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5-HT3 Receptor Ligands and Their Effect on Psychomotor Stimulants

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5-HT_3 RECEPTOR LIGANDS AND THEIR EFFECT ON PSYCHOMOTOR STIMULANTS

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

By

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List of Abbreviations

AC	Adenylyl cyclase
AChBP	Acetylcholine-binding protein
ADHD	Attention Deficit Hyperactivity Disorder
BBB	Blood Brain Barrier
cAMP	3',5'-Cyclic adenosine monophosphate
CoMFA	Comparative Molecular Field Analysis
CoMSIA	Comparative Moleuclar Similarity Indices Analysis
CSA	Controlled Substance Act
DA	Dopamine
DAT	Dopamine transporter
DD	Drug discrimination
DEA	Drug Enforcement Administration
DOB	1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane
DOI	1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane
DOM	1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane
Du	Aryl Centroid (Dummy Atom)
$GABA_A$	γ-Aminobutyric acid type A receptor
G_i	G-Protein inhibitory
GPCR	G-Protein coupled receptors
G_s	G-Protein stimulatory
5-HT	5-Hydroxytryptamine/Serotonin
i.p.	Intraperitoneal
i.v.	Intravenous
LGIC	Ligand gated ion channel
LSD	(+)Lysergic acid diethylamide
MAO	Monoamine Oxidase Isozyme
*m*CPBG	*meta*-Chlorophenylbiguanide
*m*CPG	*meta*-Chlorophenylguanidine (MD-354)
MD-354	*meta*-Chlorophenylguanidine
MDA	1-(3,4-Methylenedioxyphenyl)-2-aminopropane
MDMA	N-Methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane
nACh	Nicotinic acetylcholine
NE	Norepinephrine
NET	Norepinephrine transporter
NMQ	N-Methylquipazine
PAA	Phenylalkylamine
PCA	*p*-Chloroamphetamine
PMMA	N-Methyl-1-(4-methoxyphenyl)-2-aminopropane

q^2	Predictability
QSAR	Quantitative structure-activity relationships
r^2	Squared correlation coefficient (variance)
s.c.	Subcutaneous
SERT	Serotonin transporter
SN	Substantia nigra
TM	Transmembrane
VMAT-2	Vesicular Monoamine Transporter-2
VTA	Ventral tegmental area

Abstract

5-HT_3 RECEPTOR LIGANDS AND THEIR EFFECT ON PSYCHOMOTOR STIMULANTS

By Jessica Nicole Worsham, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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Drug abuse and addiction are considered to be a result, at least in part, of the rewarding effects produced by increasing dopamine levels. 5-HT_3 serotonin receptors have been shown to indirectly affect dopamine levels. Therefore, the effect of the 5-HT_3 receptor partial agonist, MD-354, on the actions of psychomotor stimulants was analyzed in mouse locomotor activity assays to determine whether MD-354 is working through a 5-HT_3 receptor agonist or antagonist mode of action. Studies with (+)amphetamine and

(+)methamphetamine in combination with MD-354 indicated MD-354 is either devoid of action or is behaving similar to the 5-HT_3 receptor antagonist, ondansetron. This effect could be occurring centrally; however peripheral effects can not be discounted. In combination with cocaine, MD-354 behaved similar to the 5-HT_3 receptor agonist, SR 57227A, known to act both centrally and peripherally. This difference between central and peripheral effects could account for the different modes of action observed with MD-354.

Studies also involved synthesis of potentially brain-penetrant carbamate analogs of MD-354, and QSAR to assist in validating a 5-HT_3 receptor agonist pharmacophore.

I. Introduction

Drug abuse and addiction are growing problems in today's society, where prevention is the only real treatment. There are various drugs available, both prescription drugs and illegal drugs, that are abused due to their positive (i.e., rewarding) effects. Some common effects that lead to abuse include feelings of euphoria and stress relief.[1] However, repetitive use, tolerance, and dependence (whether psychic or physical) lead to abuse, addiction, and the unfortunate withdrawal that follows when an addict no longer has access to the drug of abuse.

