M., Kume, T. & Akaike, A. (2009) Long-term treatment with nicotine suppresses neurotoxicity of, and microglial activation by, thrombin in cortico-striatal slice cultures. *Eur J Pharmacol*, **602**, 288-293.

Ojaniemi, M., Glumoff, V., Harju, K., Liljeroos, M., Vuori, K. & Hallman, M. (2003) Phosphatidylinositol 3-kinase is involved in Toll-like receptor 4-mediated cytokine expression in mouse macrophages. *Eur J Immunol*, **33**, 597-605.

Orio, L., Llopis, N., Torres, E., Izco, M., O'Shea, E. & Colado, M.I. (2010) A study on the mechanisms by which minocycline protects against MDMA ('ecstasy')-induced neurotoxicity of 5-HT cortical neurons. *Neurotoxicity research*, **18**, 187-199.

Orio, L., O'Shea, E., Sanchez, V., Pradillo, J.M., Escobedo, I., Camarero, J., Moro, M.A., Green, A.R. & Colado, M.I. (2004) 3,4-Methylenedioxymethamphetamine increases interleukin-1beta levels and activates microglia in rat brain: studies on the relationship with acute hyperthermia and 5-HT depletion. *J Neurochem*, **89**, 1445-1453.

Overton, D.A. (1979) Influence of shaping procedures and schedules of reinforcement on performance in the two-bar drug discrimination task: a methodological report. *Psychopharmacology (Berl)*, **65**, 291-298.

Page, G., Barc-Pain, S., Pontcharraud, R., Cante, A., Piriou, A. & Barrier, L. (2004) The up-regulation of the striatal dopamine transporter's activity by cAMP is PKA-, CaMK II- and phosphatase-dependent. *Neurochemistry international*, **45**, 627-632.

Park, H.J., Lee, P.H., Ahn, Y.W., Choi, Y.J., Lee, G., Lee, D.Y., Chung, E.S. & Jin, B.K. (2007) Neuroprotective effect of nicotine on dopaminergic neurons by anti-inflammatory action. *The European journal of neuroscience*, **26**, 79-89.

Park, S.Y., Bae, Y.S., Ko, M.J., Lee, S.J. & Choi, Y.W. (2013) Comparison of anti-inflammatory potential of four different dibenzocyclooctadiene lignans in microglia; action via activation of PKA and Nrf-2 signaling and inhibition of MAPK/STAT/NF-kappaB pathways. *Molecular nutrition & food research*.

Pascual, M., Balino, P., Alfonso-Loeches, S., Aragon, C.M. & Guerri, C. (2011) Impact of TLR4 on behavioral and cognitive dysfunctions associated with alcohol-induced neuroinflammatory damage. *Brain Behav Immun*, **25 Suppl 1**, S80-91.

Paterson, G.J., Ohashi, Y., Reynolds, G.P., Pratt, J.A. & Morris, B.J. (2006) Selective increases in the cytokine, TNFalpha, in the prefrontal cortex of PCP-treated rats and human

schizophrenic subjects: influence of antipsychotic drugs. *J Psychopharmacol*, **20**, 636-642.

Peachey, E., Rogers, B., Brien, J.F., Maclean, A. & Rogers, D. (1976) Measurement of acute and chronic behavioural effects of methamphetamine in the mouse. *Psychopharmacology (Berl)*, **48**, 271-275.

Peachey, J.E., Rogers, B. & Brien, J.F. (1977) A comparative study of the behavioural responses induced by chronic administration of methamphetamine and amphetamine in mice. *Psychopharmacology (Berl)*, **51**, 137-140.

Picker, M.J., Yarbrough, J., Hughes, C.E., Smith, M.A., Morgan, D. & Dykstra, L.A. (1993) Agonist and Antagonist Effects of Mixed Action Opioids in the Pigeon Drug Discrimination Procedure - Influence of Training Dose, Intrinsic Efficacy and Interanimal Differences. *Journal of Pharmacology and Experimental Therapeutics*, **266**, 756-767.

