EFFECT OF SA 4503 ON THE LOCOMOTOR STIMULATORY AND DISCRIMINATIVE STIMULUS PROPERTIES OF PSYCHOSTIMULANTS

A Dissertation presented to the Faculty of the Graduate School

University of Missouri

In Partial Fulfillment

Of the Requirements for the Degree

Doctor of Philosophy

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by

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MAY 2010

The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

EFFECT OF SA 4503 ON THE LOCOMOTOR STIMULATORY AND DISCRIMINATIVE STIMULUS PROPERTIES OF PSYCHOSTIMULANTS

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And hereby certify that in their opinion it is worthy of acceptance.

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I would like to dedicate this dissertation to my family. My journey has been long and the road has had many obstacles. Through all of the challenges that I have faced, you have been there to support me and guide me back to my path. Without your love, support and guidance throughout this process, I could not have succeeded. THANK YOU AND I LOVE YOU!

Additionally, I would like to thank my friends and peers. They have spent countless hours listening and offering their advice. And when I was inconsolable, they wisely distracted me in order for me to regain my perspective. THANK YOU AND MAY YOU BE CLOSE BEHIND!

ACKNOWLEDGEMENTS

There are many people that I would like to thank; without them this project could not have been completed. First, I would like to thank my committee members Dr. Dennis K. Miller, Dr. Todd R. Schachtman, Dr. Matthew J. Will, Dr. Thomas M. Piasecki, and Dr. Susan Z. Lever for all the time and encouragement that you have given me and this project. Thank you for your guidance and support throughout my years in graduate school. I would also like to give a special thank you to Gretchen Hendrickson for her countless hours of statistical advice.

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Food and Drug Administration	FDA
Gamma-aminobutyric acid	GABA
Dopamine transporter	DAT
Serotonin transporter	SERT
Norepinephrine transporter	NET
Vesicular monoamine transporter	VMAT
N,N-dimethyltryptamine	DMT
Dehyroepianderosterone	DHEA
Inositol triphosphate	IP_3
Di-o-tolylguanidine	DTG
N-methyl-D-aspartic acid	NMDA
Repeated measures analysis of variance	RM-ANOVA
Vesicular acetylcholine transporter	VAChT
Fixed ratio	FR

ABSTRACT

Methamphetamine and cocaine exhibit affinity (K_i value ≈ 2.2 and 2.0 μM, respectively) for σ_1 sigma receptors. Sigma receptors mediate the neurotoxic properties of methamphetamine, and the convulsive and lethal effects of cocaine. However, much less is known about the interaction of sigma receptors and the behavioral properties of these psychostimulants. The development of novel, selective sigma receptor ligands have been invaluable to elucidate the interaction of these receptors with methamphetamine's behavioral properties. SA 4503 exhibits high, preferential affinity for σ_1 sigma receptors. The present study investigated the effect of SA 4503 on the locomotor stimulatory properties of methamphetamine, and discriminative stimulus properties of methamphetamine and cocaine. In the locomotor activity study, SA 4503 dose-dependently potentiated and attenuated methamphetamine-induced hyperactivity. In the drug discrimination study, SA 4503 pretreatment augmented the effect of methamphetamine to substitute for the methamphetamine and cocaine discriminative stimuli. Our findings suggest sigma receptors mediate the locomotor stimulatory properties, and the discriminative stimulus properties of methamphetamine and cocaine. Together, these observations indicate that while SA 4503 may not be a particularly effective pharmacotherapy, sigma receptors may be important targets for investigating the mechanism underlying methamphetamine- and cocaine-induced behaviors.

INTRODUCTION

Illicit psychostimulant use, specifically cocaine and methamphetamine, in the United States is among the top major health concerns in our nation. In 2007, 2.1 million and ~530,000 Americans aged 12 or older reported using cocaine and methamphetamine, respectively (SAMHSA, 2008). Illicit substance dependence, as defined by the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV), soared in 2007 at an estimated 22.3 million Americans aged 12 or older (SAMHSA, 2008). The number of Americans who met criteria for cocaine dependence reached ~1.6 million and ~406,000 for methamphetamine dependence. Thus, psychostimulant dependence is a major public health concern in the United States and there is an urgent need for viable, effective treatments for cocaine and methamphetamine addiction.

Currently, there are no Food and Drug Administration (FDA) approved and effective pharmacotherapies for cocaine and methamphetamine addiction. Based on the known pharmacology of cocaine and methamphetamine, several existing compounds have been investigated for their ability to effectively treat addiction to these psychostimulants. Among the earliest investigations for cocaine and methamphetamine treatments were dopamine agonist substitution/replacement therapies. Several dopamine agonists failed to produce clinical effectiveness for the treatment of cocaine addiction (Preti, 2007). One study employing randomized controlled trials with dextroamphetamine substitution for methamphetamine addiction resulted in a trend toward efficacy; however, it was

not statistically significant (Barr et al., 2006). Dopamine receptor antagonists were investigated for cocaine addiction treatments with positive efficacy; however, these drugs are poorly tolerated by patients (Preti, 2007). Additionally, antidepressant drugs have been examined due to their ability to increase levels of monoamine neurotransmitters. Selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and tricyclic antidepressant drugs were not efficacious for cocaine or methamphetamine addiction treatment (Barr et al., 2006; Preti, 2007). Taken together, compounds targeting dopamine receptors and monoamine neurotransmitters have not yielded successful pharmacotherapies, thus it is essential to examine other receptors and neurotransmitter systems. Presently, numerous pharmacological targets for psychostimulant addiction are being investigated, including serotonin receptors, monoamine transporters, CB_1 cannabinoid receptors, metabotropic and ionotropic glutamate receptors, GABA receptors, and sigma receptors (Elkashef et al., 2008; Preti, 2007). The focus of the current study will be on investigating sigma receptors as potential treatment targets for cocaine and methamphetamine addiction.

Psychostimulant Neurobiology

Psychostimulants readily cross the blood-brain barrier, due to their high lipid solubility, and activate the central nervous system, thus producing their various neurochemical and behavioral effects. Both cocaine and methamphetamine increase synaptic levels of monoamine neurotransmitters (dopamine, norepinephrine, and serotonin) in the brain, however, their

mechanisms differ. Cocaine increases monoamines by preventing neurotransmitter reuptake by blocking their respective plasma membrane transporters (Izenwasser, 2004). Cocaine has moderate affinity for the dopamine (DAT), serotonin (SERT), and norepinephrine (NET) transporters (K_i = 0.3, 0.4, and 1.6 µM, respectively) (Eshleman et al., 1999). On the other hand, methamphetamine elevates monoamine levels via multiple processes. Similar to cocaine, methamphetamine reverses monoamine transporters resulting in increased synaptic levels (Khoshbouei et al., 2003). Methamphetamine has moderate/weak affinity for DAT, SERT, and NET (K_i = 5.2, 122, and 1.5 µM, respectively) (Eshleman et al., 1999). Additionally, the amphetamines increase cytosolic monoamine levels by reversal of the vesicular monoamine transporter (VMAT) (K_i = 0.14 µM) (Brown et al., 2001; Peter et al., 1994) and inhibition of monoamine oxidase activity (Mantle et al., 1976). Other mechanisms include preventing the cell surface expression of monoamine transporters (Saunders et al., 2000) and increasing the activity and expression of tyrosine hydroxylase, the enzyme responsible for synthesizing catecholamines (Mandell & Morgan, 1970). Thus, psychostimulants influence multiple processes in the central nervous system; however, both cocaine and methamphetamine produce their effects by elevating extracellular levels of monoamine neurotransmitters.

As with the neurochemical effects of cocaine and methamphetamine, the behavioral effects of these psychostimulants are also very similar. Cocaine and methamphetamine's acute effects manifest as feelings of euphoria, increased mental alertness, wakefulness and energy, and loss of appetite (NIDA, 2004;

2006). The acute physiological effects of cocaine and methamphetamine both include elevated heart rate, blood pressure and body temperature along with dilated pupils and constricted blood vessels (NIDA, 2004; 2006). Repeated use of cocaine and methamphetamine can result in substance dependence/addiction (tolerance to euphoric properties, physical and psychological withdrawal and sensitization to anesthetic and convulsant effects), paranoid psychosis (auditory hallucinations, delusions, paranoia and stereotypic movements), irritability and mood disturbances (NIDA, 2004; 2006). Thus, cocaine and methamphetamine produce numerous similar negative behavioral and physiological effects which further emphasize the need for the development of effective treatments.

Although cocaine and methamphetamine have many similarities, there are inherent differences among the two drugs. Both cocaine and methamphetamine increase levels of locomotor activity in rodents, but their potencies differ. Locomotor activity is traditionally used as an initial screening tool for the pharmacological effects predictive of drug efficacy. In the laboratory, locomotion is assessed via an automated locomotor activity monitor. In a typical experiment, animals are administered a drug, placed in an open field arena and allowed to explore for a period of time. In mice, methamphetamine produced maximum hyperactivity at a dose of 6.7 µmol/kg, while cocaine produced maximum hyperactivity at a dose of 59 µmol/kg (Katz et al., 2003; Matsumoto et al., 2008). Thus, methamphetamine is more potent than cocaine at producing changes in locomotor activity.

In humans, psychoactive drugs can serve as a discriminative stimulus (S^D). The presentation of the stimulus triggers a behavioral response, and if a behavior is produced, a reinforcer is obtained. Hence, drug discrimination procedures were developed based on operant conditioning. This paradigm can be used to elucidate the underlying mechanisms of action of psychoactive drugs. Additionally, the S^D properties of drugs have been suggested to be homologous to the subjective effects of drugs experienced in humans (Colpaert, 1999). In a typical experiment, animals are trained to discriminate between a particular dose of a drug (training drug) and saline. During training sessions, food-deprived animals press the appropriate-paired lever (either saline-paired or drug-paired depending on the training session type) in order to obtain food reinforcers. Once the animal reliably achieves stimulus control, test sessions commence where generalization and/or pretreatment tests are performed. To this end, the subjective effects and neurobiological mechanisms of psychoactive drugs can be assessed in animals using the drug discrimination assay. The cross-substitution of cocaine and methamphetamine demonstrates similar, but not identical interoceptive properties for these drugs. In rats trained to discriminate between cocaine and saline, methamphetamine fully substituted for the cocaine S^D, but was more potent than cocaine (ED_{50} = 2.2 vs. 8.8 µmol/kg) (Li et al., 2006). Additionally, in rats trained to discriminate methamphetamine from saline, cocaine fully substituted for the methamphetamine S^D, but was less potent than methamphetamine (ED_{50} = 12.9 vs. 3.4 µmol/kg) (Munzar & Goldberg, 2000).

Thus, the interoceptive properties of cocaine and methamphetamine differ, so it is important to examine both drugs.

Sigma Receptors

The lack of viable pharmacotherapies for psychostimulant addiction has led researchers to investigate sigma receptors as potential targets for treatment development. Martin and colleagues first identified sigma receptors in the mid-1970s (Martin et al., 1976). Sigma receptors represent a unique class of receptors distinct from other identified receptor classes (Walker et al., 1990). Since their discovery, two subtypes have been characterized, namely σ_1 and σ_2 (Hellewell & Bowen, 1990), which have been found in portions of the limbic system and brain stem (Walker et al., 1990). More specifically, the σ_1 sigma receptors were found to be expressed in several midbrain subregions, including striatum, substantia nigra, nucleus accumbens, and ventral tegmental area (Hayashi et al., 2010). The σ_1 sigma receptor has been cloned (Hanner et al., 1996); however, the σ_2 sigma receptor has not been cloned, which has important implications for investigating the relationship between the two distinct receptor subtypes and psychostimulant addiction. While the receptor has been identified, the endogenous ligand has not, although there are candidates.

Recently, it has been suggested that N,N-dimethyltryptamine (DMT) is an endogenous σ_1 sigma receptor agonist (Fontanilla et al., 2009). However, DMT has weak affinity for the σ_1 sigma receptor (K_d = 14 µM) (Fontanilla et al., 2009). Historically, the neuroactive steroids progesterone and dehyroepianderosterone (DHEA) have been implicated as endogenous sigma receptor agonist and

antagonist, respectively (Monnet et al., 1995). Progesterone has high affinity (K_i = 0.03 μM) and DHEA has moderate affinity (K_i = 0.7 μM) for the σ_1 sigma receptor (Waterhouse et al., 2007). The σ_2 sigma receptor endogenous ligand remains to be identified. Thus, there is a need to fully characterize the endogenous ligands in order to identify the function of these receptors in the central nervous system.

Currently, the downstream signal transduction pathways regulated by sigma receptors and the mechanism by which sigma receptors modulate downstream neurotransmitter systems, like dopamine, are unknown. Sigma receptors are thought to modulate intracellular signaling via a classic second messenger, inositol triphosphate, (IP_3). Research suggests sigma receptor agonists trigger calcium release by inducing dissociation of sigma receptors from IP_3 receptors (Hayashi & Su, 2001). Conversely, sigma receptor antagonists prevent sigma receptors from dissociating from IP_3 receptors (Hayashi & Su, 2001). It has been hypothesized that amplification of neurotransmitter systems, such as dopamine, may be caused by this excess calcium (Su & Hayashi, 2003). As such, activation of sigma receptors is sufficient to induce dopamine synthesis and release (Booth & Baldessarini, 1991; Patrick et al., 1993). Application of pentazocine increased tyrosine hydroxylase activity in rat striatal nerve terminals (Booth & Baldessarini, 1991). Furthermore, both sigma receptor agonists di-o-tolylguanidine (DTG) and pentazocine dose-dependently increased extracellular dopamine levels in striatum (Patrick et al., 1993). Thus, these receptors may

modulate brain dopamine pathways which are the primary targets for psychostimulants, such as cocaine and methamphetamine.

Both cocaine and methamphetamine have affinity for sigma receptors with preferential affinity for the σ_1 sigma receptor compared to the σ_2 sigma receptor subtype (see Table 1) (Matsumoto et al., 2002; Nguyen et al., 2005). The affinity for sigma receptors suggests that methamphetamine and cocaine's effects may be partially mediated by these receptors. σ_1 Sigma receptors were upregulated in midbrain regions in rats actively self-administering methamphetamine (Stefanski et al., 2004), including ventral tegmental area and substantia nigra (Hayashi et al., 2010). These studies indicate that sigma receptors are involved in the reinforcing properties of methamphetamine. Additionally, the σ_1 sigma receptor has been implicated in the reinforcing properties of cocaine. Acquisition and expression of cocaine-induced conditioned place preference was prevented by pretreatment with selective σ_1 sigma receptor antagonists NE-100 and BD1047 (Romieu et al., 2002). In a later study, repeated treatment with an antisense oligodeoxynucleotide targeting the σ_1 sigma receptor blocked reactivation of place-conditioning (Romieu et al., 2004). Antisense oligodeoxynucleotides decrease the number of σ_1 sigma receptors that are accessible to cocaine. Additionally, pretreatment with an antisense oligodeoxynucleotide protected animals against the convulsive and locomotor stimulatory effects of cocaine (Matsumoto et al., 2001a; Matsumoto et al., 2002). Thus, σ_1 sigma receptors appear to play an important role in cocaine- and methamphetamine-induced behaviors.

Table 1: Psychostimulant binding at sigma receptors

Psychostimulant	Affinity (K_i in μM)	
	σ_1	σ_2
Cocaine	2.0 ± 0.2	31 ± 4.0
Methamphetamine	2.2 ± 0.25	46.7 ± 10.3

Selective σ_1 sigma receptor agonist SA 4503

Our knowledge of the mechanism of sigma receptors was limited due to the lack of selective ligands available. The development of novel, selective sigma receptor ligands have been invaluable to elucidate the interaction of these receptors with psychostimulant addiction. The focus of the present studies is to investigate the effect of selective σ_1 sigma receptor agonist SA 4503 on psychostimulant-induced behavioral effects. SA 4503 exhibits high affinity for sigma receptors with preference for the σ_1 sigma receptor (K_i = 0.004 μM) compared to the σ_2 sigma receptor (K_i = 0.06 μM) (Lever et al., 2006). Matsuno and colleagues suggest that SA 4503 is a potent and selective σ_1 sigma receptor agonist. SA 4503 showed no affinity (K_i > 10 μM) for 36 receptors, ion channels, and second messengers systems assessed in this study (Matsuno et al., 1996). Thus, SA 4503 is likely acting on sigma receptors to produce its effects.