Some of the most commonly abused psychomotor stimulants on the market are the Schedule II drugs amphetamine, usually in the form of prescription medications, methamphetamine, which is more commonly found on the street in an impure form, or in prescription medications, and cocaine, most commonly a street drug. All three drugs have been shown to have an effect on the monoamines dopamine (involved with cognition, memory, and reward), norepinephrine (involved with memory, mood, and arousal), and serotonin (involved with mood, appetite, anxiety, reward, and aggression).[2]

DOM is a phenylalkylamine hallucinogen that produces euphoric and stimulant effects at low doses, but more hallucinogenic effects at higher doses.[3] DOM is structurally similar to amphetamine and could possibly behave in a similar manner even though it is not classified as a psychomotor stimulant.

The phenylalkylamine psychomotor stimulant amphetamine, and its more potent analog methamphetamine, increase synaptic dopamine levels through several different methods: blocking dopamine reuptake and releasing dopamine from vesicles in the presynaptic terminal.[4,5] Cocaine, a non-phenylalkylamine stimulant, works by blocking reuptake of dopamine into the presynaptic terminal.[2] The positive reinforcing effects of cocaine are due to increased synaptic dopamine levels, known as the "dopamine hypothesis".[6] The positive subjective effects, such as the "high" observed with stimulants could be due to differences in levels of norepinephrine, known as the "noradrenergic hypothesis".[7]

Serotonin has also been shown to play a role in the rewarding effect of psychomotor stimulants. Although there are numerous 5-HT receptor populations and subpopulations, the receptor population of interest when describing drugs of abuse is the 5-HT_3 receptor. Of the seven 5-HT receptor families, 5-HT_3 receptors are the only serotonin receptor population that is a ligand-gated ion channel (LGIC) receptor as opposed to the others that are G-protein coupled receptors (GPCR).[8] Activation of serotonin systems, more commonly 5-HT_3 receptor systems, have been shown to modulate dopamine release. Grant reviewed that 5-HT_3 receptor agonists may indirectly increase dopamine levels whereas 5-HT_3 receptor antagonists indirectly decrease dopamine levels.[9] This could be due, in part, to the location of serotonin receptors in different regions of the brain relative to dopamine receptors, more specifically location in the mesolimbic dopaminergic pathway.[9] Further support from Tecott *et al.* indicated that GABAergic input on dopamine neurons due to the quantity and distribution of 5-HT_3 receptors in the CNS could account for the indirect effects on dopamine levels.[10]

MD-354 is a unique 5-HT_3 receptor partial agonist, as it can behave as either an agonist or an antagonist, that binds at 5-HT_3 receptors with relatively high affinity (K_i = 35 nM).[11] When administered alone, MD-354 does not produce a locomotor stimulant effect, or might not penetrate the BBB; the latter is a possibility due to its low Log P value of -0.64.[12,13]

The effects of psychomotor stimulants can be evaluated through locomotor activity assays. Although locomotor activity assays can analyze various parameters, some of the more common parameters involved in locomotor stimulation include increases in movement distance and movement time, and decreased movement episodes.

Since MD-354 is a 5-HT_3 receptor partial agonist, and 5-HT_3 receptors have been shown to affect dopamine levels, MD-354 may either potentiate or antagonize the effect of psychomotor stimulants in locomotor activity assays. However, it is possible that MD-354 may have no effect on the psychomotor stimulants because it might not readily penetrate the BBB.

The purpose of this study is to determine the effect that MD-354 might have on the psychomotor stimulants, and to characterize this effect as working through either a 5-HT_3 receptor agonist or 5-HT_3 receptor antagonist mode of action. Also, since MD-354 may or may not cross the BBB, more lipophilic prodrugs will be synthesized and tested in locomotor activity assays to determine the effect. QSAR studies will be conducted on a set of arylguanidine and arylbiguanide 5-HT_3 receptor agonists and partial agonists in order to improve Dukat's current working 5-HT_3 receptor pharmacophore model.[14] Conformationally-constrained analogs will be incorporated into the model to account for

rotameric binding and CoMFA studies will be conducted to predict the binding affinities of novel arylguanidine derivatives.