Pollack, A. (2004) Coactivation of D1 and D2 dopamine receptors: in marriage, a case of his, hers, and theirs. *Science's STKE : signal transduction knowledge environment*, **2004**, pe50.

Poulsen, E.J., Mannis, M.J. & Chang, S.D. (1996) Keratitis in methamphetamine abusers. *Cornea*, **15**, 477-482.

Price, K.L., Saladin, M.E., Baker, N.L., Tolliver, B.K., DeSantis, S.M., McRae-Clark, A.L. & Brady, K.T. (2010) Extinction of drug cue reactivity in methamphetamine-dependent individuals. *Behaviour research and therapy*, **48**, 860-865.

Puffenbarger, R.A., Boothe, A.C. & Cabral, G.A. (2000) Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia*, **29**, 58-69.

Ranaldi, R. & Poeggel, K. (2002) Baclofen decreases methamphetamine self-administration in rats. *Neuroreport*, **13**, 1107-1110.

Rashid, A.J., So, C.H., Kong, M.M., Furtak, T., El-Ghundi, M., Cheng, R., O'Dowd, B.F. & George, S.R. (2007) D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc Natl Acad Sci U S A*, **104**, 654-659.

Rawson, R.A., Gonzales, R. & Brethen, P. (2002) Treatment of methamphetamine use disorders: an update. *J Subst Abuse Treat*, **23**, 145-150.

Rawson, R.A., Shoptaw, S.J., Obert, J.L., McCann, M.J., Hasson, A.L., Marinelli-Casey, P.J., Brethen, P.R. & Ling, W. (1995) An intensive outpatient approach for cocaine abuse treatment. The Matrix model. *J Subst Abuse Treat*, **12**, 117-127.

Reichel, C.M., Murray, J.E., Grant, K.M. & Bevins, R.A. (2009) Bupropion attenuates methamphetamine self-administration in adult male rats. *Drug Alcohol Depend*, **100**, 54-62.

Richardson, N.R. & Roberts, D.C. (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *Journal of neuroscience methods*, **66**, 1-11.

Robinson, T.E. & Berridge, K.C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain research. Brain research reviews*, **18**, 247-291.

Roger, T., David, J., Glauser, M.P. & Calandra, T. (2001) MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature*, **414**, 920-924.

Rojanathammanee, L., Puig, K.L. & Combs, C.K. (2013) Pomegranate polyphenols and extract inhibit nuclear factor of activated T-cell activity and microglial activation in vitro and in a transgenic mouse model of Alzheimer disease. *The Journal of nutrition*, **143**, 597-605.

Rolan, P., Gibbons, J.A., He, L., Chang, E., Jones, D., Gross, M.I., Davidson, J.B., Sanftner, L.M. & Johnson, K.W. (2008) Ibudilast in healthy volunteers: safety, tolerability and pharmacokinetics with single and multiple doses. *Br J Clin Pharmacol*, **66**, 792-801.

Rolan, P., Hutchinson, M. & Johnson, K. (2009) Ibudilast: a review of its pharmacology, efficacy and safety in respiratory and neurological disease. *Expert Opin Pharmacother*, **10**, 2897-2904.

Roll, J.M., Petry, N.M., Stitzer, M.L., Brecht, M.L., Peirce, J.M., McCann, M.J., Blaine, J., MacDonald, M., DiMaria, J., Lucero, L. & Kellogg, S. (2006) Contingency management for the treatment of methamphetamine use disorders. *The American journal of psychiatry*, **163**, 1993-1999.

Ruiz-Medina, J., Ledent, C., Carreton, O. & Valverde, O. (2011) The A2a adenosine receptor modulates the reinforcement efficacy and neurotoxicity of MDMA. *J Psychopharmacol*, **25**, 550-564.

Russo, S.J., Wilkinson, M.B., Mazei-Robison, M.S., Dietz, D.M., Maze, I., Krishnan, V., Renthal, W., Graham, A., Birnbaum, S.G., Green, T.A., Robison, B., Lesselyong, A., Perrotti, L.I., Bolanos, C.A., Kumar, A., Clark, M.S., Neumaier, J.F., Neve, R.L., Bhakar, A.L., Barker, P.A. & Nestler, E.J. (2009) Nuclear factor kappa B signaling regulates neuronal morphology and cocaine reward. *J Neurosci*, **29**, 3529-3537.