In parallel with these receptor binding studies, research has aimed to elucidate the physiological functions of sigma receptors in the central nervous system by using this selective agonist. Recent work from our laboratory has investigated the effects of SA 4503 on cocaine-induced locomotor activity and methamphetamine-induced dopamine release. In the former study, mice were placed in an automated locomotor activity monitor for 45 min, injected with SA

4503 or saline, and returned to the monitor for 15 min. Subsequently, mice were administered cocaine or saline and returned to the monitor for 60 min. SA 4503 dose-dependently attenuated cocaine-induced hyperactivity. In the latter study, rat striatal slices were preloaded with [^{3}H]dopamine and superfused with concentrations of SA 4503 alone and then in the presence of methamphetamine (3 or 10 μM). SA 4503 did not evoke dopamine release, but blocked methamphetamine-induced dopamine release in striatal slices in a concentration-dependent manner. Thus, preliminary data from our laboratory suggest that SA 4503 is sufficient to prevent cocaine- and methamphetamine-induced behavioral and neurochemical effects, respectively.

Previous research has shown that SA 4503 exhibits antireinforcing, antiamnesic, and neuroprotective effects. Pretreatment with SA 4503 attenuated the conditioned place preference response to nicotine (Horan et al., 2001). Nicotine produced place-conditioning, indicating the reinforcing properties of the psychostimulant (Horan et al., 2001). In addition to the antireinforcing effects, SA 4503 significantly reduced scopolamine- and dizocilpine-induced working memory impairments (Senda et al., 1996; Zou et al., 2000). Moreover, the beneficial effects of SA 4503 were reversed by putative σ_1 sigma receptor antagonist NE-100, suggesting that σ_1 sigma receptors are important for working memory (Senda et al., 1996; Zou et al., 2000). Activation of σ_1 sigma receptor by SA 4503 suppressed hypoxia/hypoglycemia-induced neurotoxicity (Nakazawa et al., 1998). The suppression of neurotoxicity was blocked by NE-100, implicating a role for σ_1 sigma receptors (Nakazawa et al., 1998). These findings indicate

that SA 4503 is beneficial to prevent the reinforcing properties of nicotine, working memory deficits, and neurotoxicity. SA 4503 is likely acting on σ_1 sigma receptors; however, the downstream mechanism of SA 4503 to produce these effects is unclear. In subsequent sections, the effect of several sigma receptor agonists (DTG, pentazocine, BD1031, and BD1052) and antagonists (rimcazole, BMY-14802, and analogs of BD1008 and YX-069) on cocaine- and methamphetamine-induced behaviors will be discussed.

Role of sigma receptor agonists on cocaine-induced behavioral effects

Sigma receptor agonism potentiates cocaine-induced behaviors. Both σ_1 and σ_2 sigma receptor selective agonists exacerbate the convulsive effects of cocaine. In humans, convulsions or seizures are a documented outcome of cocaine intoxication (Pascual-Leone et al., 1990). As such, cocaine-induced convulsions represent a measure of behavioral toxicity in rodents. Pretreatment with putative sigma receptor agonists, BD1031, BD1052, and DTG, worsens cocaine-induced convulsions; however, pentazocine was ineffective to alter the response to the convulsive effects of cocaine (Matsumoto et al., 2001a; Matsumoto et al., 2001b). Additionally, DTG, pentazocine, BD1031, and BD1052 pretreatment exacerbates cocaine-induced lethality (Matsumoto et al., 2001a; Matsumoto et al., 2001b). In humans, death can be produced by cocaine overdose; hence, administration of a lethal cocaine dose represents the behavioral intoxication endpoint.

In addition to cocaine's toxic effects in humans, acute cocaine administration can produce behavioral changes that manifest as psychomotor

activation. Animals administered cocaine systemically were 300% more active than animals in the control group (Skuza, 1999). Sigma receptor agonist DTG did not alter locomotor activity intrinsically (Skuza, 1999). However, pretreatment with DTG dose-dependently potentiates the locomotor stimulatory effects of cocaine (Skuza, 1999). In a drug discrimination study, rats were trained to discriminate cocaine from saline. Sigma receptor agonist DTG did not substitute for cocaine, but shifted the cocaine dose-response curve to the left (Ukai et al., 1997). These results suggest that DTG alone produced a subjective state different from cocaine, but augmented the S^D properties of cocaine.

It appears that sigma receptor agonists DTG, pentazocine, BD1031, and BD1052 facilitate the convulsive, lethal, locomotor stimulatory and S^D effects of cocaine. These observations are in contrast to the effects of SA 4503 on the convulsive and locomotor stimulatory properties of cocaine. Skuza and colleagues reported that SA 4503 did not significantly alter cocaine-induced convulsions and hyperactivity (Skuza, 1999). Thus, it is necessary to elucidate the role of SA 4503 on the behavioral properties of psychostimulants.

Role of sigma receptor antagonists on cocaine-induced effects

Sigma receptor antagonism attenuates cocaine-induced behaviors. Both σ_1 and σ_2 sigma receptor selective antagonists attenuate the convulsive effects of cocaine. Matsumoto and colleagues pretreated rodents with sigma receptor antagonists followed by a convulsive dose of cocaine. Several compounds from the ethylenediamines, the largest characterized class of sigma receptor antagonists, significantly attenuate cocaine-induced convulsions (Matsumoto et

al., 2004a; Matsumoto et al., 2001a; Matsumoto et al., 2001b; Matsumoto et al., 2002). These compounds include BD1008 and its analogs (BD1018, BD1060, BD1063, BD1067, LR132, YX-011, YZ-027, YZ-032, UMB 100, UMB 101, and UMB 103). Other sigma receptor antagonists found to significantly attenuate the convulsive effects of cocaine include YZ-069 and its analogs (YZ-067, YZ-184, and YZ-185), (±)-SM 21, UMB 24, and BMY-14802 (Matsumoto et al., 2001b; Matsumoto et al., 2004b; Matsumoto et al., 2007). Similarly, sigma receptor antagonists prevent cocaine-induced lethality when administered both prior to and after a lethal dose of cocaine. Matsumoto and colleagues pretreated rodents with σ_1 sigma receptor selective antagonists followed by a lethal cocaine dose. The following σ_1 sigma receptor selective antagonists prevented cocaine-induced lethality: BD1008, BD1018, BD1060, BD1063, BD1067, LR132, YX-011, YZ-027, YZ-032, UMB 100, UMB 101, and UMB 103 (Matsumoto et al., 2004a; Matsumoto et al., 2001a; Matsumoto et al., 2001b; Matsumoto et al., 2002). Additionally, cocaine-induced lethality is attenuated by LR132 and YZ-011 post-treatment (Matsumoto et al., 2001a; Matsumoto et al., 2002). Conversely, pretreatment with σ_2 sigma receptor preferring antagonists, (±)-SM 21 and UMB 24, failed to prevent cocaine-induced lethality (Matsumoto et al., 2007), suggesting that cocaine-induced lethality may be mediated by σ_1 sigma receptors.

Additionally, sigma receptor antagonists attenuate cocaine's locomotor stimulatory effects. Sigma receptor antagonist pretreatment blocks cocaine-induced hyperactivity in rodents (Katz et al., 2003; Liu et al., 2007; Matsumoto et

al., 2001a; Matsumoto et al., 2002; Matsumoto et al., 2007; Menkel et al., 1991). Rimcazole and its analogues SH 3-24, and SH 3-28 dose-dependently decreased locomotor activity (Katz et al., 2003). Pretreatment with rimcazole, SH 3-24, and SH 3-28 prevented cocaine-induced hyperactivity (Katz et al., 2003). Pretreatment with behaviorally inactive doses of BD1018, BD1063, and LR132 significantly attenuated cocaine-induced hyperactivity (Matsumoto et al., 2001a). YZ-011, YZ-027, and YZ-032 pretreatment (at doses ineffective to alter activity alone) prevented the locomotor stimulatory properties of cocaine (Matsumoto et al., 2002). Doses of BMY-14802 and rimcazole that were ineffective to alter locomotor activity intrinsically blocked cocaine-induced hyperactivity (Menkel et al., 1991). Pretreatment with selective σ_1 sigma receptor antagonist TC1 prevented cocaine's locomotor stimulatory properties; however, this TC1 dose significantly decreased locomotor activity when administered alone (Liu et al., 2007). In contrast, σ_2 sigma receptor selective antagonist TC4 produced hyperactivity when administered alone, and pretreatment with TC4 did not prevent cocaine-induced hyperactivity (Liu et al., 2007). Subchronic cocaine exposure can produce locomotor sensitization in which repeated cocaine administration enhances psychomotor activity. Co-administration of sigma receptor antagonists, BMY-14802, rimcazole, or SR-31742A, and cocaine significantly attenuated the development of cocaine-induced locomotor sensitization (Ujike et al., 1996).

In drug discrimination studies, sigma receptor antagonists produce differential effects on a cocaine S^D. In rats trained to discriminate cocaine from

saline injections, rimcazole, SH 3-24, and SH 3-28 produced responding on the saline-paired lever (Katz et al., 2003). Co-administration of rimcazole or SH 3-24 and cocaine did not significantly alter the S^D properties of cocaine, however, co-administration of SH 3-28 and cocaine attenuated the S^D properties of cocaine (Katz et al., 2003). Additionally, TC1 partially substituted, while TC4 failed to substitute for a cocaine S^D, but neither TC1 nor TC4 altered cocaine-appropriate responding when co- administered with cocaine (Liu et al., 2007). Together, these results indicate that sigma receptor antagonists produce a subjective state different from cocaine, but decrease cocaine-appropriate responding when administered concomitantly with cocaine.

Overall, sigma receptor antagonists inhibit cocaine-induced convulsions, lethality, hyperactivity, behavioral sensitization and S^D properties of cocaine. However, there is a degree of inconsistency among the sigma receptor antagonists, particularly across compound classes and selectivity for the sigma receptor subtypes.

Role of sigma receptor antagonists on methamphetamine-induced effects

Sigma receptor antagonism attenuates methamphetamine-induced neurotoxic and behavioral effects. In humans, methamphetamine administration can produce losses of dopamine neuron terminal regions which results in reductions of presynaptic dopamine markers (Davidson et al., 2001). As such, methamphetamine-induced neurotoxicity in animals can be evaluated by measuring tyrosine hydroxylase activity, dopamine content and DAT expression in dopamine neuron-rich brain regions. Pretreatment with BMY-14802 attenuated

methamphetamine-induced reductions in tyrosine hydroxylase activity, dopamine content, and D_1 and D_2 dopamine receptor number (Terleckyj & Sonsalla, 1994). Selective σ_1 sigma receptor antagonist AC927 pretreatment prevented methamphetamine-induced reductions in striatal DAT expression (Matsumoto et al., 2008). Additionally, co-incubation of AC927 and methamphetamine inhibited the cytotoxic effects of methamphetamine in NG108-15 cells (Matsumoto et al., 2008). Furthermore, sigma receptor antagonism attenuates methamphetamine-induced hyperthermia. Hyperthermia or elevated body temperature often accompanies methamphetamine's neurotoxic effects in humans and nonhumans (Davidson et al., 2001). Pretreatment with AC927 prevented methamphetamine-induced increases in body temperature in mice (Matsumoto et al., 2008).

Sigma receptor antagonists also inhibit methamphetamine-induced hyperactivity. Like cocaine, methamphetamine produces behavioral changes that manifest as psychomotor activation in humans and animals. Pretreatment with selective σ_1 sigma receptor antagonists, BD1047, BD1063, and AC927, attenuate methamphetamine's locomotor stimulatory effects (Matsumoto et al., 2008; Nguyen et al., 2005). Also, sigma receptor antagonism prevents the development of locomotor sensitization induced by repeated treatment with methamphetamine. Like cocaine, repeated methamphetamine exposure enhances psychomotor activity known as locomotor sensitization. Co-administration of sigma receptor antagonist BMY-14802 and methamphetamine significantly attenuated the development of methamphetamine-induced locomotor sensitization (Ujike et al., 1992).

Overall, sigma receptor antagonists prevent methamphetamine-induced neurotoxicity, hyperthermia, hyperactivity and behavioral sensitization. Taken together, these studies suggest a role for sigma receptors in psychostimulant addiction and further studies are needed to evaluate the involvement of sigma receptors in psychostimulant-induced behaviors.

Purpose and Hypotheses

Psychostimulant addiction is a serious health concern in the United States. Cocaine and methamphetamine interact with sigma receptors, suggesting their involvement in psychostimulant-induced behavioral and neurochemical effects. Overall, previous research indicates that sigma receptor antagonism attenuates the effects of psychostimulants, while sigma receptor agonism potentiates these effects.

The purpose of the proposed study was to investigate the role of sigma receptors in the behavioral properties of psychostimulants. Experiment 1 investigated the effect of SA 4503 on the locomotor stimulatory properties of methamphetamine. Locomotor activity investigates the effects of drugs on spontaneous motor activity. Measurements of locomotion can be used as an initial screen for the pharmacological effects predictive of drug efficacy in humans. Thus, the locomotor activity paradigm was used to determine the involvement of sigma receptors in methamphetamine-induced locomotion.

Experiment 2 examined the effect of sigma receptor agonist SA 4503 pretreatment on the S^D properties of methamphetamine and cocaine in rodents. Drug discrimination investigates the S^D properties of drugs in animals which have

been considered to be homologous to the subjective effects of drugs in humans (Colpaert, 1999). As such, the neurobiological mechanisms of these experiences can be investigated experimentally in animals. Thus, the drug discrimination paradigm was used to investigate the role of sigma receptors in methamphetamine and cocaine's subjective effects.

Since SA 4503 is a selective σ_1 sigma receptor agonist the hypothesis for Experiment 1 is pretreatment with SA 4503 will exacerbate methamphetamine's locomotor stimulatory properties. Thus, SA 4503 will augment methamphetamine-induced hyperactivity. Additionally, the hypothesis for Experiment 2 is pretreatment with SA 4503 will potentiate the S^D properties of cocaine and methamphetamine. Thus, based on the binding literature, SA 4503 pretreatment will enhance the subjective effects of methamphetamine and cocaine in rodents. These hypotheses are in line with previous locomotor activity and drug discrimination studies investigating the effect of sigma receptor agonist DTG on cocaine (Matsumoto et al., 2001a; Skuza, 1999; Ukai et al., 1997).

EXPERIMENT 1: EFFECT OF SA 4503 ON THE LOCOMOTOR STIMULATORY PROPERTIES OF METHAMPHETAMINE

Design

The purpose of Experiment 1 was to determine the effect of sigma receptor agonist SA 4503 pretreatment on methamphetamine-induced locomotor activity. Rats were given a pretreatment injection of either SA 4503 or vehicle, then placed into a standard locomotor activity monitor for 10 min. Subsequently, rats were administered an injection of either methamphetamine or vehicle, then placed into the locomotor activity monitor for 80 min. Distance traveled (cm) was recorded in 5-min intervals.

Materials and Methods

Subjects. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri. Male and female Wistar rats (Harlan, Indianapolis, IN; 250-350 g upon experiment commencement) were housed 2 rats per cage in clear polycarbonate cages in a temperature and humidity controlled room. During the entire behavioral study, rats had ad libitum access to water and standard rat chow. Prior to the start of the experiments, rats were weighed and handled daily. The colony was maintained under a 12-hr/12-hr light/dark cycle and the experiments were conducted during the light phase of the cycle.

Apparatus. Locomotor activity was monitored automatically using Med Associates' (Georgia VT) Open Field Test Environments (ENV-515), comprised

of a 16×16 horizontal grid of infrared sensors and a bank of 16 vertical sensors. Each monitor surrounded an acrylic cage (43.2×43.2×30.5 cm), and each monitor and cage was housed in a large sound-resistant cubicle (ENV-017M). Locomotor activity data was collected in 5 min intervals using Med Associates' Open Field Activity Software (SOF-811) that records the number of sensor breaks throughout the monitor and computes these data as measures of distance traveled (cm).

Procedures. On the first three days of the experiment, three acclimation sessions were performed. For these acclimation sessions, rats were placed in the locomotor activity monitor for 30 min, but were not administered drug. Subsequently, rats were assigned to a drug treatment condition based on their activity on the acclimation days, such that there was comparable basal locomotor activity among the treatment conditions. On the fourth day of the experiment a test session commenced. For the test session, rats were injected (IP) with SA 4503 (2.7 – 81 µmol/kg) or vehicle, placed in the monitor for 10 min, injected (SC) with methamphetamine (3.3 µmol/kg) or saline, and returned to the monitor for 80 min. Drug doses are presented in µmol/kg to provide more acute comparisons between and across drug class (see Table 2 for conversions to mg/kg). The SA 4503 doses were chosen based on previous research in which they did not intrinsically alter locomotor activity (Skuza, 1999). In the design of the experiments, eight treatment conditions were formed—Vehicle–Saline, Vehicle–Methamphetamine (3.3 µmol/kg), SA 4503 (2.7 µmol/kg)–Saline, SA 4503 (2.7 µmol/kg)–Methamphetamine (3.3 µmol/kg), SA 4503 (27 µmol/kg)–

Saline, SA 4503 (27 µmol/kg)–Methamphetamine (3.3 µmol/kg), SA 4503 (81 µmol/kg)–Saline, SA 4503 (81 µmol/kg)–Methamphetamine (3.3 µmol/kg).