II. Background

A. Drug Abuse/Drug Addiction

Drug abuse has social, medical, economic, and criminal impacts costing Americans millions of dollars per year, making treatment pertinent. The term "drug abuse" is very vague, as its definition is based on social perceptions. Some of the more common definitions for drug abuse entail any use of an illegal substance, use of legal substances in excessive amounts, or even use of legal substances regardless of the amount.[1] A drug's positive effects such as euphoric feelings, stress relief, and improvement of performance, are key factors in why drugs readily become abused.[1] Tolerance occurs when the rewarding effects are no longer achieved by the initial dose; therefore a higher dose is necessary to achieve an earlier effect. Drug abuse and tolerance can lead to addiction and psychic dependence (i.e., continuous administration is necessary to achieve a euphoric feeling, regardless of the harmful consequences).[1] This is not to be confused with physical dependence, which involves a physiological reaction, such as withdrawal symptoms, to the absence of the drug.[1] Withdrawal symptoms are negative physical symptoms such as irritability, anxiety, fatigue, and even nausea.[1] Drug addiction and dependence are not synonymous, because not all addicts are dependent upon the drug. [1]

The extent to which a drug has pharmacological properties that are able to predict a drug's likelihood of abuse and dependence is termed "abuse liability".[15] If a drug is determined to have abuse potential, it can then be regulated under the Controlled Substances Act (CSA) as a scheduled drug. This legislation was created in 1970, as a reorganization of government agencies, to regulate these drugs. Schedule I drugs have a high potential for abuse, with no accepted medical use or safety for use. Substances in Schedule II – V have medical use, with differences in abuse potential.[16] Schedule II drugs have high abuse potential resulting in high psychological and/or physical dependence, whereas substances in Schedules III-V have lower abuse potential, as well as moderate to low physical or psychological dependence.[16] Some countries and agencies further divide controlled substances into a sixth schedule that can be broken into three parts: Part 1 includes Class A precursors, Part 2 includes Class B precursors, and Part 3 contains any preparation or mixture set out in Part 1 or Part 2.[17] Some examples of Schedule VI Part 1 substances include acetic anhydride, lysergic acid, piperonal, and potassium permanganate.[17] Part 2 substances include acetone, ethyl ether, hydrochloric acid, sulfuric acid, and toluene.[17]

In addition to the abuse of legal substances such as alcohol and nicotine, and the abuse of prescription drugs, some of the most widely abused substances include opioids, stimulants, hallucinogens, and related designer drugs.[18] Even though abused drugs are easily classified on the basis of abuse potential, they might act by different mechanisms. By understanding the mechanisms of action of each drug, it might be easier to find effective treatments for drug abuse and addiction and, possibly, drug abuse prevention.

B. Classification

1. Opioids

The use and abuse of opium was reported as far back as 200 B.C.; opium is a substance occurring naturally from poppy seeds of *Papaver somniferum.*[19] Opioids can be defined as opium alkaloids, their synthetic derivatives, and peptides with morphine-like pharmacological effects that are antagonized by an opioid antagonist.[19] These agents are thought to work through one or more of three major opioid receptors: mu (μ), kappa (κ), and delta (δ), to help with postoperative analgesia, myocardial infarction, trauma, burns, and orthopedic pain.[20] Most opioids, such as morphine, the principle alkaloid of opium, codeine, hydrocodone, and oxycodone, are Schedule II drugs that help with moderate to severe pain, and that have abuse and addiction potential.[21] Opioids are commonly abused due to their euphoric, analgesic, and sedative effects.[20] However, an opioid overdose can lead to respiratory failure.[20] Heroin, an acetylated form of morphine, is currently a Schedule I drug that is highly addictive.[21] However, once heroin is no longer available to a heroin addict, intense withdrawal symptoms occur, such as nausea, vomiting, diarrhea, anxiety, and depression.[20] Currently there are two drug replacement therapies for heroin addicts: methadone and buprenorphine.[20,22]

With most other drugs of abuse, prevention is the only treatment for addiction, as no other medications are available. Because "replacement therapy" is available here, and because replacement therapy might be possible for other types of drug abuse, drug replacement therapy and drug abuse treatment are a major area of interest to help treat drug addiction.