SAMHSA, S.A.a.M.H.S.A. (2010) Results From the 2010 National Survey on Drug Use and Health: Summary of National Findings. In Services, U.D.o.H.a.H. (ed), Maryland.

Saunders, C., Ferrer, J.V., Shi, L., Chen, J., Merrill, G., Lamb, M.E., Leeb-Lundberg, L.M., Carvelli, L., Javitch, J.A. & Galli, A. (2000) Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism. *Proc Natl Acad Sci U S A*, **97**, 6850-6855.

Sawaya, B.E., Deshmane, S.L., Mukerjee, R., Fan, S. & Khalili, K. (2009) TNF alpha production in morphine-treated human neural cells is NF-kappaB-dependent. *J Neuroimmune Pharmacol*, **4**, 140-149.

Sax, K.W. & Strakowski, S.M. (2001) Behavioral sensitization in humans. *J Addict Dis*, **20**, 55-65.

Schechter, M.D. (1997a) Discrete versus cumulative dosing in dose-response discrimination studies. *Eur J Pharmacol*, **326**, 113-118.

Schechter, M.D. (1997b) Discriminative characteristics of high and low cocaine administration: effect of other psychostimulants. *Pharmacol Biochem Behav*, **56**, 457-463.

Schmitz, Y., Lee, C.J., Schmauss, C., Gonon, F. & Sulzer, D. (2001) Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. *J Neurosci*, **21**, 5916-5924.

Schuster, C.R. (1976) Behavioral methods for the study of drug interactions. *Ann N Y Acad Sci*, **281**, 64-73.

Schuster, C.R. & Thompson, T. (1969) Self administration of and behavioral dependence on drugs. *Annu Rev Pharmacol*, **9**, 483-502.

Schwarz, J.M., Hutchinson, M.R. & Bilbo, S.D. (2011) Early-life experience decreases drug-induced reinstatement of morphine CPP in adulthood via microglial-specific epigenetic programming of anti-inflammatory IL-10 expression. *J Neurosci*, **31**, 17835-17847.

Sekine, Y., Ouchi, Y., Sugihara, G., Takei, N., Yoshikawa, E., Nakamura, K., Iwata, Y., Tsuchiya, K.J., Suda, S., Suzuki, K., Kawai, M., Takebayashi, K., Yamamoto, S., Matsuzaki, H., Ueki, T., Mori, N., Gold, M.S. & Cadet, J.L. (2008) Methamphetamine causes microglial activation in the brains of human abusers. *J Neurosci*, **28**, 5756-5761.

Sevak, R.J., Stoops, W.W., Hays, L.R. & Rush, C.R. (2009) Discriminative stimulus and subject-rated effects of methamphetamine, d-amphetamine, methylphenidate, and triazolam in methamphetamine-trained humans. *J Pharmacol Exp Ther*, **328**, 1007-1018.

Shah, A., Silverstein, P.S., Singh, D.P. & Kumar, A. (2012) Involvement of metabotropic glutamate receptor 5, AKT/PI3K signaling and NF-kappaB pathway in methamphetamine-mediated increase in IL-6 and IL-8 expression in astrocytes. *J Neuroinflammation*, **9**, 52.

Shames, B.D., McIntyre, R.C., Jr., Bensard, D.D., Pulido, E.J., Selzman, C.H., Reznikov, L.L., Harken, A.H. & Meng, X. (2001) Suppression of tumor necrosis factor alpha production by cAMP in human monocytes: dissociation with mRNA level and independent of interleukin-10. *The Journal of surgical research*, **99**, 187-193.

Sharma, H.S. & Ali, S.F. (2008) Acute administration of 3,4-methylenedioxymethamphetamine induces profound hyperthermia, blood-brain barrier disruption, brain edema formation, and cell injury. *Ann N Y Acad Sci*, **1139**, 242-258.