Table 2: Experiment 1 Drug Dose Conversions

SA 4503		Methamphetamine	
µmol/kg	mg/kg	µmol/kg	mg/kg
81	30	3.3	0.5
8.1	3		
2.7	1		
0.81	0.3		

Drugs and chemicals. (±) methamphetamine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO) and diluted in 0.9% (w/v) saline. SA 4503 was synthesized by Dr. Susan Lever's laboratory in the Department of Chemistry at the University of Missouri. SA 4503 and methamphetamine were administered IP and SC, respectively. All injections were administered at a volume of 1.0 ml solution/kg body weight. All drug doses represent the free base weight.

Data Analysis. Distance traveled was analyzed via three-way repeated measures analysis of variance (RM-ANOVA) with SA 4503 Dose and Methamphetamine Dose as between-group factors and Time as a within-subjects factor. Post hoc comparisons were performed when necessary via Tukey tests because observations being tested are independent and multiple comparisons are being made.

Results

In order to determine the effect of SA 4503 pretreatment on methamphetamine-induced hyperactivity, rats were injected with either SA 4503 (2.7 – 81 µmol/kg) or vehicle, then placed into a standard locomotor activity

monitor for 10 min. Subsequently, rats were administered an injection of either methamphetamine or saline, then placed into the locomotor activity monitor for 80 min. Analysis of the time course revealed a significant main effect of SA 4503 dose [$F(3,54) = 16.88$, $P<0.0001$] and Methamphetamine dose [$F(1,54) = 55.34$, $P<0.0001$]. Additionally, the SA 4503 dose x Methamphetamine dose interaction [$F(3,54) = 11.10$, $P<0.0001$] was significant. Regarding the time course, the main effect of Time [$F(6,358) = 74.0$, $P<0.0001$], the Time x SA 4503 dose interaction [$F(19,358) = 4.87$, $P<0.0001$], the Time x Methamphetamine interaction [$F(6,358) = 15.14$, $P>0.0001$] and the Time x SA 4503 dose x Methamphetamine dose interaction [$F(19,358) = 4.59$, $P<0.0001$] were all significant.

Post hoc analyses of the time course indicated significant differences between the Vehicle-Saline and Vehicle-Methamphetamine groups at all time points between 20 – 90 min, indicating that methamphetamine induces hyperactivity. At time points 20 – 40 min, there were significant differences between the Vehicle-Methamphetamine and 2.7 µmol/kg SA 4503-Methamphetamine groups, suggesting that pretreatment with 2.7 µmol/kg SA 4503 augmented methamphetamine-induced hyperactivity (Figure 1, Panel A). There were also significant differences between the Vehicle-Methamphetamine and 27 µmol/kg SA 4503-Methamphetamine groups at time points 15 – 50 and 65 min, indicating that pretreatment with 27 µmol/kg SA 4503 attenuated methamphetamine-induced hyperactivity (Figure 1, Panel B). Additionally, there was a significant difference between the Vehicle-Methamphetamine and

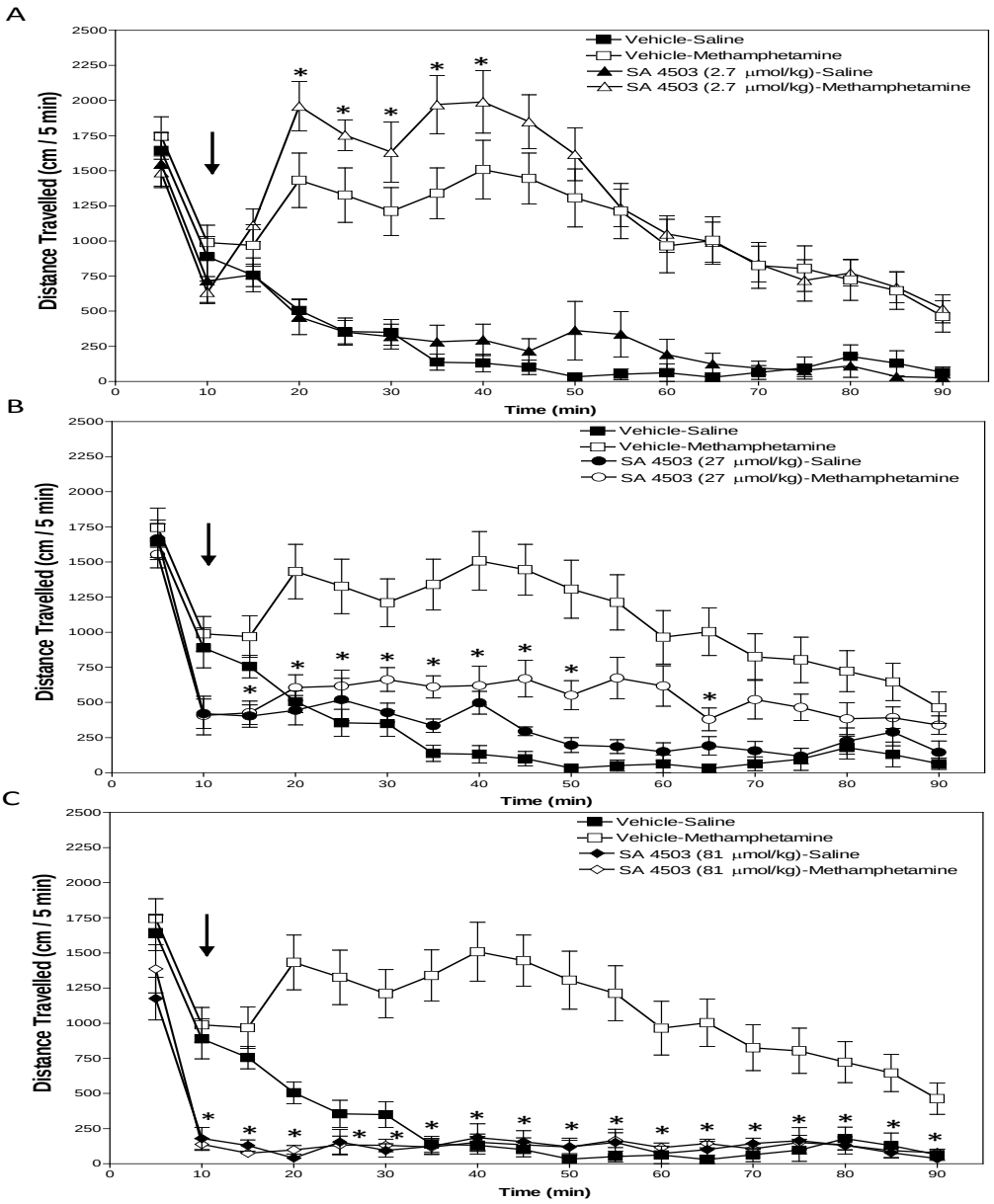

Figure 1. SA 4503 dose-dependently potentiates and attenuates methamphetamine-induced hyperactivity: time course analysis. Rats were administered SA 4503 (2.7 – 81 µmol/kg) or vehicle, placed in the locomotor activity monitor for 10 min, injected with methamphetamine (3.3 µmol/kg) or saline, then activity was monitored for 80 min. The data are represented as mean (± S.E.M) distance traveled. Panel A depicts locomotor activity for the Vehicle–Saline, Vehicle–Methamphetamine, SA 4503 (2.7 µmol/kg)–Saline, and SA 4503 (2.7 µmol/kg)–Methamphetamine groups. Panel B depicts locomotor activity for the Vehicle–Saline, Vehicle–Methamphetamine, SA 4503 (27 µmol/kg)–Saline, and SA 4503 (27 µmol/kg)–Methamphetamine groups. Panel C depicts locomotor activity for the Vehicle–Saline, Vehicle–Methamphetamine, SA 4503 (81 µmol/kg)–Saline, and SA 4503 (81 µmol/kg)–Methamphetamine groups. The arrow designates the second injection. Asterisks represent a significant ($P<0.05$) difference from the Vehicle-Methamphetamine group. (n = 6-10 rats/group).

81 µmol/kg SA 4503-Methamphetamine groups at all the time points after the methamphetamine injection (15 – 90 min), suggesting that 81 µmol/kg SA 4503 attenuated methamphetamine-induced hyperactivity (Figure 1, Panel C). However, analysis of the 10-min period after SA 4503 injection and before methamphetamine injection revealed significant differences in activity among the groups of rats administered saline or SA 4503 (2.7 – 81 µmol/kg). The main effects of Time [$F(1,53) = 434$, $P<0.0001$] and SA 4503 dose [$F(3,53) = 11.8$, $P<0.0001$] were significant. Additionally, the Time x SA 4503 dose interaction was significant [$F(3,53) = 7.18$, $P<0.0001$]. Rats administered 81 µmol/kg SA 4503 were less active than rats administered Vehicle, 2.7, and 27 µmol/kg SA 4503 (all $P<0.05$), indicating that 81 µmol/kg SA 4503 produced hypoactivity. Taken together, the effect of 81 µmol/kg SA 4503 on methamphetamine-induced hyperactivity cannot be interpreted because it reduced activity when administered alone. Thus, analysis of the time course indicates that SA 4503 pretreatment dose-dependently potentiates and augments methamphetamine-induced hyperactivity.

Total distance traveled for the 80-min period after methamphetamine or saline administration is presented in Figure 2. There were no differences in activity among the Vehicle-Saline and SA 4503 (2.7 – 81 µmol/kg)-Saline groups, suggesting that activity levels for these rats did not differ for the 80-min period after the saline injection. Rats in the Vehicle-Methamphetamine group were more active than rats in the Vehicle-Saline group ($P<0.0001$), indicating that this dose of methamphetamine produces hyperactivity. Activity was greater for the

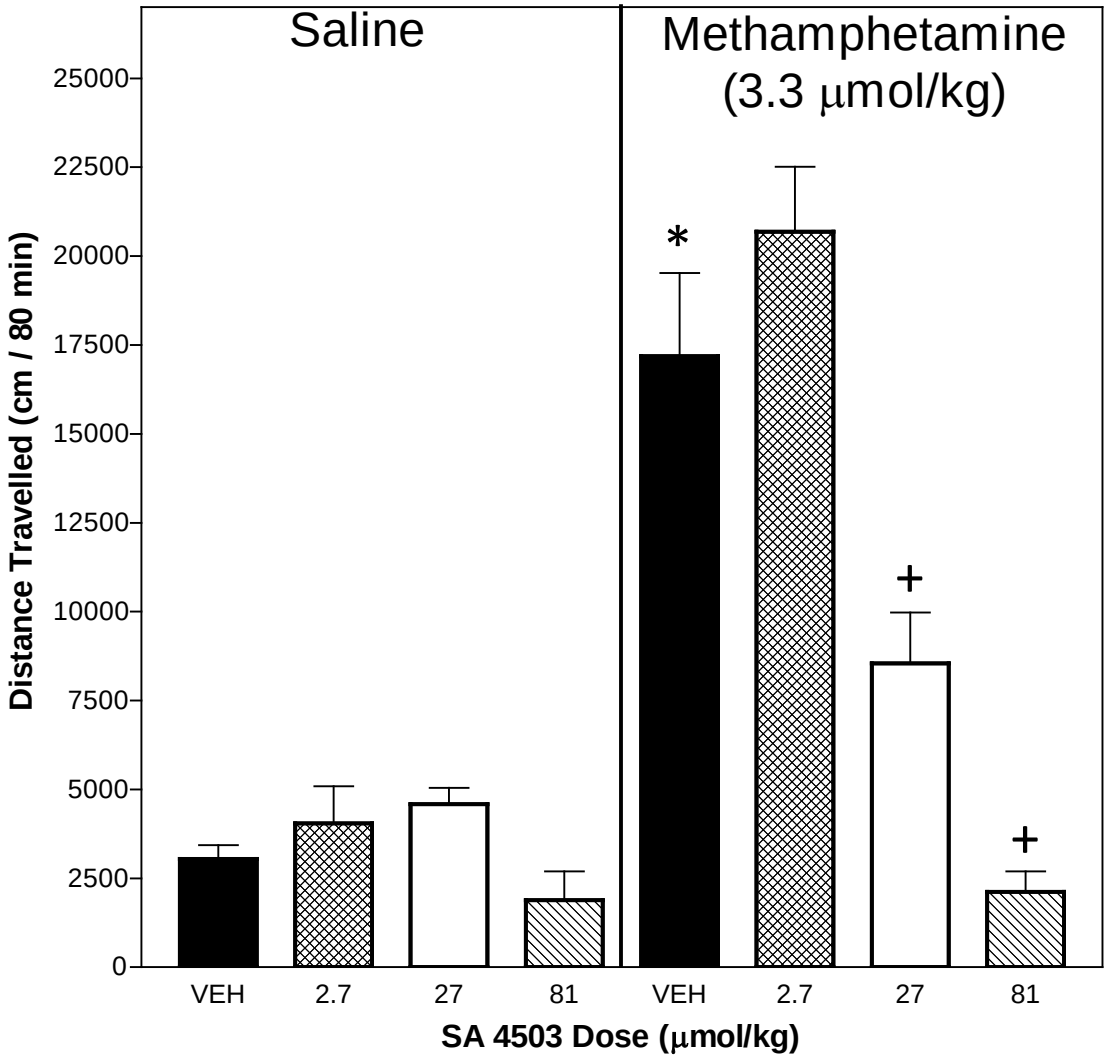

Figure 2. SA 4503 attenuates methamphetamine-induced hyperactivity; analysis of total distance travelled. Rats were administered SA 4503 (2.7, 27, or 81 µmol/kg) or vehicle, placed in an automated activity monitor for 10 min, the injected with methamphetamine (3.3 µmol/kg) or saline, and returned to the monitor for 80 min. Data represent total distance traveled during the 80-min period after the second injection. The asterisk designates a significant ($P<0.05$) difference from the Vehicle-Saline group and the plus signs designate differences from the Vehicle-Methamphetamine group. VEH, vehicle. (n = 6-10 rats/group).

Vehicle-Methamphetamine group than for 27 and 81 µmol/kg SA 4503-Methamphetamine groups ($P<0.05$ and $P<0.0001$, respectively), indicating that these SA 4503 doses prevented methamphetamine-induced hyperactivity. However, as stated above, the 81 µmol/kg dose of SA 4503 produce hypoactivity intrinsically. There were no significant differences between the Vehicle-Methamphetamine and 2.7 µmol/kg SA 4503-Methamphetamine groups. There was a significant difference in activity levels for rats in the 2.7 µmol/kg SA 4503-Saline group and rats in the 2.7 µmol/kg SA 4503-Methamphetamine group ($P<0.0001$), but no significant differences between the 27 µmol/kg SA 4503-Saline and 27 µmol/kg SA 4503-Methamphetamine groups or between the 81 µmol/kg SA 4503-Saline and 81 µmol/kg SA 4503-Methamphetamine groups. Together, analysis of the total distance traveled during the 80 min period after the second injection indicates that 27 µmol/kg SA 4503 attenuated methamphetamine-induced hyperactivity.

Discussion

The major finding of the present study was that SA 4503 dose-dependently potentiated and reduced methamphetamine-induced hyperactivity, indicating a behavioral interaction of SA 4503 and methamphetamine. Additionally, higher doses of SA 4503 significantly altered basal locomotor activity. During the 10-min period prior to methamphetamine injection, the high dose of SA 4503 decreased locomotor activity, indicating that SA 4503 dose-dependently changed levels of activity intrinsically.

The results of the present study demonstrate that SA 4503 augments and blocks the locomotor stimulatory properties of methamphetamine, a novel finding. This pattern is in line with recent observations from our laboratory in which a similar dose of SA 4503 prevented cocaine-induced hyperactivity. In contrast, previous research has shown that SA 4503 did not alter cocaine-induced hyperactivity and did not have intrinsic activity to alter locomotion (Skuza, 1999). However, SA 4503 is beneficial to prevent the reinforcing properties of nicotine, working memory deficits, and neurotoxicity. Administration of nicotine, but not SA 4503, produced significant place-conditioning (Horan et al., 2001). Pretreatment with SA 4503 blocked the acquisition of nicotine-induced conditioned place preference (Horan et al., 2001), suggesting SA 4503 is sufficient to attenuate the reinforcing properties of nicotine. Systemic administration of NMDA receptor antagonist dizocilpine produced spatial working memory deficits as assessed in a radial arm maze task (Zou et al., 2000). SA 4503 improved dizocilpine-induced working memory impairments (Zou et al., 2000), indicating that SA 4503 ameliorates memory impairments. Furthermore, SA 4503 attenuated the hypoxia/hypoglycemia-induced neurotoxicity (Nakazawa et al., 1998), suggesting SA 4503 is neuroprotective. In the working memory and neurotoxicity studies, the beneficial effects of SA 4503 were reversed by selective σ_1 sigma receptor antagonist NE-100 (Nakazawa et al., 1998; Zou et al., 2000), indicating these effect were mediated by σ_1 sigma receptors. Together, the present results and previous research indicate σ_1 sigma receptors are involved in locomotor activity, drug reinforcement, working memory, and neuroprotection.