2. Stimulants

a) Analeptics

Psychostimulants are drugs that have a direct neurological effect such as increased alertness and energy, appetite suppression, and euphoria.[23] There are two main categories of stimulants: analeptics and behavioral stimulants. Analeptic stimulants are not as readily abused as behavioral stimulants; analeptics work primarily on autonomic centers generally affecting respiration and circulation.[18] Some examples of analeptics include strychnine and caffeine. Strychnine is considered an analeptic stimulant because it increases respiration and blood pressure, but is a convulsant that can be lethal at high doses.[24] Sometimes, "street drugs" such as cocaine and (+)lysergic acid diethylamide (LSD) are "laced" with small amounts of strychnine for respiratory stimulation.[24] Caffeine, a xanthine, is considered the most widely used psychoactive drug in the world because it reduces drowsiness and fatigue while increasing heart rate and blood pressure.[1] It is found in tea from *Thea sinensis* (and other species), and in coffee from *Coffea Arabica* (and other species).[25] Interestingly, although craving is observed regardless of its lack of euphoric effects upon withdrawal of the caffeinated beverage, caffeine is not considered addicting.[1]

b) Behavioral Stimulants

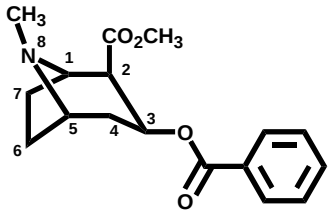
i. Non-Phenylalkylamines

Behavioral stimulants have more of a central stimulatory effect than analeptics, resulting in changes in motor activity. The class of behavioral stimulants can further be divided into non-phenylalkylamine and phenylalkylamine (PAA) stimulants. The most common non-phenylalkylamine stimulant is cocaine, a psychotropic drug used for the past 2000 years with 25.6% of 26-34 year olds using cocaine at least once in their lifetime;[26] about five million people, just in the United States, use cocaine each year.[27] Cocaine, a naturally occurring drug, is found as a constituent of the *Erythoxylon coca* species of plants in South America.[18] In the 1860's, cocaine was used in several different tonics, which gave them "magic properties".[26] It was even found as the main stimulant in Coca Cola.[18] In the late 1800's cocaine served medical purposes such as treatment for morphine addiction and as a topical anesthetic in ophthalmology.[26] Due to its stimulant properties, as well as the fact that it alleviated hunger, and allowed people to forget reality, it became a highly abused drug, eventually labeled as a Schedule II substance.[26] Cocaine has many different street names, such as "blow", "crack", "rock", and "coke", along with different forms: hydrochloride salt and free base. Cocaine, a positive reinforcer, has a high abuse potential due to its rapid onset of action with "rush" central stimulatory effects including intense euphoria, psychic energy, heightened sexual excitement, and increased self-confidence.[26,28,29] However, cocaine abusers can also experience paranoia, hallucinations, and dysphoria, during their subsequent "crash", with toxic concentrations potentially leading to fatal cardiac arrhythmias.[26]

Cocaine works through dopamine (DA), norepinephrine (NE), and serotonin (5-HT) mechanisms, with its stimulant effect mainly a result of the dopaminergic mechanism. Dopamine nerve terminals contain a dopamine transporter (DAT), located perisynaptically, which terminates the actions of dopamine by a transport mechanism.[2] Cocaine works by blocking the DAT, preventing reuptake of dopamine into the presynaptic nerve terminal.[2] This increases extracellular dopamine levels, producing a heightened state of euphoria.[2] It is important to note that although cocaine blocks the dopamine transporter, it itself is not transported into the nerve terminal.[2]