Shelton, K.L. & Beardsley, P.M. (2008) Effect of drug-paired exteroceptive stimulus presentations on methamphetamine reinstatement in rats. *Pharmacol Biochem Behav*, **90**, 434-440.

Shelton, K.L., Hendrick, E.S. & Beardsley, P.M. (2013) Efficacy of buspirone for attenuating cocaine and methamphetamine reinstatement in rats. *Drug Alcohol Depend*, **129**, 210-216.

Shetty, V., Mooney, L.J., Zigler, C.M., Belin, T.R., Murphy, D. & Rawson, R. (2010) The relationship between methamphetamine use and increased dental disease. *Journal of the American Dental Association*, **141**, 307-318.

Shi, F.D., Piao, W.H., Kuo, Y.P., Campagnolo, D.I., Vollmer, T.L. & Lukas, R.J. (2009) Nicotinic attenuation of central nervous system inflammation and autoimmunity. *J Immunol*, **182**, 1730-1739.

Shoptaw, S., Reback, C.J., Larkins, S., Wang, P.C., Rotheram-Fuller, E., Dang, J. & Yang, X. (2008) Outcomes using two tailored behavioral treatments for substance abuse in urban gay and bisexual men. *J Subst Abuse Treat*, **35**, 285-293.

Shoptaw, S., Reback, C.J., Peck, J.A., Yang, X., Rotheram-Fuller, E., Larkins, S., Veniegas, R.C., Freese, T.E. & Hucks-Ortiz, C. (2005) Behavioral treatment approaches for methamphetamine dependence and HIV-related sexual risk behaviors among urban gay and bisexual men. *Drug Alcohol Depend*, **78**, 125-134.

Smout, M.F., Longo, M., Harrison, S., Minniti, R., Wickes, W. & White, J.M. (2010) Psychosocial treatment for methamphetamine use disorders: a preliminary randomized controlled trial of cognitive behavior therapy and Acceptance and Commitment Therapy. *Substance abuse : official publication of the Association for Medical Education and Research in Substance Abuse*, **31**, 98-107.

Snider, S.E., Vunck, S.A., van den Oord, E.J., Adkins, D.E., McClay, J.L. & Beardsley, P.M. (2012) The glial cell modulators, ibudilast and its amino analog, AV1013, attenuate methamphetamine locomotor activity and its sensitization in mice. *Eur J Pharmacol*, **679**, 75-80.

Sofuoglu, M., Mooney, M., Kosten, T., Waters, A. & Hashimoto, K. (2011) Minocycline attenuates subjective rewarding effects of dextroamphetamine in humans. *Psychopharmacology (Berl)*, **213**, 61-68.

Solinas, M., Ferre, S., You, Z.B., Karcz-Kubicha, M., Popoli, P. & Goldberg, S.R. (2002) Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *J Neurosci*, **22**, 6321-6324.

Solinas, M., Panlilio, L.V., Justinova, Z., Yasar, S. & Goldberg, S.R. (2006) Using drug-discrimination techniques to study the abuse-related effects of psychoactive drugs in rats. *Nature protocols*, **1**, 1194-1206.

Somjen, G.G. (1988) Nervenkitt: notes on the history of the concept of neuroglia. *Glia*, **1**, 2-9.

Sriram, K., Miller, D.B. & O'Callaghan, J.P. (2006) Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: role of tumor necrosis factor-alpha. *J Neurochem*, **96**, 706-718.

Stafford, D. & Branch, M.N. (1998) Effects of step size and break-point criterion on progressive-ratio performance. *Journal of the experimental analysis of behavior*, **70**, 123-138.

Steketee, J.D. & Kalivas, P.W. (2011) Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. *Pharmacological reviews*, **63**, 348-365.

Stellwagen, D. & Malenka, R.C. (2006) Synaptic scaling mediated by glial TNF-alpha. *Nature*, **440**, 1054-1059.

Strakowski, S.M. & Sax, K.W. (1998) Progressive behavioral response to repeated d-amphetamine challenge: further evidence for sensitization in humans. *Biological psychiatry*, **44**, 1171-1177.