Studies have suggested an interaction of sigma receptors and downstream dopamine systems. SA 4503 administration significantly decreased the firing pattern of dopamine neurons in the substantia nigra (Minabe et al., 1999), suggesting that sigma receptors may play a role in the motor effects mediated by dopamine neurons in the nigrostriatal pathway. Recently, our laboratory determined the effect of SA 4503 to evoke dopamine release intrinsically and in the presence of methamphetamine. Application of SA 4503 to rat striatal slices was ineffective to evoke [^{3}H]dopamine release. However, when SA 4503 was applied to rat striatal slices in the presence of methamphetamine, SA 4503 blocked methamphetamine-induced [^{3}H]dopamine release in a concentration-dependent manner. Our present findings and previous research with SA 4503 suggest that it could be acting as a mixed agonist-antagonist at sigma receptors. From a molecular level, it has been proposed that low and high doses of sigma receptor ligands can produce agonist- and antagonist-like effects, respectively (Su et al., 2009). Low concentrations of sigma receptor ligands may be increasing intracellular calcium levels and producing amplification of downstream dopamine systems, such that a potentiation may occur. In contrast, higher concentrations of sigma receptor ligands may be inhibiting plasmalemmal ion channels and blocking downstream dopamine systems such that attenuation may be produced. Thus, the unique effects of differential doses on these receptors help to explain our present locomotor activity results.

SA 4503 showed weak to no affinity for many receptors, ion channels, and second messenger systems (Matsuno et al., 1996) including the DAT (Ishiwata et

al., 2001), supporting the notion that it produces its effects through sigma receptors. However, more recently SA 4503 exhibited high affinity (K_i = 0.05 µM) for the vesicular acetylcholine transporter (VAChT) (Ishiwata et al., 2006). To this author's knowledge, methamphetamine's affinity for the VAChT has yet to be determined. However, the VAChT is localized in cholinergic nerve terminals of several brain regions including striatum (Gilmor et al., 1996). Moreover, striatal VAChT levels were increased in human methamphetamine users (Siegal et al., 2004), suggesting that VAChT could play a role in methamphetamine's locomotor stimulatory properties. This observation exposes our lack of knowledge about the affinity and interaction of SA 4503 with the VMAT. Methamphetamine reverses VMAT in order to increase cytosolic monoamines. It is possible that SA 4503 may be altering the behavioral effects of methamphetamine by acting on the VMAT. Thus, future studies are needed to elucidate the role of VAChT and VMAT in SA 4503-induced changes in locomotor activity produced by psychostimulants.

In summary, SA 4503 dose-dependently augmented and prevented the locomotor stimulatory effects of methamphetamine. This finding supports previous research showing the controversial effects of sigma receptor drugs. Researchers have proposed involvement of sigma receptors and downstream dopamine pathways. As such, SA 4503 may be increasing and decreasing the firing pattern of dopamine neurons by modulating intracellular calcium levels and plasmalemmal ion channels, respectively, which could result in potentiation and attenuation of methamphetamine-induced hyperactivity.

EXPERIMENT 2: EFFECT OF SA 4503 ON THE DISCRIMINATIVE STIMULUS PROPERTIES OF METHAMPHETAMINE AND COCAINE

Design

The purpose of Experiment 2 was to investigate the effect of sigma receptor agonist SA 4503 pretreatment on the subjective effects of methamphetamine and cocaine. Rats were trained to discriminate between either methamphetamine and vehicle or cocaine and vehicle (2 groups of rats). Upon acquisition of stimulus control, substitution and pretreatment test sessions commenced. Rats were given injections of drug, followed by a delay, then placed into standard operant chambers where testing commenced. Dose-response curves for methamphetamine and cocaine were determined to show a stimulus generalization gradient. Amphetamine and nicotine substitution tests were performed to determine generalization to other stimulants. Procaine substitution tests were performed to investigate whether the S^D properties of methamphetamine and cocaine are mediated solely by the central nervous system. SA 4503 substitution tests were completed in order to determine generalization to methamphetamine and cocaine.

Following the SA 4503 substitution tests, doses of SA 4503 were chosen to administer in the pretreatment test sessions. Rats were given a SA 4503 injection followed by a delay. Subsequently, rats were given a methamphetamine, cocaine or amphetamine injection, followed by a delay, and then placed into standard operant chambers for testing.

Materials and Methods

Subjects. Prior to the start of the study, male Sprague-Dawley rats (Harlan, Indianapolis, IN; ~200 g upon arrival to the laboratory) were weighed and handled daily. During the entire behavioral study, rats had ad libitum access to tap water. Rats were given access to a limited amount of standard rat chow (~25 g) after the completion of the behavioral session and body weights were maintained at approximately 300 - 350 g. All other animal and colony procedures and maintenance were similar to those as described for Experiment 1.

Apparatus. Standard operant chambers (ENV-001; Med Associates, Georgia VT) were used. The chambers' side walls consisted of aluminum, and the front and back walls were made of Plexiglas. The operant chamber floor consisted of stainless steel rods. A house light was located on one of the side walls in the top-center. Reinforcers were delivered into a recessed receptacle (5 cm x 4.2 cm) located in the bottom-center of the side wall opposite the house light, while the response levers were located on each side of the receptacle. Responses made on the active lever were reinforced, and responses made on the inactive lever were recorded but not reinforced. Achievement of the response requirement resulted in delivery of a reinforcer, food pellet (20 mg; Bio-Serv, Frenchtown, NJ), into the receptacle. All stimulus and response events were controlled and recorded by a computer running Med Associates' Med PC-IV Software. Operant chambers were washed with a 10% bleach and water solution after each rat's behavioral session.

Training procedures. Upon arrival, all rats were allowed to acclimate to the colony room and were handled by researchers for approximately 5 min each day prior to the start of training. Initially, rats were trained to respond on both levers in the operant chambers. Rats were placed in the operant chambers and responding on either lever was maintained by a fixed-ratio (FR)-1 schedule. During each FR-1 shaping session, rats were reinforced with a maximum of 30 food pellets. FR-1 shaping sessions continued until each rat successfully completed the sessions. Subsequently, the ratio requirement for each shaping session was systematically increased to FR-10. Following the successful completion of the FR-10 shaping sessions, rats were randomly assigned to one of two groups in which the S^D was 3.3 μmol/kg methamphetamine (n = 10 rats) or 16 μmol/kg cocaine (n = 10 rats). At this time, discrimination training commenced.

During the training sessions, rats were injected with drug or saline, put back into their home cage for 10 min, and then placed in the operant chamber. In the discrimination training program, one lever was active (delivered food reinforcement) and the other was inactive (responses recorded, but no food reinforcement). After administration of drug, one lever was active (the opposite lever was inactive) and following saline administration, the other lever was active (the opposite lever was inactive). The drug- and saline-paired levers were counterbalanced within each group. The session was terminated after 30 min or 300 responses, whichever occurred first. Only one discrimination training session was administered daily and the presentation of drug or saline across sessions

was maintained by the following pattern – saline (S), drug (D), D, S, S, D, S, D, S, S, D, D.

Within a discrimination training session, performance was assessed on the first completed ratio. A correct lever selection was recorded when the rat made 10 responses on the active lever with 5 or less responses on the inactive lever. The criterion for acquisition of the discrimination (stimulus control) for each rat was correct lever selection on 8 out of 10 successive daily sessions.

Test procedures. After all rats had reached stimulus control, substitution test sessions began using a discrete-trials procedure. In each test session only one drug dose and post-injection interval was assessed. In addition, only one test session was completed in a day and at least two training sessions followed each test session. In the test program, both levers were active and 10 responses on either lever delivered a food pellet. The test session was terminated after delivery of food reinforcement or 15 min, whichever occurred first.

In the substitution tests, rats were administered a single dose of methamphetamine (0.00003 µmol/kg – 3.3 µmol/kg), cocaine (0.01 – 33 µmol/kg), *d*-amphetamine (0.002 – 2.2 µmol/kg), nicotine (0.03 – 3.7 µmol/kg), SA 4503 (0.81 – 81 µmol/kg) or procaine (4.23 – 127.1 µmol/kg). Drug doses are presented in µmol/kg to provide more acute comparisons between and across drug class (see Table 3 for conversions to mg/kg). Rats were returned to the home cage for a 10 min delay, and then placed in the operant chamber when a test session commenced. Additional SA 4503 (0.81 – 8.1 µmol/kg) substitution

tests were performed with a 20 min delay between injection time and test session commencement.

Table 3: Experiment 2 Drug Dose Conversions

Methamphetamine		Cocaine		Amphetamine		Nicotine		Procaine		SA 4503	
µmol/kg	mg/kg	µmol/kg	mg/kg	µmol/kg	mg/kg	µmol/kg	mg/kg	µmol/kg	mg/kg	µmol/kg	mg/kg
3.3	0.5	33	10	2.2	0.3	3.7	0.6	127.1	30	81	30
0.3	0.05	16	5	0.2	0.03	0.37	0.06	42.3	10	8.1	3
0.03	0.005	1.6	0.5	0.02	0.003	0.11	0.019	4.23	1	2.7	1
0.003	0.0005	0.16	0.05	0.002	0.0003	0.03	0.006			0.81	0.3
0.0003	0.00005	0.016	0.005								
0.00003	0.000005										

In the pretreatment tests, rats were administered a single dose of sigma receptor agonist SA 4503 (0.81 – 2.7 µmol/kg), returned to the home cage for a 10 min delay, injected with either methamphetamine (0.00003 µmol/kg – 3.3 µmol/kg), cocaine (0.01 – 16 µmol/kg) or amphetamine (0.002 – 2.2 µmol/kg), returned to the home cage for a 10 min delay, and then placed in the operant chamber when a test session commenced.

Drugs and chemicals. Cocaine hydrochloride, ± methamphetamine hydrochloride, *d*-amphetamine sulfate, nicotine hemisulfate, and procaine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO) and diluted in 0.9% (w/v) saline. SA 4503 was synthesized by Dr. Susan Lever's laboratory in the Department of Chemistry at the University of Missouri. Cocaine, procaine, and SA 4503 were administered IP and all other drugs were administered SC. All injections were administered at a volume of 1.0 ml solution/kg body weight. All drug doses represent the free base weight.

Data analysis. Analyses were conducted on the session numbers in which the rats reached stimulus control in order to determine if there were differences

among the groups in the acquisition of stimulus control. An independent samples t-test was used to determine group differences in acquisition of stimulus control.

For substitution and pretreatment test sessions, the percentage of responses on the drug-paired lever was calculated. From the doses tested in this study, “partial” substitution was defined as 40 - 80% of responses made on the drug-paired lever and “full” substitution was defined as > 80% of responses made on the drug-paired lever. A nonlinear regression analysis was performed on the mean dose response curve when “full” substitution was observed and an ED_{50} value was calculated. Dose-response curves were considered to be significantly different when 95% confidence intervals of their ED_{50} values did not overlap. Also for substitution and pretreatment test sessions, response rates were calculated. Response rates were determined by dividing the total number of responses on both (drug- and saline-paired) levers by the session latency. For substitution tests, a one-way RM-ANOVA was performed on response rate data with drug dose as the within-subjects factor. For pretreatment tests, a two-way RM-ANOVA was performed on response rate data with pretreatment condition and drug dose as within-subjects factors. Post hoc paired comparisons were performed when necessary. Animals that exhibited poor training (i.e. did not respond on the appropriate-paired lever during two training sessions consecutively before a test session) were removed from analyses.

Results

Training. All rats reached the criteria for stimulus control (3.3 µmol/kg methamphetamine S^D group median = 16 sessions, range = 14-26 sessions; 16 µmol/kg cocaine S^D group median = 41 sessions, range = 16-53 sessions). Rats in the methamphetamine S^D group achieved stimulus control faster than the cocaine S^D group ($T(18) = 5.54$, $P<0.0001$).

Substitution tests with cocaine. Rats in the methamphetamine S^D group were injected with cocaine and placed in the operant chamber after a 10 min post-injection interval. None of the cocaine doses significantly altered response rates ($P>0.05$) in the methamphetamine S^D group (Figure 3, panel C).

Panel A of figure 3 shows the percentage of responses on the methamphetamine-paired lever. Full substitution was evident at 16 and 33 µmol/kg doses of cocaine whereas, partial substitution was evident at the 1.6 µmol/kg cocaine dose in the methamphetamine S^D group. The ED_{50} value was 0.89 µmol/kg (95% C.I. = 0.46-1.71 µmol/kg) for the methamphetamine S^D group.

Rats in the cocaine S^D group were injected with cocaine and placed in the operant chamber after a 10 min post-injection interval. The 0.016 µmol/kg cocaine dose decreased response rates significantly [$F(3,27) = 4.43$, $P<0.05$] in the cocaine S^D group. Post hoc analyses determined a significant difference between the 0.016 µmol/kg and 33 µmol/kg cocaine doses ($P<0.05$) (Figure 3, panel D).

Panel B of figure 3 shows the percentage of responses on the cocaine-paired lever. Full substitution was evident at 16 and 33 µmol/kg doses of cocaine

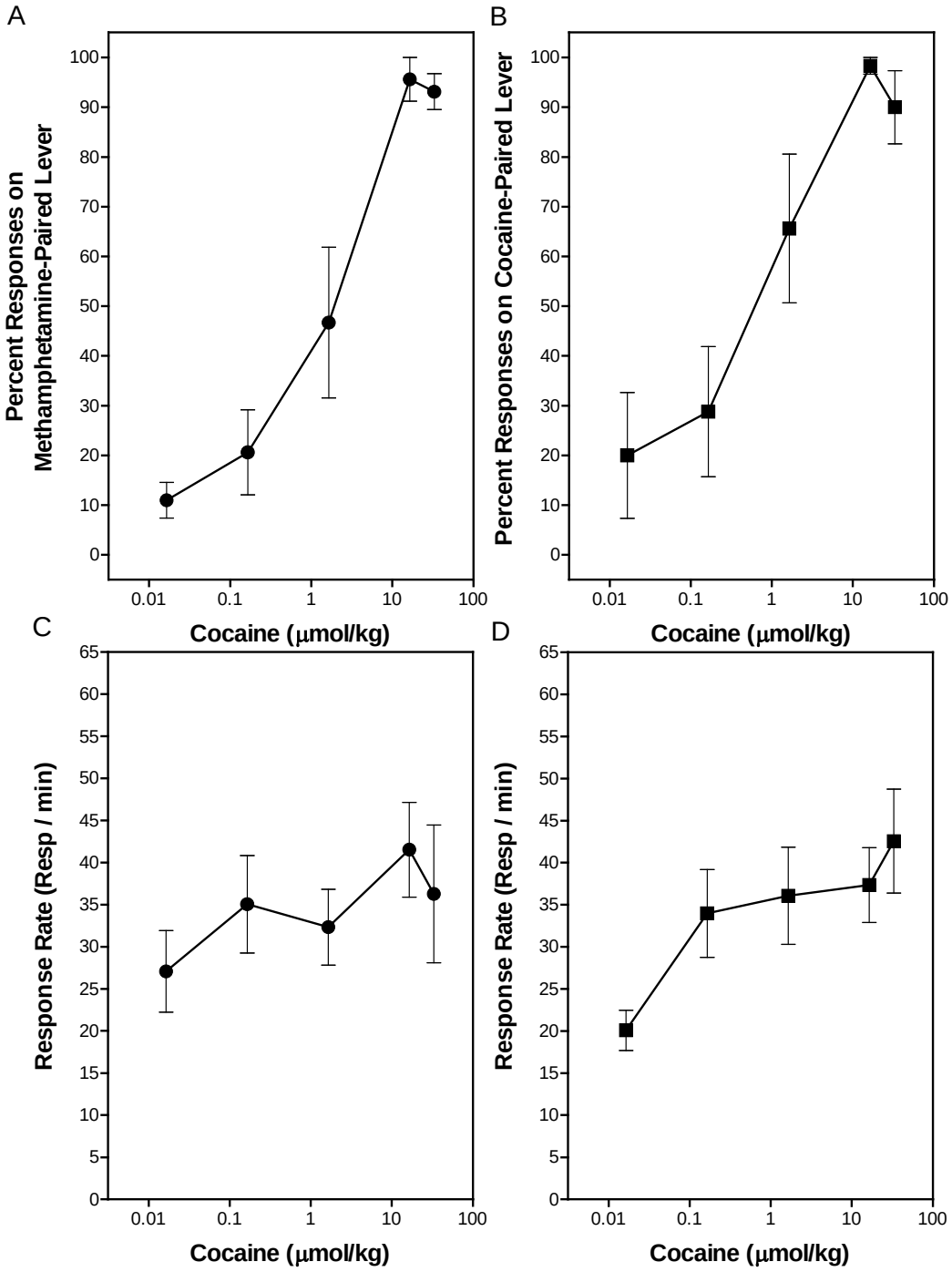

Figure 3. Cocaine substitutes for the methamphetamine and cocaine S^D in a dose-dependent manner. Rats were administered cocaine, returned to the home cage for a 10 min delay, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

whereas, partial substitution was evident at the 1.6 µmol/kg cocaine dose in the cocaine S^D group. The ED_{50} value was 0.46 µmol/kg (95% C.I. = 0.16-1.15 µmol/kg) for the cocaine S^D group.