Cocaine's reinforcing effects are due to its ability to bind to the dopamine transporter and block reuptake of dopamine into the presynaptic terminal.[6] The better able an agent to bind to the dopamine transporter, the greater the blockade, allowing more dopamine in the synapse, which accounts for the euphoric effect. Structure-activity studies show that there are some key aspects to the structure of cocaine that can enhance or decrease its activity. By simply switching the configuration of cocaine from R to S, by switching the carbomethoxy group from the C-2 position to the C-4 position, the activity of cocaine decreases more than 100-fold (**1**; Figure 1).[30] The presence of the carbomethoxy group at the 2-position is optimal for cocaine-like activity.[30] Removal of this group or replacement with most other substituents decreases activity, with one exception; different ester groups can replace the carbomethoxy substituent with only small alterations in activity.[30] Also, at this position stereochemistry is important: epimerization from β to α substantially decreases activity.[30] Similar decreases in activity are observed with the same epimerization at the C-3 position.[30] Changes at the nitrogen

position can have varying effects. For example, changing the length of the substituent chain has little to no effect on activity, whereas changing the functional group such as from an amine to an amide or even conversion from a tertiary amine to a quaternary amine significantly reduces activity.[30] Interestingly, it was observed that the nitrogen atom can be moved from the 8-position to the 6- or 7-position without decreasing binding affinity to the dopamine transporter.[30] These, as well as other, structural changes allow for a better understanding of the binding profile of cocaine.

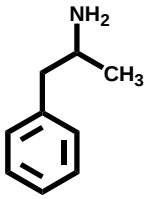


Cocaine (**1**)

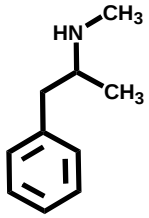
Figure 1. Structure of cocaine (**1**) labeled for discussion of structure-activity relationship studies.

ii. Phenylalkylamines

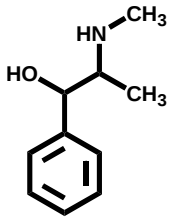
Several different drugs make up the stimulant class of phenylalkylamines: agents that contain a phenyl group, an alkyl chain, and an amine.[18] The most widely recognized stimulant is amphetamine (**2**; Figure 2), a central stimulant, anorectic, and sympathomimetic agent.[18] Sympathomimetic drugs are drugs that mimic the actions of endogenous neurotransmitters stimulating the sympathetic nervous system.[24] Of the stimulant phenylalkylamines, amphetamine is considered the prototypical drug, with others referred to as "amphetamine-like" or "amphetaminergic".[18] Unlike many drugs of abuse, amphetamine is not naturally occurring. It was first synthesized in 1877[24] as an optical isomer; (+)amphetamine is the more potent of the two isomers.[31] In 1930, amphetamine began to be used therapeutically in the treatment of narcolepsy and depression.[24] However, its positive effects of mood elevation, euphoria, alleviation of fatigue, and improving task performance, resulted in abuse.[24] Finally, in 1970 the Drug Enforcement Administration (DEA) labeled amphetamine as a Schedule II drug.[21]



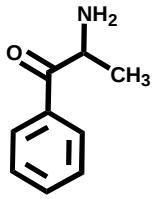
Amphetamine (**2**)



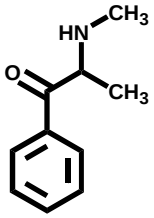
Methamphetamine (**3**)



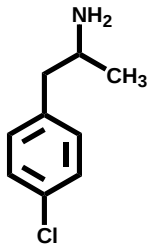
Ephedrine/Pseudoephedrine (**4**)



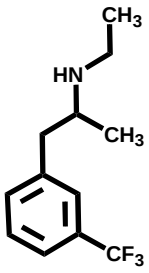
Cathinone (**5**)



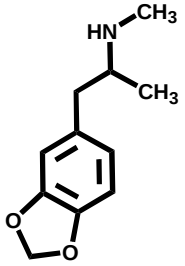
Methcathinone (**6**)



p-Chloroamphetamine (**7**)



Fenfluramine (**8**)



MDMA (**9**)

Figure 2. Structures of amphetamine and some important derivatives.