Suzumura, A., Ito, A., Yoshikawa, M. & Sawada, M. (1999) Ibudilast suppresses TNFalpha production by glial cells functioning mainly as type III phosphodiesterase inhibitor in the CNS. *Brain Res*, **837**, 203-212.

Svenningsson, P., Nairn, A.C. & Greengard, P. (2005) DARPP-32 mediates the actions of multiple drugs of abuse. *The AAPS journal*, **7**, E353-360.

Svenningsson, P., Tzavara, E.T., Carruthers, R., Rachleff, I., Wattler, S., Nehls, M., McKinzie, D.L., Fienberg, A.A., Nomikos, G.G. & Greengard, P. (2003) Diverse psychotomimetics act through a common signaling pathway. *Science*, **302**, 1412-1415.

Szabo, G., Dolganiuc, A., Dai, Q. & Pruett, S.B. (2007) TLR4, ethanol, and lipid rafts: a new mechanism of ethanol action with implications for other receptor-mediated effects. *J Immunol*, **178**, 1243-1249.

Tai, K., Iwasaki, H., Ikegaya, S. & Ueda, T. (2013) Minocycline modulates cytokine and chemokine production in lipopolysaccharide-stimulated THP-1 monocytic cells by inhibiting IkappaB kinase alpha/beta phosphorylation. *Translational research : the journal of laboratory and clinical medicine*, **161**, 99-109.

Tanibuchi, Y., Shimagami, M., Fukami, G., Sekine, Y., Iyo, M. & Hashimoto, K. (2010) A case of methamphetamine use disorder treated with the antibiotic drug minocycline. *General hospital psychiatry*, **32**, 559 e551-553.

Tatsuta, T., Kitanaka, N., Kitanaka, J., Morita, Y. & Takemura, M. (2006) Lobeline attenuates methamphetamine-induced stereotypy in adolescent mice. *Neurochem Res*, **31**, 1359-1369.

Theodore, S., Cass, W.A. & Maragos, W.F. (2006) Involvement of cytokines in human immunodeficiency virus-1 protein Tat and methamphetamine interactions in the striatum. *Experimental neurology*, **199**, 490-498.

Thomas, D.M., Dowgiert, J., Geddes, T.J., Francescutti-Verbeem, D., Liu, X. & Kuhn, D.M. (2004a) Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. *Neurosci Lett*, **367**, 349-354.

Thomas, D.M., Walker, P.D., Benjamins, J.A., Geddes, T.J. & Kuhn, D.M. (2004b) Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *J Pharmacol Exp Ther*, **311**, 1-7.

Thomas, D.M., Walker, P.D., Benjamins, J.A., Geddes, T.J. & Kuhn, D.M. (2004c) Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *J Pharmacol Exp Ther*, **311**, 1-7.

Thompson, T. & Pickens, R. (1970) Stimulant self-administration by animals: some comparisons with opiate self-administration. *Federation proceedings*, **29**, 6-12.

Tidey, J.W. & Bergman, J. (1998) Drug discrimination in methamphetamine-trained monkeys: Agonist and antagonist effects of dopaminergic drugs. *Journal of Pharmacology and Experimental Therapeutics*, **285**, 1163-1174.

Tominaga, Y., Nakamura, Y., Tsuji, K., Shibata, T. & Kataoka, K. (1996) Ibudilast protects against neuronal damage induced by glutamate in cultured hippocampal neurons. *Clin Exp Pharmacol Physiol*, **23**, 519-523.

Torres, E., Gutierrez-Lopez, M.D., Borcel, E., Peraile, I., Mayado, A., O'Shea, E. & Colado, M.I. (2010) Evidence that MDMA ('ecstasy') increases cannabinoid CB2 receptor expression in microglial cells: role in the neuroinflammatory response in rat brain. *J Neurochem*, **113**, 67-78.

Tourino, C., Zimmer, A. & Valverde, O. (2010) THC Prevents MDMA Neurotoxicity in Mice. *PLoS One*, **5**, e9143.

Toussi, S.S., Joseph, A., Zheng, J.H., Dutta, M., Santambrogio, L. & Goldstein, H. (2009) Short communication: Methamphetamine treatment increases in vitro and in vivo HIV replication. *AIDS Res Hum Retroviruses*, **25**, 1117-1121.