Substitution tests with methamphetamine. A similar test was performed to determine the S^D properties of methamphetamine. Rats in the methamphetamine S^D group were injected with methamphetamine and placed in the operant chamber after a 10 min post-injection interval. None of the methamphetamine doses significantly altered response rates ($P>0.05$) in the methamphetamine S^D group (Figure 4, panel C). In the methamphetamine S^D group, only data from eight of the ten rats were included for the 0.00003 µmol/kg and 0.0003 µmol/kg methamphetamine doses due to poor training.

The percentage of responses on the methamphetamine-paired lever are presented in figure 4, panel A. For rats in the methamphetamine S^D group, partial substitution was evident at 0.3 µmol/kg methamphetamine and full substitution was evident at 3.3 µmol/kg methamphetamine. Regression analyses determined an ED_{50} value of 0.13 µmol/kg (95% C.I. = 0.06-0.33 µmol/kg) for the methamphetamine S^D group.

Similarly, rats in the cocaine S^D group were injected with methamphetamine and placed in the operant chamber after a 10 min post-injection interval. None of the methamphetamine doses significantly altered response rates ($P>0.05$) in the cocaine S^D group (Figure 4, panel D). In the cocaine S^D group, data from one of the ten rats was excluded due to poor training for the 0.00003 and 0.0003 µmol/kg methamphetamine doses.

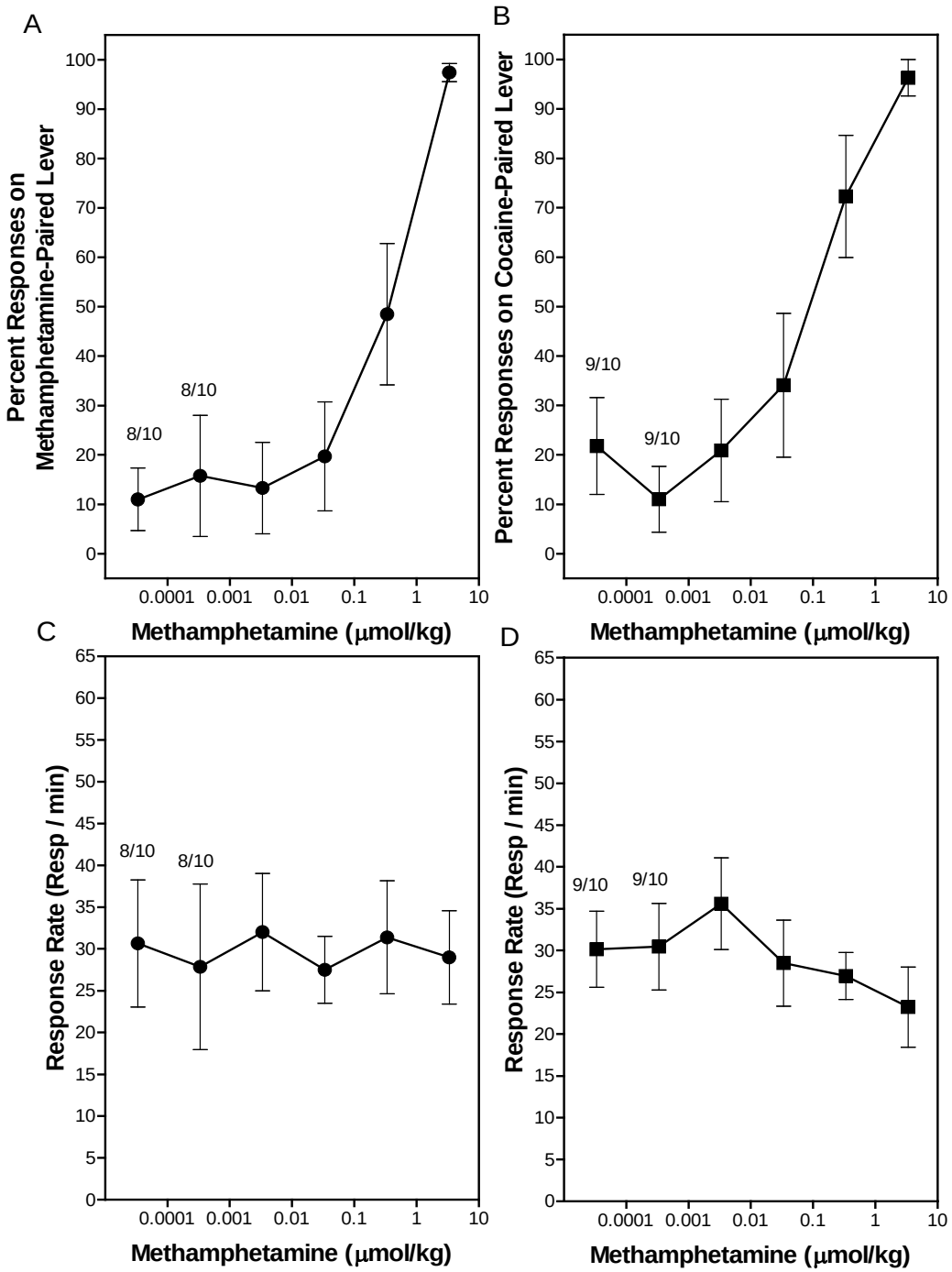

Figure 4. Methamphetamine substitutes for the methamphetamine and cocaine S^D in a dose-dependent manner. Rats were administered methamphetamine, returned to the home cage for a 10 min delay, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

The percentage of responses on the cocaine-paired lever are presented in figure 4, panel B. For rats in the cocaine S^D group, partial substitution was evident at 0.3 µmol/kg methamphetamine and full substitution was evident at 3.3 µmol/kg methamphetamine. Regression analyses determined an ED_{50} value of 0.06 µmol/kg (95% C.I. = 0.02-0.13 µmol/kg) for the cocaine S^D group.

Substitution tests with amphetamine. Rats in the methamphetamine S^D group were injected with amphetamine and placed in the operant chamber after a 10 min post-injection interval. Figure 5, panel C illustrates the 2.2 µmol/kg amphetamine dose increased response rates significantly [$F(3,22) = 4.01$, $P<0.05$] in the methamphetamine S^D group. Post hoc analyses determined a significant difference between the 2.2 µmol/kg amphetamine dose and the 0.2 and 0.002 µmol/kg doses of amphetamine (both $P<0.05$). Data from two of the ten rats were excluded for the 0.002 µmol/kg amphetamine dose due to poor training.

The proportion of responses on the methamphetamine-paired lever are shown in figure 5, panel A. In the methamphetamine S^D group, full substitution was evident at 2.2 µmol/kg amphetamine and partial substitution was evident at 0.2 µmol/kg amphetamine. Analysis of the regression line determined an ED_{50} value of 0.06 µmol/kg (95% C.I. = 0.02-0.2 µmol/kg) for the methamphetamine S^D group.

Rats in cocaine S^D group were injected with amphetamine and placed in the operant chamber after a 10 min post-injection interval. In the cocaine S^D group, none of the amphetamine doses significantly altered response rates

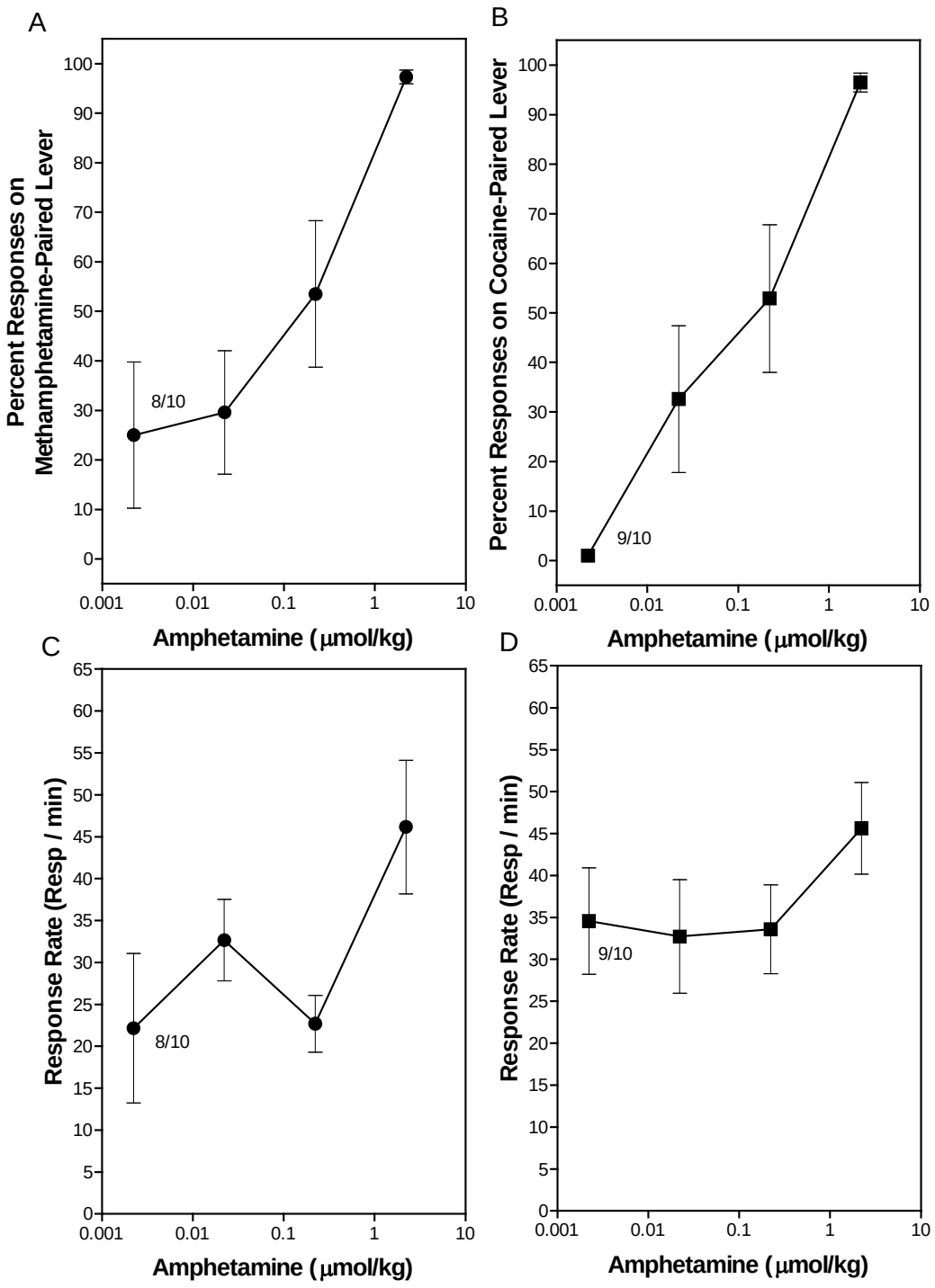

Figure 5. Amphetamine substitutes for the methamphetamine and cocaine S^D in a dose-dependent manner. Rats were administered amphetamine, returned to the home cage for a 10 min delay, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

(P>0.05) (Figure 5, panel D). Only data from nine of the ten rats was included for the 0.002 µmol/kg amphetamine dose due to poor training.

The proportion of responses on the cocaine-paired lever are shown in figure 5, panel B. In the cocaine S^D group, full substitution was evident at 2.2 µmol/kg amphetamine and partial substitution was evident at 0.2 µmol/kg amphetamine. Analysis of the regression line determined an ED_{50} value 0.09 µmol/kg (95% C.I. = 0.04-0.2 µmol/kg) for the cocaine S^D group.

Substitution tests with nicotine. In order to determine cross generalization to nicotine, rats in the methamphetamine S^D group were injected with nicotine and placed in the operant chamber after a 10 min post-injection interval. None of the nicotine doses significantly altered response rates (P>0.05) in the methamphetamine S^D group (Figure 6, panel C).

The proportion of responses on the methamphetamine-paired lever are presented in figure 6, panel A. For rats in the methamphetamine S^D group, only partial substitution (40 - 80%) was achieved and no ED_{50} value was determined.

Similarly, rats in the cocaine S^D group were injected with nicotine and placed in the operant chamber after a 10 min post-injection interval. None of the nicotine doses significantly altered response rates (P>0.05) in the cocaine S^D group (Figure 6, panel D). The 3.7 µmol/kg nicotine dose was omitted from analyses because only four out of ten rats in the cocaine S^D group were tested. During this test session, rats did not make any responses and the test session was aborted.

The proportion of responses on the cocaine-paired lever are presented in

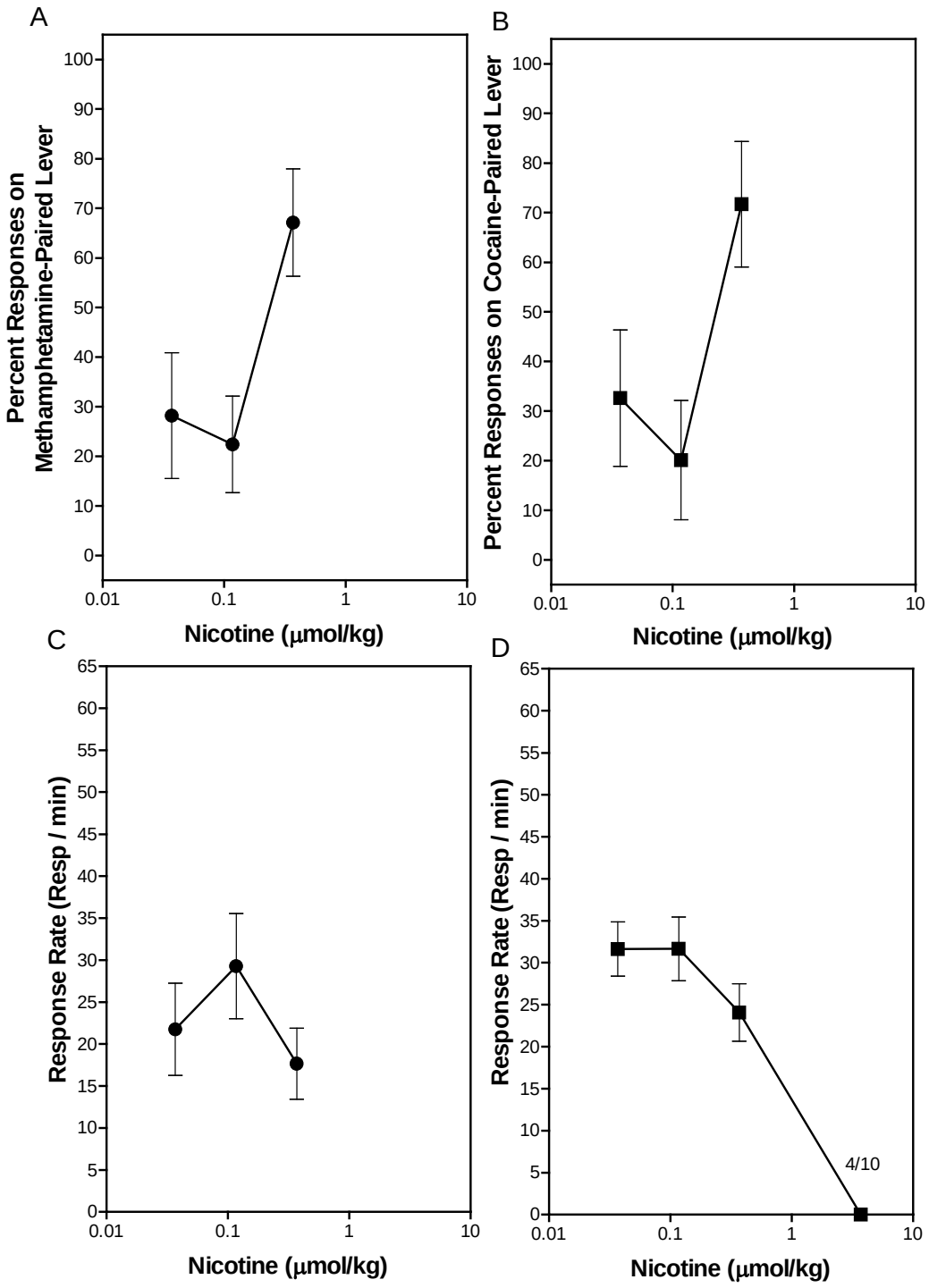

Figure 6. Nicotine only partially substitutes for the methamphetamine and cocaine S^D. Rats were administered nicotine, returned to the home cage for a 10 min delay, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

figure 6, panel B. For rats in the cocaine S^D group, only partial substitution (40 - 80%) was achieved and no ED_{50} value was determined.