Currently, amphetamines, such as Dexedrine®, Ferndex®, and Oxydess II® are used to treat narcolepsy, attention deficit hyperactivity disorder (ADHD), and obesity.[24] Both with prescribed and illicit amphetamines, toxic levels can create "amphetamine psychosis", aggression, delusions, arrhythmias, and convulsions.[32] "Amphetamine psychosis" is a paranoid-hallucinatory psychosis in a setting of clear consciousness in which formal aspects of thought are relatively intact, but in which delusions evoke intense fear.[32] Sometimes, patients with amphetamine psychosis are misdiagnosed as having schizophrenia, a disease for which the symptoms are indistinguishable.[23] Amphetamine withdrawal can result in a dysphoric state resulting in anhedonia, depression, anxiety, and social inhibition.[33,34] Therefore, some attempts have been made to treat amphetamine addicts with antidepressants such as mirtazapine, fluoxetine, and imipramine.[35,36] Amphetamine abuse poses a severe problem as there currently is no effective treatment on the market for addicts, whose withdrawal symptoms could be detrimental.

Methamphetamine (**3**; Figure 2), a derivative of amphetamine with a methylated amine, is a more popular street drug than amphetamine, as it generally has the same type of effects as amphetamine, but with a higher potency.[18] Methamphetamine's popularity is due to its ease of synthesis, with over 50% of clandestine labs seized in 1981 being "meth" labs.[24] Methamphetamine synthesis is so easy it can be made in the trunk of a car using the "special ingredients" and two-liter bottles.[37] Methamphetamine can be synthesized by numerous routes, the most common using (-)-ephedrine or (+)-pseudoephedrine, ingredients in over-the-counter decongestants and bronchodilators.[24]

For this reason, over-the-counter medications with these ingredients have recently been under strict control. The therapeutic forms of methamphetamine are Desoxyn®, Adipex®, and Methedrine® which are used for ADHD and obesity.[2,24] However, street versions of methamphetamine include "meth", "speed", "crank", "go", "crystal", and "ice", all of which are hydrochloride salts for greater bioavailability.[24] Methamphetamine is a Schedule II drug.[21] Like amphetamine, methamphetamine use causes euphoria, increased alertness, self-confidence, and suppresses fatigue.[38] However, withdrawal from methamphetamine use also causes a depressive state, with 49.4% of arrested "meth" addicts stating they had thoughts of suicide.[39] As with amphetamine, treatment for methamphetamine addicts is also elusive.

Stimulatory effects of phenylalkylamines mainly result from their effect on dopamine levels, primarily D_1 receptors.[40] Amphetamine, and amphetamine-like phenylalkylamines that have a stimulant effect, such as methamphetamine, work by increasing the release of dopamine by affecting vesicular monoamine transporter-2 (VMAT-2), preventing reuptake of dopamine into presynaptic terminals, and affecting monoamine oxidase isozymes MAO-A and MAO-B.[4,5] This increase of dopamine levels causes central stimulant actions. Also, the activity of dopamine receptors can be modulated by NE and 5-HT.[40] Some of the non-stimulant phenylalkylamines do not readily effect dopamine, but have a greater effect on 5-HT levels.[41] The different effects of phenylalkylamine stimulants on DA, NE, and 5-HT can be largely attributed to minor structural changes.