Valles, S.L., Blanco, A.M., Pascual, M. & Guerri, C. (2004) Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. *Brain pathology*, **14**, 365-371.

Vanderschuren, L.J. & Everitt, B.J. (2005) Behavioral and neural mechanisms of compulsive drug seeking. *Eur J Pharmacol*, **526**, 77-88.

Volterra, A. & Meldolesi, J. (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nature reviews. Neuroscience*, **6**, 626-640.

Wagner, H.N., Jr., Burns, H.D., Dannals, R.F., Wong, D.F., Langstrom, B., Duelfer, T., Frost, J.J., Ravert, H.T., Links, J.M., Rosenbloom, S.B., Lukas, S.E., Kramer, A.V. & Kuhar, M.J. (1983) Imaging dopamine receptors in the human brain by positron tomography. *Science*, **221**, 1264-1266.

Walker, E.A. & Young, A.M. (1993) Discriminative-Stimulus Effects of the Low Efficacy Mu Agonist Nalbuphine. *Journal of Pharmacology and Experimental Therapeutics*, **267**, 322-330.

Wang, X., Loram, L.C., Ramos, K., de Jesus, A.J., Thomas, J., Cheng, K., Reddy, A., Somogyi, A.A., Hutchinson, M.R., Watkins, L.R. & Yin, H. (2012a) Morphine activates neuroinflammation in a manner parallel to endotoxin. *Proc Natl Acad Sci U S A*, **109**, 6325-6330.

Wang, X., Wang, Y., Ye, L., Li, J., Zhou, Y., Sakarcan, S. & Ho, W. (2012b) Modulation of intracellular restriction factors contributes to methamphetamine-mediated enhancement of acquired immune deficiency syndrome virus infection of macrophages. *Current HIV research*, **10**, 407-414.

Watanabe-Galloway, S., Ryan, S., Hansen, K., Hullsiek, B., Muli, V. & Malone, A.C. (2009) Effects of methamphetamine abuse beyond individual users. *J Psychoactive Drugs*, **41**, 241-248.

Wilms, H., Claasen, J., Rohl, C., Sievers, J., Deuschl, G. & Lucius, R. (2003) Involvement of benzodiazepine receptors in neuroinflammatory and neurodegenerative diseases: evidence from activated microglial cells in vitro. *Neurobiology of disease*, **14**, 417-424.

Winslow, B.T., Voorhees, K.I. & Pehl, K.A. (2007) Methamphetamine abuse. *American family physician*, **76**, 1169-1174.

Wires, E.S., Alvarez, D., Dobrowolski, C., Wang, Y., Morales, M., Karn, J. & Harvey, B.K. (2012) Methamphetamine activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) and induces human immunodeficiency virus (HIV) transcription in human microglial cells. *Journal of neurovirology*, **18**, 400-410.

Wolf, M.E. (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Progress in neurobiology*, **54**, 679-720.

Woolverton, W.L. & Balster, R.L. (1982) Behavioral pharmacology of local anesthetics: reinforcing and discriminative stimulus effects. *Pharmacol Biochem Behav*, **16**, 491-500.

Wu, Y., Lousberg, E.L., Moldenhauer, L.M., Hayball, J.D., Robertson, S.A., Coller, J.K., Watkins, L.R., Somogyi, A.A. & Hutchinson, M.R. (2011) Attenuation of microglial and IL-1 signaling protects mice from acute alcohol-induced sedation and/or motor impairment. *Brain Behav Immun*, **25 Suppl 1**, S155-164.

Xie, Z. & Miller, G.M. (2007) Trace amine-associated receptor 1 is a modulator of the dopamine transporter. *J Pharmacol Exp Ther*, **321**, 128-136.

Yamaguchi, T., Kuraishi, Y., Minami, M., Nakai, S., Hirai, Y. & Satoh, M. (1991) Methamphetamine-induced expression of interleukin-1 beta mRNA in the rat hypothalamus. *Neurosci Lett*, **128**, 90-92.