Substitution tests with procaine. In order to understand the contribution of peripheral activation on the S^D properties of methamphetamine, rats in the methamphetamine S^D group were injected with the peripheral sodium channel blocker procaine and placed in the operant chamber after a 10 min post-injection interval. For the methamphetamine S^D group, none of the procaine doses significantly altered response rates ($P>0.05$) (Figure 7, panel C).

In figure 7, panel A, the proportion of responses on the methamphetamine-paired lever are presented. Only partial substitution (40 - 80%) was achieved for the methamphetamine S^D group and no ED_{50} value was determined.

Similarly, rats in the cocaine S^D group were injected with the peripheral sodium channel blocker procaine and placed in the operant chamber after a 10 min post-injection interval. For the cocaine S^D group, none of the procaine doses significantly altered response rates ($P>0.05$) (Figure 7, panel D).

In figure 7, panel B, the proportion of responses on the cocaine-paired lever are presented. Only partial substitution (40 - 80%) was achieved for the cocaine S^D group and no ED_{50} value was determined.

Substitution tests with SA 4503. Substitution tests were conducted in which rats in the methamphetamine S^D group were administered SA 4503 and placed in the operant chamber 10 min later. Panel C of figure 8 represents the response rates for the SA 4503 substitution tests. With a 10 min post- injection

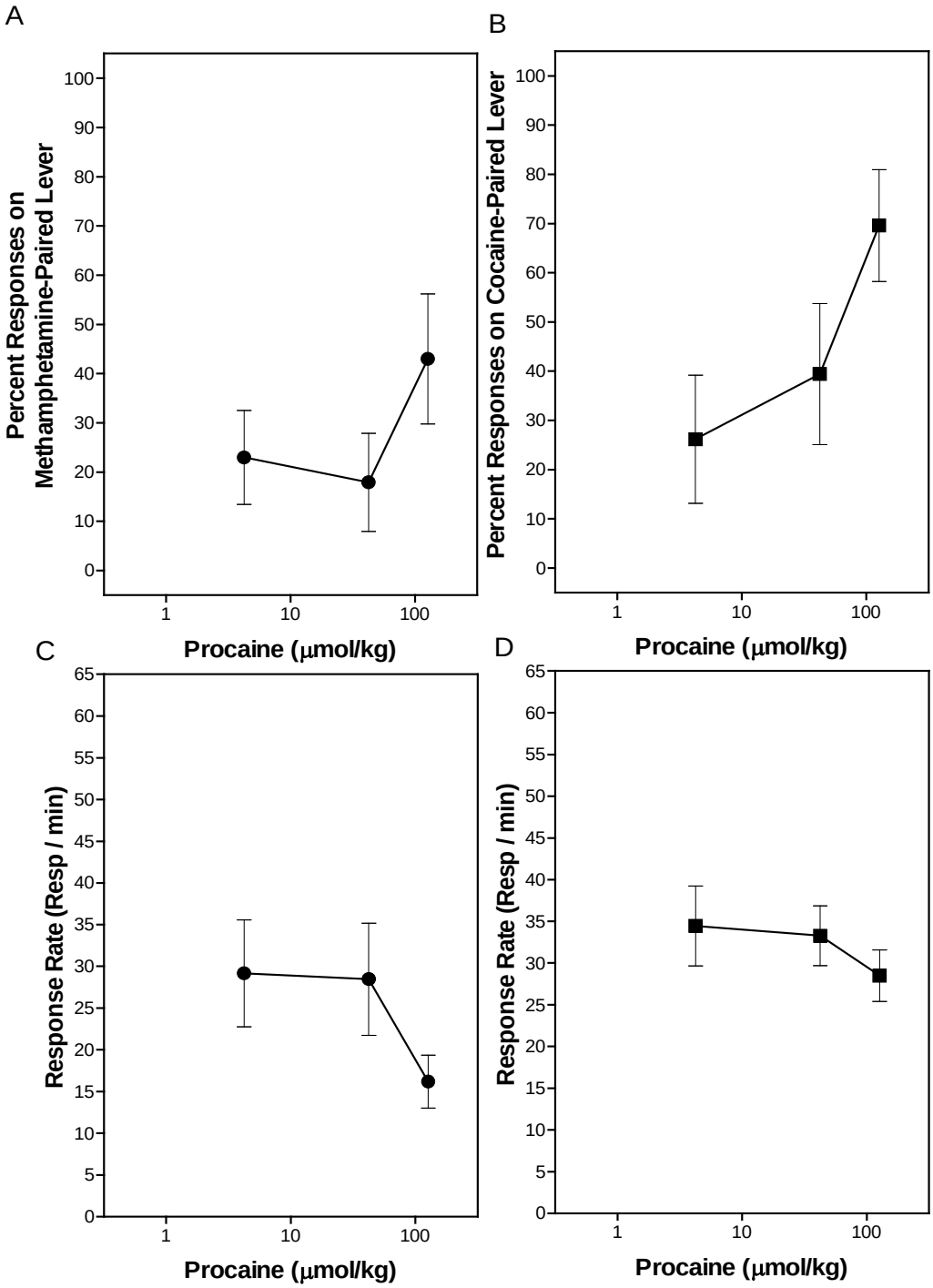

Figure 7. Procaine partially substitutes for the methamphetamine and cocaine S^D. Rats were administered procaine, returned to the home cage for a 10 min delay, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

interval, 8.1 µmol/kg SA 4503 produced a significant decrease in response rates [$F(2,14) = 5.53$, $P<0.05$] for the rats in the methamphetamine S^D group (Figure 8, panel C). Post hoc analyses indicate a marginally significant difference between 2.7 µmol/kg and 8.1 µmol/kg SA 4503 doses ($P=0.052$). The percentage of responses on the methamphetamine-paired lever are presented in figure 8, panel A. For rats in the methamphetamine S^D group, only partial substitution was evident at the 8.1 µmol/kg SA 4503 dose after a 10 min delay and no ED_{50} value was calculated (Figure 8, panel A).

Additionally, rats in the methamphetamine S^D group were injected with SA 4503 and placed in the operant chamber after a 20 min post-injection interval. None of the SA 4503 doses significantly altered response rates ($P>0.05$) in the methamphetamine S^D group (Figure 8, panel C). In the methamphetamine S^D group, data for two of the ten rats was excluded due to poor training for the 0.81 µmol/kg SA 4503 dose. The percentage of responses on the methamphetamine-paired lever are presented in figure 8, panel A. When the delay was 20 min, no SA 4503 doses substituted for the methamphetamine S^D and no ED_{50} value was calculated (Figure 8, panel A).

Substitution tests were conducted in which rats in the cocaine S^D group were administered SA 4503 and placed in the operant chamber 10 min later. Panel D of figure 8 represents the response rates for the SA 4503 substitution tests. In the cocaine S^D group, 8.1 µmol/kg SA 4503 decreased response rates significantly [$F(2,17) = 6.42$, $P<0.05$] (Figure 8, panel D). Post hoc analyses

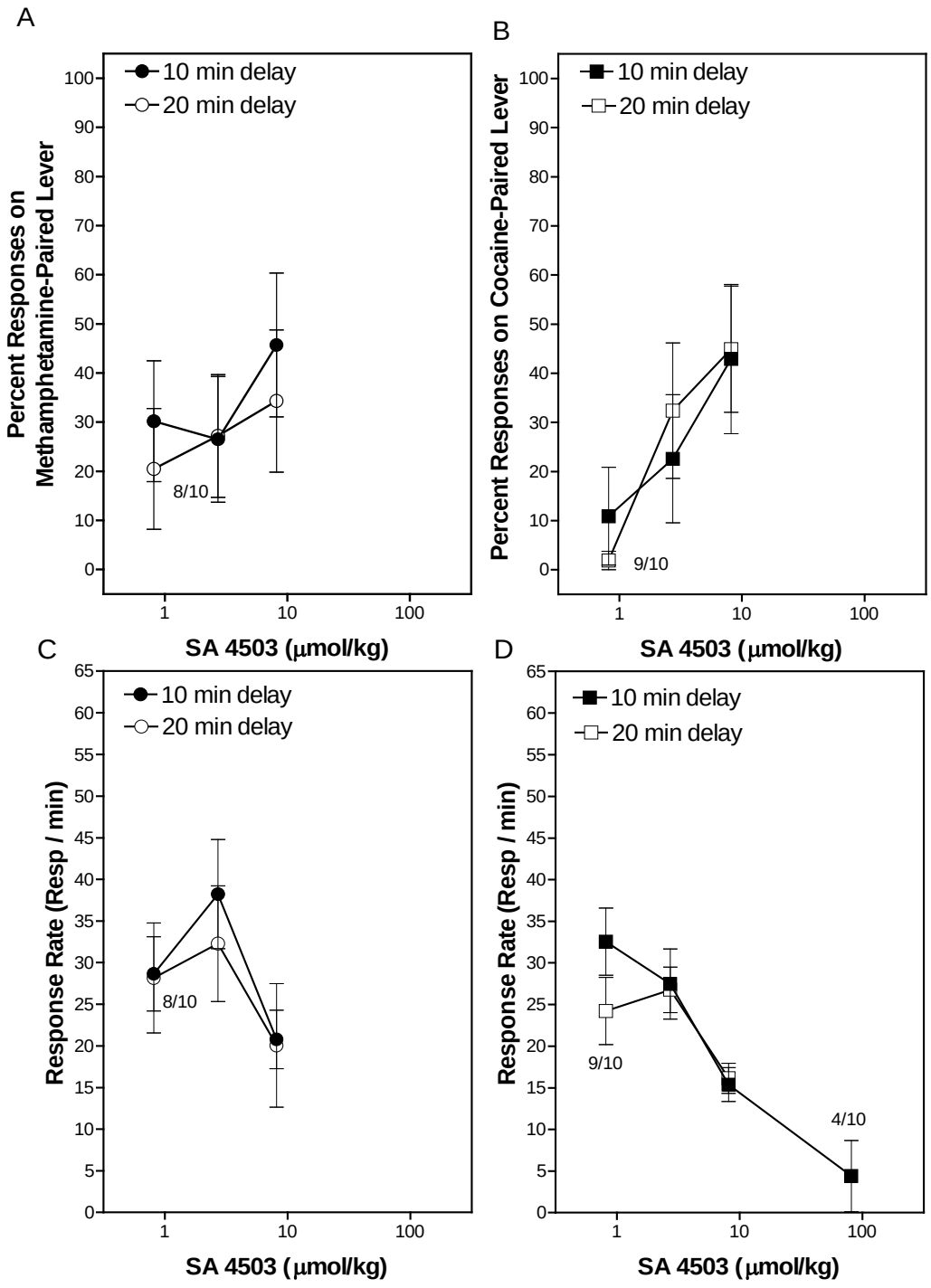

Figure 8. SA 4503 partially substitutes for the methamphetamine and cocaine S^D in a time-dependent manner. Rats were administered SA 4503, returned to the home cage for a 10 or 20 min delay, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

indicate a significant difference between 2.7 µmol/kg and 8.1 µmol/kg SA 4503 doses ($P<0.05$). The 81 µmol/kg SA 4503 dose was omitted from analyses because only four out of ten rats were tested. During this test session, rats were unresponsive and the test session was aborted. The percentage of responses on the cocaine-paired lever are presented in figure 8, panel B. In the cocaine S^D group, only partial substitution was evident at the 8.1 µmol/kg dose of SA 4503 after the 10 min post-injection interval and no ED_{50} value was calculated (Figure 8, panel B).

Additionally, rats in the cocaine S^D group were injected with SA 4503 and placed in the operant chamber after a 20 min post-injection interval. None of the SA 4503 doses significantly altered response rates ($P>0.05$) in the cocaine S^D group (Figure 8, panel D). In the cocaine S^D group, data for one of the ten rats was excluded for the 0.81 µmol/kg SA 4503 dose due to poor training. The percentage of responses on the cocaine-paired lever are presented in figure 8, panel B. In the cocaine S^D group, only partial substitution was evident at the 8.1 µmol/kg dose of SA 4503 after the 20 min post-injection interval and no ED_{50} value was calculated (Figure 8, panel B).

SA 4503 pretreatment tests with cocaine. Rats in the methamphetamine S^D group were pretreated with 2.7 µmol/kg SA 4503 (a dose that did not substitute for the S^D) prior to administration of cocaine to determine the effect of SA 4503 on cocaine. Response rates are presented in panel C of figure 9. In the methamphetamine S^D group, pretreatment with 2.7 µmol/kg SA 4503 decreased response rates [$F(1,9) = 19.23$, $P<0.05$] at the 1.6 µmol/kg and 16 µmol/kg

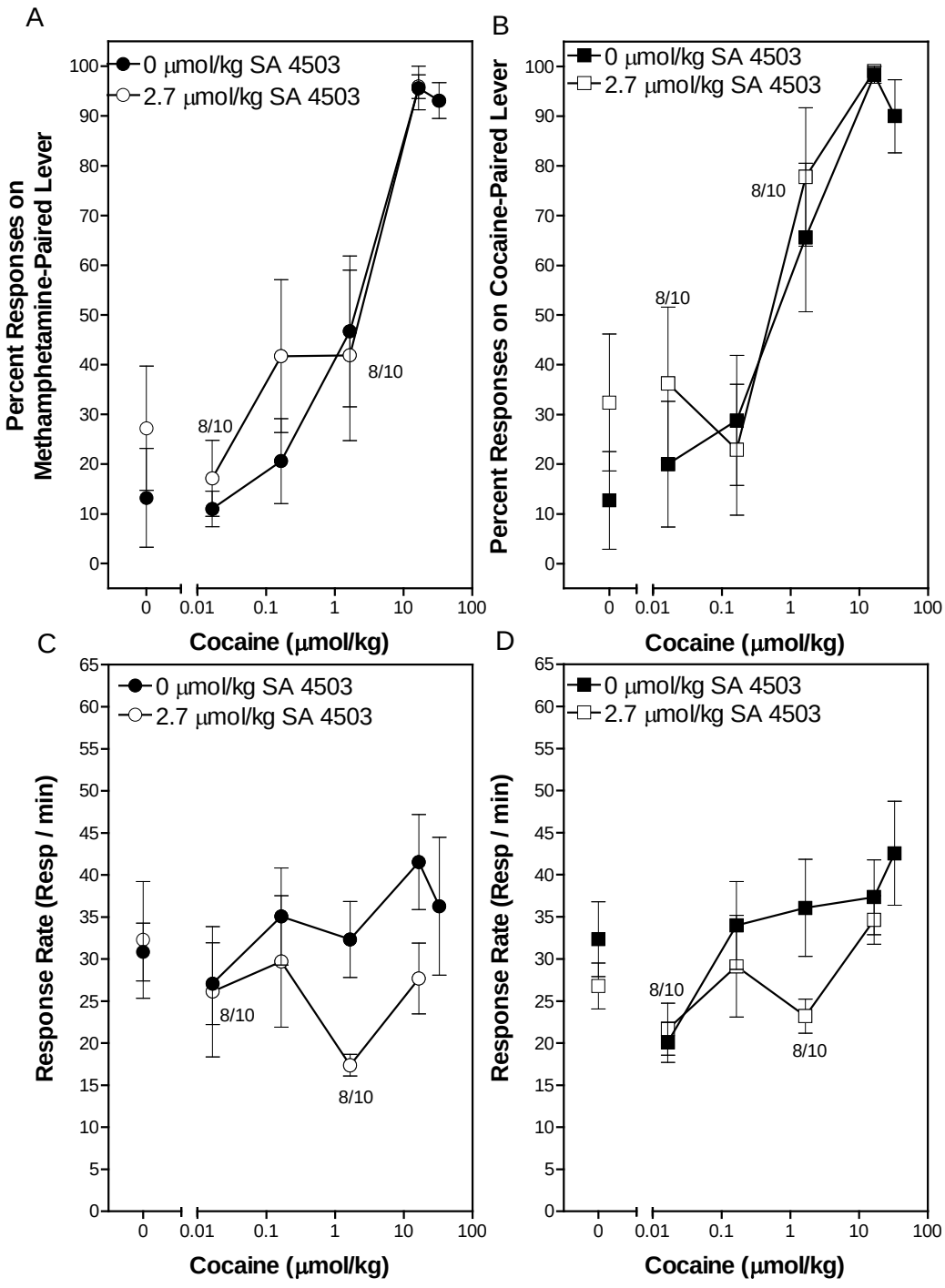

Figure 9. SA 4503 pretreatment does not alter the effect of cocaine to substitute for the methamphetamine and cocaine S^D. Rats were administered SA 4503, returned to the home cage for a 10 min delay, injected with cocaine, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

cocaine doses (both $P<0.05$). In the methamphetamine S^D group, data for two of the ten rats was excluded due to poor training for the 0.016 and 1.6 µmol/kg cocaine doses for the 2.7 µmol/kg SA 4503 pretreatment condition.