It is observed that small modifications to the structure of amphetamine, the prototypical phenylalkylamine stimulant, can cause vast changes in the activity, potency, effects, and even mechanism of action, of the drug. When analyzing structure-activity relationships of phenylalkylamine stimulants, there are five key points to be considered: terminal amine, chiral center, α-methyl group, β-position, and aromatic substitution. With the amine group, it is observed that primary amines are more potent than secondary amines (with the exception of methamphetamine), which are more potent than tertiary amines.[18] Length of the amine substituent has an effect on secondary amines, with a decrease in activity as the length of the substituent is increased.[18] There is a limit of bulk tolerance as larger substituents usually result in agents having little to no stimulant character.[18]

The α–methyl group seems pertinent for effect as demethylation to phenylethylamine results in loss of stimulant activity.[18] Also, homologation to larger, bulkier substituents decreases stimulant effect.[18] The presence of an α-methyl group creates a chiral center; the S(+) isomer is more potent than the R(-) isomer.[18] Changes at the β-position have varying effects. Hydroxylation of the β-carbon to norephedrine and norpseudoephedrine decreases stimulant actions.[18] Ephedrine and pseudoephedrine (**4**; Figure 2) are β-oxidized analogs of methamphetamine.[18] The abuse potential of ephedrine and pseudoephedrine is low but dependence can occur.[25] Some side effects of these drugs include anxiety, headache, tachyarrhythmia, and hypertensive crisis.[25]

However, oxidation of norephedrine to cathinone results in an agent that retains equal to or greater potency as compared to amphetamine.[18,42] Cathinone (**5**; Figure 2) is

a constituent of *Catha edulis*, the "Khat" shrub which is predominantly found in East Africa.[25] Its stimulant effects are similar to that of amphetamine, including euphoria, excessive talkativeness, focus, excitement, elimination of hunger, and insomnia, with a high chance of tolerance occurring.[41,43] The toxic effects of cathinone are limited, but include aggressive behavior, hallucinatory psychosis, and fatal hyperthermina.[25] The *N*-monomethyl analog of cathinone, methcathinone (**6**; Figure 2), a name originally coined in the Glennon laboratories,[44] is a designer drug synthesized from ephedrine, linking it to its common name ephedrone.[25] S(-)Methcathinone, which is found more often in the clandestine market than the R(+)-isomer or the racemic mixture is referred to as "CAT".[45] As cathinone's effects are similar to those of amphetamine, methcathinone is equally similar to methamphetamine.[25]

Lastly is manipulation of the ring by adding substituents. For the most part, adding substituents to the ring decreases and may even abolish amphetamine-like stimulant action as seen with *p*-chloroamphetamine (PCA) and fenfluramine (**7** and **8**, respectively; Figure 2).[18] PCA depletes serotonin levels in the brain, and produces a neurotoxic effect.[46] Instead of the euphoric effect caused by amphetaminergic agents, PCA causes aggression, anxiety, and panic disorders.[46] (±)Fenfluramine, marketed as Pondimin®, and its more potent stereoisomer (+)fenfluramine (Redux®), are non-amphetaminergic phenylalkylamine derivatives once used clinically for appetite suppression.[41] However, due to the side effects of cardiovalvulopathy, as well as primary pulmonary hypertension, these two drugs were withdrawn from the market in 1997.[41] Like PCA, fenfluramine also depletes serotonin levels in the brain.[46] However, this

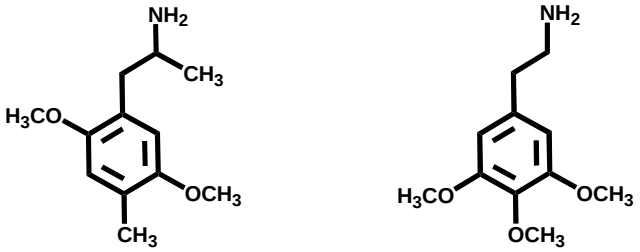
decrease is selective and may account for the development of tolerance to fenfluramine. Some, negative effects of fenfluramine include depression and aggravated psychosis.[46]