Yamamoto, B.K. & Zhu, W. (1998) The effects of methamphetamine on the production of free radicals and oxidative stress. *J Pharmacol Exp Ther*, **287**, 107-114.

Yan, Y., Mizuno, T., Nitta, A., Yamada, K. & Nabeshima, T. (2004) Nefiracetam attenuates methamphetamine-induced discriminative stimulus effects in rats. *Ann N Y Acad Sci*, **1025**, 274-278.

Yan, Y., Nitta, A., Mizuno, T., Nakajima, A., Yamada, K. & Nabeshima, T. (2006) Discriminative-stimulus effects of methamphetamine and morphine in rats are attenuated by cAMP-related compounds. *Behav Brain Res*, **173**, 39-46.

Yan, Y., Yamada, K., Niwa, M., Nagai, T., Nitta, A. & Nabeshima, T. (2007) Enduring vulnerability to reinstatement of methamphetamine-seeking behavior in glial-cell-line-derived neurotrophic factor mutant mice. *Faseb J*, **21**, 1994-2004.

Yan, Z., Feng, J., Fienberg, A.A. & Greengard, P. (1999) D(2) dopamine receptors induce mitogen-activated protein kinase and cAMP response element-binding protein phosphorylation in neurons. *Proc Natl Acad Sci U S A*, **96**, 11607-11612.

Yang, F.L., Li, C.H., Hsu, B.G., Tsai, N.M., Lin, S.Z., Harn, H.J., Chen, H.I., Liao, K.W. & Lee, R.P. (2007) The reduction of tumor necrosis factor-alpha release and tissue damage by pentobarbital in the experimental endotoxemia model. *Shock*, **28**, 309-316.

Yao, H., Yang, Y., Kim, K.J., Bethel-Brown, C., Gong, N., Funa, K., Gendelman, H.E., Su, T.P., Wang, J.Q. & Buch, S. (2010) Molecular mechanisms involving sigma receptor-mediated induction of MCP-1: implication for increased monocyte transmigration. *Blood*, **115**, 4951-4962.

Yu, B., Becnel, J., Zerfaoui, M., Rohatgi, R., Boulares, A.H. & Nichols, C.D. (2008) Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency. *J Pharmacol Exp Ther*, **327**, 316-323.

Yui, K., Goto, K., Ikemoto, S. & Ishiguro, T. (2000) Stress induced spontaneous recurrence of methamphetamine psychosis: the relation between stressful experiences and sensitivity to stress. *Drug Alcohol Depend*, **58**, 67-75.

Yui, K., Goto, K., Ishiguro, T. & Ikemoto, S. (1997) Noradrenergic activity and spontaneous recurrence of methamphetamine psychosis. *Drug Alcohol Depend*, **44**, 183-187.

Zhang, L., Kitaichi, K., Fujimoto, Y., Nakayama, H., Shimizu, E., Iyo, M. & Hashimoto, K. (2006) Protective effects of minocycline on behavioral changes and neurotoxicity in mice after administration of methamphetamine. *Prog Neuropsychopharmacol Biol Psychiatry*, **30**, 1381-1393.

Zhang, X.Q., Cui, Y., Cui, Y., Chen, Y., Na, X.D., Chen, F.Y., Wei, X.H., Li, Y.Y., Liu, X.G. & Xin, W.J. (2012) Activation of p38 signaling in the microglia in the nucleus accumbens contributes to the acquisition and maintenance of morphine-induced conditioned place preference. *Brain Behav Immun*, **26**, 318-325.

Zhang, Y., Li, H., Li, Y., Sun, X., Zhu, M., Hanley, G., Lesage, G. & Yin, D. (2011) Essential role of toll-like receptor 2 in morphine-induced microglia activation in mice. *Neurosci Lett*, **489**, 43-47.

Zhu, J.P., Xu, W. & Angulo, J.A. (2006) Distinct mechanisms mediating methamphetamine-induced neuronal apoptosis and dopamine terminal damage share the neuropeptide substance p in the striatum of mice. *Ann N Y Acad Sci*, **1074**, 135-148.

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