The percentage of responses on the methamphetamine-paired lever are presented in figure 9, panel A. For rats in the methamphetamine S^D group, full substitution was evident at the 16 µmol/kg cocaine dose and partial substitution was evident at the 1.6 and 0.16 µmol/kg cocaine doses (Figure 9, panel A). Regression analyses determined an ED_{50} value of 0.52 µmol/kg (95% C.I. = 0.16-1.6 µmol/kg) for the methamphetamine S^D group. As presented above, the ED_{50} value calculated from the cocaine dose-response curve for the 0 µmol/kg SA 4503 pretreatment condition was 0.89 µmol/kg for the methamphetamine S^D group. These ED_{50} values did not differ between the 0 and 2.7 µmol/kg SA 4503 pretreatment conditions for the methamphetamine S^D group, indicating that pretreatment with 2.7 µmol/kg SA 4503 does not alter the effect of cocaine to substitute for the methamphetamine S^D.

Rats in the cocaine S^D group were pretreated with 2.7 µmol/kg SA 4503 (a dose that did not substitute for the S^D) prior to administration of cocaine to determine the effect of SA 4503 on cocaine. Response rates are presented in panel D of figure 9. In the cocaine S^D group, analysis of response rates indicated a significant main effect of cocaine dose [$F(2,18) = 5.19$, $P<0.05$]. Post hoc tests showed a significant decrease in responding between pretreatment with 2.7 µmol/kg SA 4503 and no SA 4503 pretreatment at the 1.6 µmol/kg cocaine dose ($P<0.05$). Data for two of the ten rats was excluded due to poor training for the

0.016 and 1.6 µmol/kg cocaine doses for the 2.7 µmol/kg SA 4503 pretreatment condition in the cocaine S^D group.

The percentage of responses on the cocaine-paired lever are presented in figure 9, panel B. In the cocaine S^D group, full substitution was evident at the 16 µmol/kg dose of cocaine and partial substitution was evident at the 1.6 µmol/kg dose of cocaine (Figure 9, panel B). Analysis of the regression line determined an ED_{50} value of 0.26 µmol/kg (95% C.I. = 0.06-0.9 µmol/kg) for the cocaine S^D group. As presented above, the ED_{50} value calculated from the cocaine dose-response curve for the 0 µmol/kg SA 4503 pretreatment condition was 0.46 µmol/kg for the cocaine S^D group. These ED_{50} values did not differ between the 0 and 2.7 µmol/kg SA 4503 pretreatment conditions for the methamphetamine S^D group, indicating that pretreatment with 2.7 µmol/kg SA 4503 does not alter the effect of cocaine to substitute for the cocaine S^D.

SA 4503 pretreatment tests with methamphetamine. In order to investigate the effect of SA 4503 on methamphetamine, rats in the methamphetamine S^D group were pretreated with 0.81 and 2.7 µmol/kg SA 4503 prior to methamphetamine administration. These SA 4503 doses did not substitute for the methamphetamine S^D. For the methamphetamine S^D group, response rates are presented in figure 10, panel C. There was a significant main effect of methamphetamine dose [$F(2,19) = 5.59$, $P<0.05$]. Pretreatment with 0.81 and 2.7 µmol/kg SA 4503 increased response rates at the 3.3 µmol/kg dose of methamphetamine (both $P<0.05$).

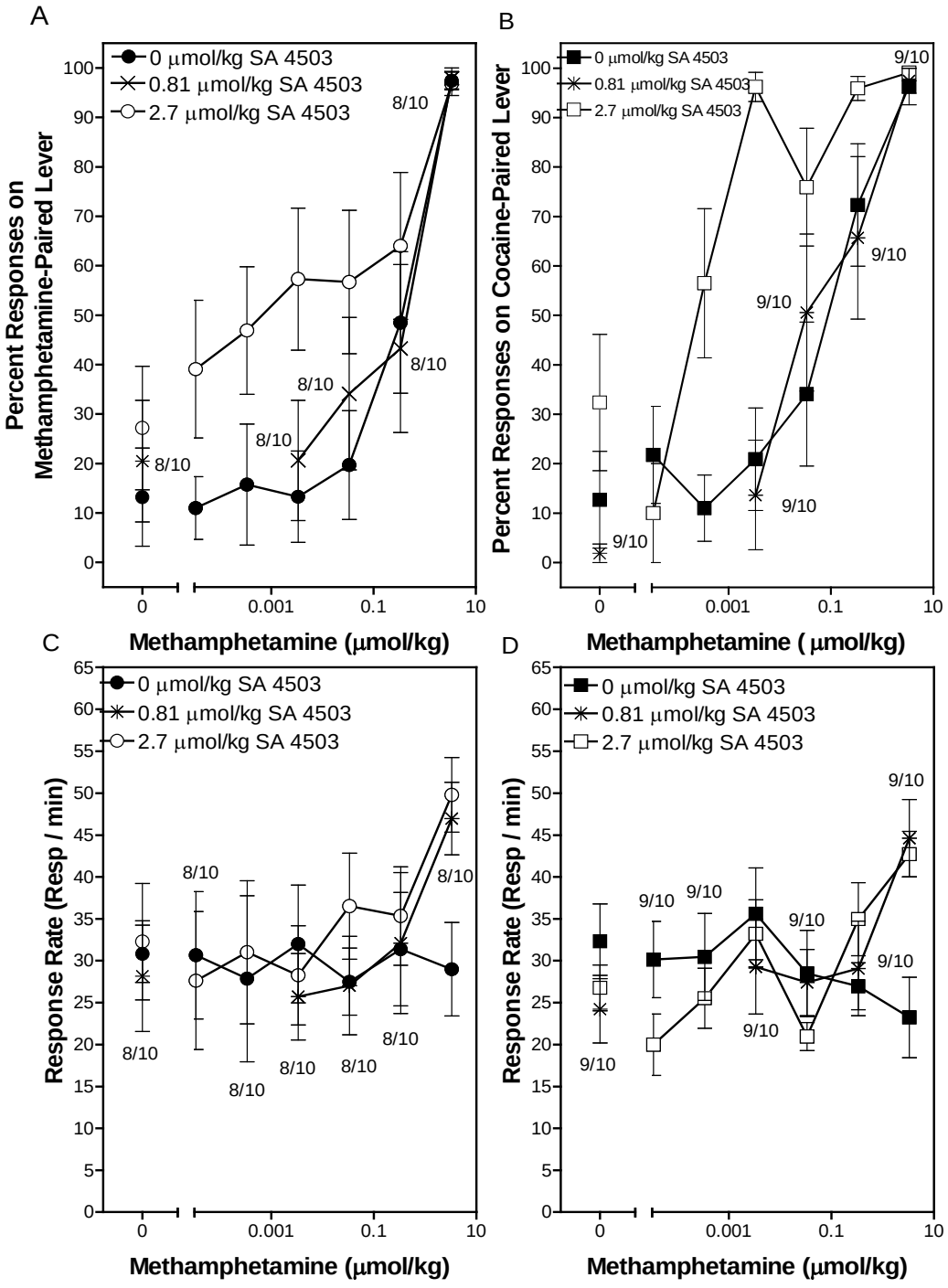

Figure 10. SA 4503 pretreatment augments the effect of methamphetamine to substitute for the methamphetamine and cocaine S^D in a dose-dependent manner. Rats were administered SA 4503, returned to the home cage for a 10 min delay, injected with methamphetamine, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

For rats in the methamphetamine S^D group, the percentage of responses on the methamphetamine-paired lever are presented in figure 10, panel A. In the 0.81 µmol/kg SA 4503 pretreatment condition, full substitution was evident at the 3.3 µmol/kg methamphetamine dose and partial substitution was evident at the 0.3 µmol/kg dose of methamphetamine. Regression analyses determined an ED_{50} value of 0.11 µmol/kg (95% C.I. = 0.02-0.40 µmol/kg) for the 0.81 µmol/kg SA 4503 pretreatment condition. In the 2.7 µmol/kg SA 4503 pretreatment condition, full substitution was evident at the 3.3 µmol/kg methamphetamine dose and partial substitution was evident at the 0.3, 0.03, 0.003, and 0.0003 µmol/kg doses of methamphetamine. Regression analyses determined an ED_{50} value of 0.0009 µmol/kg (95% C.I. = 0.00004-0.013 µmol/kg) for the 2.7 µmol/kg SA 4503 pretreatment condition. As mentioned previously, the ED_{50} value calculated from the methamphetamine dose-response curve for the 0 µmol/kg SA 4503 pretreatment condition was 0.13 µmol/kg for the methamphetamine S^D group. These ED_{50} values did not differ between the 0 and 0.81 µmol/kg SA 4503 pretreatment conditions for the methamphetamine S^D group, indicating that pretreatment with 0.81 µmol/kg SA 4503 does not alter the effect of methamphetamine to substitute for the methamphetamine S^D. However, the ED_{50} value for the 2.7 µmol/kg SA 4503 condition was different from the 0 µmol/kg SA 4503 condition. Thus, pretreatment with 2.7 µmol/kg SA 4503 shifted the methamphetamine dose-response curve 144-fold to the left in the methamphetamine S^D group, indicating that SA 4503 (2.7 µmol/kg) pretreatment

was sufficient to potentiate the effect of methamphetamine to substitute for the methamphetamine S^D.

Similarly, rats in the cocaine S^D group were pretreated with 0.81 and 2.7 µmol/kg SA 4503 prior to methamphetamine administration. These SA 4503 doses did not substitute for the cocaine S^D. For the cocaine S^D group, response rates are presented in figure 10, panel D. The methamphetamine dose x SA 4503 pretreatment interaction was significant [$F(3,31) = 3.89$, $P<0.05$]. Additionally, there was a significant main effect of methamphetamine dose [$F(2,23) = 3.59$, $P<0.05$]. Post hoc analyses confirmed that pretreatment with 0.81 and 2.7 µmol/kg SA 4503 increased response rates at the 3.3 µmol/kg dose of methamphetamine (both $P<0.05$).

For rats in the cocaine S^D group, the percentage of responses on the cocaine-paired lever are presented in figure 10, panel B. In the 0.81 µmol/kg SA 4503 pretreatment condition, full substitution was evident at the 3.3 µmol/kg methamphetamine dose and partial substitution was evident at the 0.3 and 0.03 µmol/kg doses of methamphetamine. Regression analyses determined an ED_{50} value of 0.05 µmol/kg (95% C.I. = 0.01-0.13 µmol/kg) for the 0.81 µmol/kg SA 4503 pretreatment condition. In the 2.7 µmol/kg SA 4503 pretreatment condition, full substitution was evident at the 0.003, 0.3, and 3.3 µmol/kg methamphetamine doses and partial substitution was evident at the 0.03 and 0.0003 µmol/kg doses of methamphetamine. Regression analyses determined an ED_{50} value of 0.0003 µmol/kg (95% C.I. = 0.00006-0.001 µmol/kg) for the 2.7 µmol/kg SA 4503 pretreatment condition. As mentioned previously, the ED_{50} value calculated from

the methamphetamine dose-response curve for the 0 µmol/kg SA 4503 pretreatment condition was 0.06 µmol/kg for the cocaine S^D group. These ED_{50} values did not differ between the 0 and 0.81 µmol/kg SA 4503 pretreatment conditions for the cocaine S^D group, indicating that pretreatment with 0.81 µmol/kg SA 4503 does not alter the effect of methamphetamine to substitute for the cocaine S^D. However, the ED_{50} value for the 2.7 µmol/kg SA 4503 condition was different from the 0 µmol/kg SA 4503 condition. Thus, pretreatment with 2.7 µmol/kg SA 4503 shifted the methamphetamine dose-response curve 200-fold to the left in the cocaine S^D group, indicating that SA 4503 (2.7 µmol/kg) pretreatment was sufficient to potentiate the effect of methamphetamine to substitute for the cocaine S^D.

SA 4503 pretreatment tests with amphetamine. Rats in the methamphetamine S^D group were pretreated with 2.7 µmol/kg SA 4503 (a dose that did not substitute for the methamphetamine S^D), placed in the home cage for 10 min, injected with amphetamine, and placed in an operant chamber after a 10 min delay. Response rates are presented in figure 11 panel C. In the methamphetamine S^D group, analysis of response rates indicated a significant main effect of amphetamine dose [$F(3,22) = 8.05$, $P<0.05$] and a significant main effect of SA 4503 pretreatment [$F(1,9) = 7.80$, $P<0.05$]. Pretreatment with 2.7 µmol/kg SA 4503 slightly decreased response rates at the 2.2 µmol/kg amphetamine dose ($P=0.071$). Data from two of the ten rats were excluded for the 0.002 µmol/kg amphetamine dose for the no SA 4503 pretreatment due to poor training. For the rats in the 2.7 µmol/kg SA 4503 pretreatment condition,

data from two of the ten rats were excluded for all amphetamine doses due to poor training.

Figure 11, panel A shows the percentage of responses on the methamphetamine-paired lever. For rats in the methamphetamine S^D group, full substitution was evident at the 2.2 µmol/kg amphetamine dose and partial substitution was evident at the 0.2 µmol/kg dose of amphetamine (Figure 11, panel A). Regression analyses determined an ED_{50} value of 0.04 µmol/kg (95% C.I. = 0.01-0.14 µmol/kg) for the methamphetamine S^D group. As mentioned previously, the ED_{50} value calculated from the amphetamine dose-response curve for the 0 µmol/kg SA 4503 pretreatment condition was 0.06 µmol/kg for the methamphetamine S^D group. These ED_{50} values did not differ between the 0 and 2.7 µmol/kg SA 4503 pretreatment conditions for the methamphetamine S^D group, indicating that pretreatment with 2.7 µmol/kg SA 4503 does not alter the effect of amphetamine to substitute for the methamphetamine S^D.

Rats in the cocaine S^D group were pretreated with 2.7 µmol/kg SA 4503 (a dose that did not substitute for the cocaine S^D), placed in the home cage for 10 min, injected with amphetamine, and placed in an operant chamber after a 10 min delay. Response rates are presented in figure 11 panel D. In the cocaine S^D group, there was a significant main effect of amphetamine dose [$F(3,22) = 3.42$, $P<0.05$] and SA 4503 pretreatment [$F(1,9) = 11.01$, $P<0.05$]. Pretreatment with 2.7 µmol/kg SA 4503 decreased response rates at the 0.02, 0.2 and 2.2 µmol/kg amphetamine doses (all $P<0.05$). Data from one of the ten rats was excluded for the 0.002 µmol/kg amphetamine dose for the no SA 4503 pretreatment due to

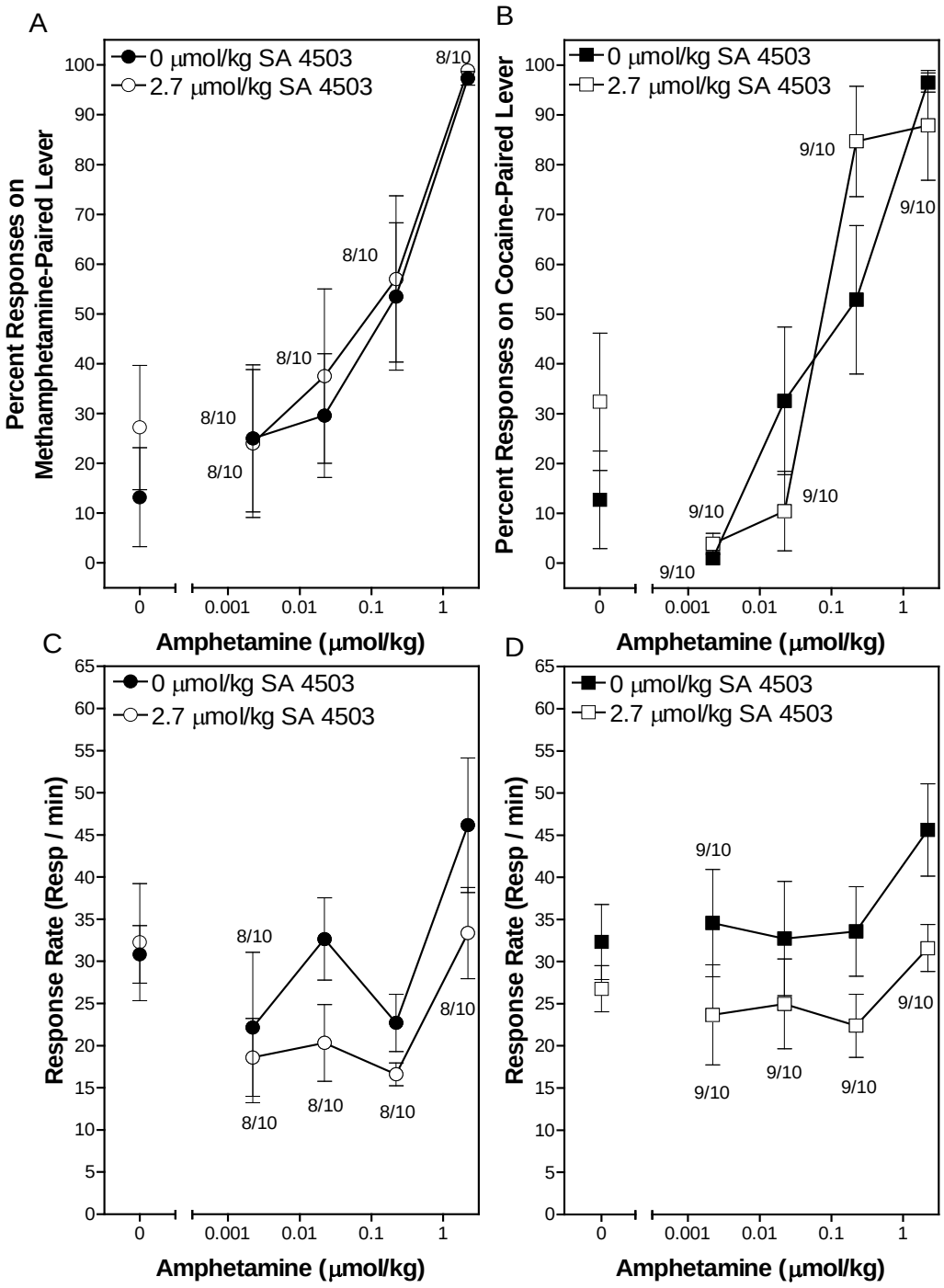

Figure 11. SA 4503 pretreatment does not alter the effect of amphetamine to substitute for the methamphetamine and cocaine S^D. Rats were administered SA 4503, returned to the home cage for a 10 min delay, injected with amphetamine, then placed in the chamber where testing commenced. Data represent mean (±SEM) percentage of responses on the drug-paired lever (panels A-B) and the mean (±SEM) response rates (panels C-D). Panels A and C represent data from the methamphetamine S^D group and B and D are data from the cocaine S^D group.

poor training. For the rats in the 2.7 µmol/kg SA 4503 pretreatment condition, data from one of the ten rats was excluded for all amphetamine doses due to poor training.

Figure 11, panel B shows the percentage of responses on the cocaine-paired lever. In the cocaine S^D group, full substitution was evident at the 0.2 and 2.2 µmol/kg doses of amphetamine (Figure 11, panel B). Analysis of the regression line determined an ED_{50} value of 0.07 µmol/kg (95% C.I. = 0.03-0.14 µmol/kg) for the cocaine S^D group. As mentioned previously, the ED_{50} value calculated from the amphetamine dose-response curve for the 0 µmol/kg SA 4503 pretreatment condition was 0.09 µmol/kg for the cocaine S^D group. These ED_{50} values did not differ between the 0 and 2.7 µmol/kg SA 4503 pretreatment conditions for the cocaine S^D group, indicating that pretreatment with 2.7 µmol/kg SA 4503 does not alter the effect of amphetamine to substitute for the cocaine S^D.

Discussion

The major finding of the present study was that SA 4503 dose-dependently shifted the stimulus-generalization curve for methamphetamine to the left, which is a novel finding. Importantly, SA 4503 produced a subjective state inherently different from the methamphetamine and cocaine S^D. Additionally, we found that methamphetamine, cocaine and amphetamine, but not nicotine, had similar subjective properties to the training doses of methamphetamine and cocaine, and these effects were likely mediated via the central nervous system.

In the present study, rats were trained to discriminate methamphetamine from saline on a two-lever operant task for food reinforcement. In a dose-response study, methamphetamine produced a dose-dependent increase in methamphetamine-appropriate responding. Moreover, cocaine produced a dose-dependent increase in methamphetamine-appropriate responding in generalization tests. These results are in line with previous studies in which methamphetamine and cocaine fully substituted for the training dose of methamphetamine (Munzar & Goldberg, 2000). In the methamphetamine S^D group, full substitution was evident at doses of amphetamine, however, only partial substitution was observed with nicotine. The results from our amphetamine substitution test are in agreement with a previous study examining the effect of amphetamine analogs in rats trained to discriminate methamphetamine from saline. In this previous study, the amphetamine analog phentermine fully substituted for the methamphetamine S^D (Munzar et al., 1999). The results from our nicotine substitution test are in line with previous research investigating the interaction of nicotine and methamphetamine. Rats were trained to discriminate methamphetamine from saline. Nicotine only partially substituted for the methamphetamine S^D (Gatch et al., 2008). The results of the present study suggest there are similar subjective properties induced by methamphetamine, cocaine and amphetamine, but not nicotine.

Cocaine also served as a S^D in the present experiment in a separate group of rats. Interestingly, a similar pattern of results were observed compared to the methamphetamine S^D group. In a dose-response study, cocaine produced

a dose-dependent increase in cocaine-appropriate responding. In addition, methamphetamine and amphetamine fully substituted for the cocaine S^D. Partial substitution only was observed in the nicotine substitution tests. These findings are in line with previous research from our laboratory investigating the S^D properties of cocaine. Rats were trained to discriminate cocaine from saline. Cocaine, amphetamine and nicotine fully substituted for the cocaine S^D (Cunningham et al., 2006). Additionally, previous research from other laboratories found that methamphetamine, cocaine, and amphetamine fully substitute for a cocaine S^D (Li et al., 2006). Like the methamphetamine S^D in the present study, these results indicated that methamphetamine, cocaine and amphetamine, but not nicotine, produce similar subjective states to the cocaine S^D.

Also in the present study, procaine did not substitute fully for the methamphetamine or cocaine S^D, indicating that the effect of 3.3 µmol/kg methamphetamine and 16 µmol/kg cocaine to serve as the S^D is likely mediated by the central nervous system. In previous studies in our laboratory, we found that procaine did not fully substitute for the cocaine S^D (Cunningham et al., 2006).

A novel finding from the present study was the inability of SA 4503 to fully substitute for the methamphetamine or cocaine S^D in either the 10- or 20-min delay SA 4503 substitution tests. This aligns with a previous study which investigated the effect of sigma receptor agonist DTG in rats trained to discriminate between cocaine and saline. In this previous study, DTG failed to

produce responding appropriate for the cocaine lever (Ukai et al., 1997). Furthermore, sigma receptor agonists igmesine and PRE-084 failed to produce a conditioned place preference (Romieu et al., 2002), supporting the lack of rewarding properties of sigma receptor agonists. This is in contrast to a recent study examining the effect of DTG on cocaine self-administration. When substituted for cocaine, DTG and PRE-084 maintained self-administration, suggesting that these compounds have intrinsic reinforcing properties (Hiranita et al., 2010). Although conditioned place preference and self-administration assess the reinforcing properties of substances, the inherent differences in behavioral methods may have important considerations for the incongruent findings across procedures. Additionally, the inconsistency in the behavioral responses to the sigma receptor agonists highlights the variation among these drugs. Thus, in the present study, SA 4503 produced a subjective state different from the training doses of methamphetamine and cocaine, suggesting that affinity for sigma receptors is not sufficient to produce substitution for the methamphetamine or cocaine S^Ds.

In the SA 4503 pretreatment tests, doses of SA 4503 that did not substitute for either the methamphetamine or the cocaine S^D were used. These tests were performed in order to determine if SA 4503 pretreatment is sufficient to alter the effect of methamphetamine, cocaine, or amphetamine to substitute for the methamphetamine or cocaine S^D. In the pretreatment tests, rats in both S^D groups were administered SA 4503, placed in the home cage for 10 min, injected with methamphetamine, cocaine, or amphetamine, placed in home cage

for 10 min, then put into the operant chamber where testing commenced. In both the methamphetamine and cocaine S^D groups, SA 4503 dose-dependently potentiated the effect of methamphetamine to substitute for the S^D. In the methamphetamine S^D group, pretreatment with 2.7 µmol/kg SA 4503 increased the ED_{50} value 144-fold compared to the ED_{50} value of the 0 µmol/kg SA 4503 pretreatment condition. In the cocaine S^D group, pretreatment with 2.7 µmol/kg SA 4503 increased the ED_{50} value 200-fold compared to the ED_{50} value of the 0 µmol/kg SA 4503 pretreatment condition. These findings indicate that pretreatment with SA 4503 caused lower doses of methamphetamine to produce similar subjective properties to both the methamphetamine and cocaine S^D. Previous studies investigating the effect of sigma receptor agonists on the S^D properties of cocaine found a similar pattern of results. DTG and PRE-084 dose-dependently produced significant leftward shifts in the cocaine dose-response function (Hiranita et al., 2010; Ukai et al., 1997). In contrast, SA 4503 pretreatment was insufficient to alter the effect of cocaine or amphetamine to substitute for the methamphetamine or cocaine S^D in the present study. It is unclear why SA 4503 was unable to alter the effect of cocaine or amphetamine since generalization was observed in both S^D groups to these stimulants. One possible explanation for the lack of effect of SA 4503 on the effect of amphetamine to substitute for the S^Ds could be due to the weak affinity (K_i > 10 µM) for σ_1 sigma receptors (Walker et al., 1990). Methamphetamine has a higher affinity for sigma receptors than amphetamine which may responsible for the augmentation of methamphetamine's effects observed after SA 4503

pretreatment. Furthermore, the doses of amphetamine tested in this study were below (<10 μM) the affinity of amphetamine for sigma receptors.

A potential interaction between sigma receptors and dopamine systems has recently been proposed which would explain the mechanism of action for SA 4503 to potentiate the effect of methamphetamine to increase methamphetamine- and cocaine-appropriate responding. The mesolimbic dopamine system is likely mediating the S^D properties of methamphetamine and cocaine (Callahan et al., 1997; Nakajima et al., 2004). As mentioned previously, activation of sigma receptors stimulates dopamine synthesis and release. Pentazocine stimulated dopamine synthesis in striatum and this effect was blocked by sigma receptor antagonist BMY-14802, suggesting a sigma receptor-mediated mechanism (Booth & Baldessarini, 1991). Additionally, extracellular striatal dopamine levels were increased by administration of DTG and pentazocine (Patrick et al., 1993). Furthermore, administration of SA 4503 increases the number of spontaneously active dopamine neurons in the ventral tegmental area (Minabe et al., 1999), the origination of the mesolimbic dopamine pathway. These studies suggest that sigma receptor activation is sufficient to alter the firing pattern of mesolimbic dopamine neurons which may contribute to the potentiation of subjective effects of psychostimulants. From a molecular perspective, a proposed mechanism for dopamine system amplification is that sigma receptor activation increases intracellular calcium (Su & Hayashi, 2003). Thus, excess intracellular calcium produced by sigma receptor activation may be

augmenting downstream dopamine systems important for modulating the subjective effects of drugs.

Recently, sigma receptor agonists DTG and PRE-084 potentiated the reinforcing effect of cocaine as observed by a significant leftward shift of the cocaine self-administration dose-response curve (Hiranita et al., 2010). In the same study, this effect of sigma receptor agonists was further potentiated by dopamine reuptake inhibitor WIN 35,428 (Hiranita et al., 2010), suggesting an interaction between these two systems. Blockade of the DAT plays a role in the S^D effects of methamphetamine and cocaine. Dopamine reuptake inhibitors fully substituted for both a methamphetamine and cocaine S^D (Cunningham & Callahan, 1991; Munzar & Goldberg, 2000), implicating a role for the DAT in the S^D effects of methamphetamine and cocaine. Additionally, DAT inhibitors can increase intracellular calcium via activation of the IP_3 second messenger pathway (Su & Hayashi, 2001). However, SA 4503 reportedly does not have affinity for the DAT (Ishiwata et al., 2001). Although, its potentiating actions may be mediated through intracellular calcium signaling and downstream increases in dopamine neuron firing. Thus, SA 4503, acting as a sigma agonist, may be regulating the IP_3 second messenger pathway to increase intracellular calcium which facilitates downstream dopamine neurons.

SUMMARY AND CONCLUSIONS

To summarize, SA 4503 dose-dependently augmented and prevented methamphetamine-induced hyperactivity. Furthermore, SA 4503 pretreatment augmented the effect of methamphetamine to substitute for both the methamphetamine and cocaine S^D. In addition, SA 4503 produced a subjective state different from the training dose of methamphetamine and cocaine. To the author's knowledge, these results are novel and have not been reported in the literature. These data support a role for sigma receptor involvement in the locomotor activating and S^D properties of methamphetamine and cocaine. The mechanism likely involves an indirect interaction between sigma receptors and dopamine systems.

Our results and previous research indicate that sigma receptors are involved in modulating the motor and subjective effects of psychostimulants (Skuza, 1999; Ukai et al., 1997). In the present study, SA 4503 dose-dependently potentiated and attenuated methamphetamine-induced hyperactivity, and augmented the effect of methamphetamine to increase methamphetamine- and cocaine-appropriate responding. The differential effect of SA 5403 seems a paradox. However, previous work investigating the effect of selective sigma receptor ligand NPC 16377 on cocaine-induced hyperactivity and methamphetamine and cocaine S^Ds showed a similar pattern of results. A behaviorally inactive dose of NPC 16377 attenuated the locomotor stimulatory properties of cocaine (Witkin et al., 1993). Additionally, NPC 16377 did not

substitute for either a cocaine S^D or a methamphetamine S^D (Witkin et al., 1993). However, NPC 16377 slightly potentiated the effects of cocaine and methamphetamine to substitute for the cocaine and methamphetamine S^D (Witkin et al., 1993). Recently, it has been proposed that sigma receptor mechanisms may be dependent on the concentration of ligand available (Su et al., 2009). For example, high concentrations of sigma receptor ligands may lead to ion channel inhibition, resulting in attenuation of downstream dopamine systems. Low concentrations of sigma receptor ligands may lead to excess intracellular calcium, resulting in potentiation of downstream dopamine systems. Together, these studies support the notion that SA 4503 can be acting as a mixed agonist-antagonist in the present study where SA 4503 produced differential effects on methamphetamine-induced hyperactivity and both the cocaine and methamphetamine S^Ds.

Although the full mechanism of action is yet to be determined, it appears that SA 4503 may produce differential effects by acting as a mixed agonist-antagonist at sigma receptors. In the locomotor activity study, SA 4503 augmented and blocked methamphetamine-induced hyperactivity, suggesting that SA 4503 may be acting a mixed agonist-antagonist. In the drug discrimination study, SA 4503 potentiated the effect of methamphetamine to produce subjective effects similar to the cocaine and methamphetamine S^D, suggesting that SA 4503 may be acting as an agonist. When acting as an agonist, SA 4503 may increase intracellular calcium levels, which would facilitate downstream dopamine systems. When acting as an antagonist, SA 4503 may

inhibit plasmalemmal ion channels, which would attenuate downstream dopamine systems. Thus, it appears that the effects of sigma receptors are dependent on the dose of ligand administered.

Presently, there are numerous pharmacological targets being investigated for psychostimulant addiction. However, there are no FDA approved and effective treatments. Many studies have examined sigma receptors as a potential target for the development of pharmacotherapies effective for treating psychostimulant addiction. SA 4503 has many beneficial properties including its effects to prevent methamphetamine-induced hyperactivity, reverse working memory impairments, and attenuate the reinforcing properties of nicotine. Since SA 4503 was sufficient to prevent methamphetamine-induced hyperactivity, perhaps it would be a beneficial pharmacotherapy at these doses. However, SA 4503 potentiated methamphetamine-induced hyperactivity and the effect of methamphetamine to produce a subjective state similar to methamphetamine and cocaine, indicating at these doses it would not be a beneficial antagonist therapy. Together, these observations indicate that while SA 4503 may not be a particularly effective pharmacotherapy, sigma receptors may be important targets for investigating the mechanism underlying psychostimulant-induced behaviors.

Future Directions

In the future, it would be beneficial to further investigate the involvement of sigma receptors in the behavioral and neurochemical effects of psychostimulants. Currently our laboratory has determined the effect of acute SA 4503 on methamphetamine- and cocaine-induced locomotor activity. It will be

essential to determine the effect of SA 4503 on methamphetamine- and cocaine-induced behavioral sensitization. The importance of these studies will be to examine the involvement of sigma receptors in the development of behavioral sensitization to cocaine and methamphetamine which involve a different mechanism than acute psychomotor activation. In the present study, we investigated the effect of SA 4503 pretreatment on the S^D properties of cocaine and methamphetamine. It will be beneficial to examine the effect of selective sigma antagonists on the S^D properties of methamphetamine and cocaine, as well as the effect of selective sigma agonists and antagonists on reinforcing properties of methamphetamine and cocaine via the drug self-administration or conditioned place preference paradigms. These studies will contribute to our understanding of the involvement of sigma receptors in the motivational aspects of drugs of abuse.

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