One of the key designer drugs on the market is N-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane, more commonly referred to as MDMA.[18] MDMA (**9**; Figure 2) was first synthesized in 1914 as an appetite suppressant and for psychotherapy to facilitate communication, but never had legal therapeutic use.[24,25] MDMA is an empathogen, in so much as it increases sociability, empathy, and feelings of well being.[24] Also, there are readily observed physical reactions, such as trismus (jaw clenching) and bruxism (teeth grinding), which account for "ravers" usually chewing gum or sucking on a pacifier.[47] Overdoses can cause panic, paranoia, psychosis and delirium with toxic (fatal) effects including convulsions, hyperthermia, behavioral changes, and acute renal failure.[24] There are several street names for MDMA, including "Ecstasy", "XTC", "M&M", "Zen", and "Adam".[18,24] Its lack of therapeutic use resulted in its placement in Schedule I.[21] The demethylated form of MDMA is 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDA), which has both hallucinogenic and stimulant character based on optical isomers.[18] Other structural changes of the phenylalkylamines result in hallucinogenic agents.

3. Non-stimulant Phenylalkylamines

Hallucinogens, upon administration of a single effective dose, consistently produce changes in thought, mood, and perception with little memory impairment, produce little stupor, narcosis, or excessive stimulation, produce minimal autonomic side effects, and are non-addicting.[48] The classical hallucinogens are hallucinogens that bind at 5-HT_2 serotonin receptors (*5-HT_2 hypothesis of hallucinogen action*)[18] and are recognized by animals trained to discriminate 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (**10**; Figure 3) from vehicle.[31,49] The family of 5-HT_2 receptors can be further divided into 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors with evidence that 5-HT_{2A} receptors play the major role in the hallucinogenic action.[50] The classical hallucinogens can further be divided into several other classes: lysergic acid derivatives (e.g. LSD), phenylethylamines (e.g. mescaline), indolealkylamines (e.g. DMT), and other indolic derivatives (β-carbolines).[18] The main focus of the present study is on phenylalkylamines, which includes both phenylisopropylamines and phenylethylamines.[18]

Mescaline (**11**; Figure 3), one of the most commonly known phenylethylamines, is naturally occurring from cactus, usually peyote (*Lophophora williamsii)*.[51] Onset of action usually has some negative effects such as nausea, vomiting, and diarrhea, but once subsided, visual hallucinations and perceptual distortions occur.[51] Some side effects include emotional instability and anxiety, with toxic effects of bradycardia, hypotension, and respiratory depression: death is a rare result of toxicity.[51] There is no therapeutic use for mescaline, it is currently classified as a Schedule I drug.[18]



DOM (**10**) Mescaline (**11**)

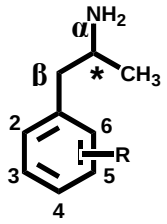
Figure 3. Structures of common phenylalkylamine hallucinogens.

DOM (**10**; Figure 3), 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane is a phenylisopropylamine used as the prototypical hallucinogen in drug discrimination studies.[18] The R(-)-isomer of DOM is more potent than the S(+)-isomer or the racemic mixture.[52] At low doses, DOM increases self-awareness, while producing feelings of anxiety, euphoria, and dysphoria, without producing a hallucinogenic effect.[3] However, at higher doses, DOM produces hallucinogenic effect similar to LSD and mescaline;[3] DOM is >80 times more potent than mescaline.[53] One of the original street names for DOM was "STP".[3] DOM has proven to be more hazardous than LSD, as it has a longer onset of action. Therefore, impatient people could take multiple doses of DOM causing an overdose and even hospitalization.[54] DOM is believed to work as an agonist at central serotonin (5-HT) sites,[55] more specifically through a 5-HT_2 mechanism.[56,57]

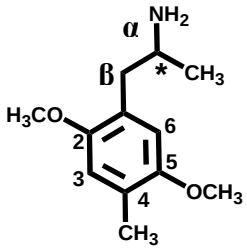
Structural changes to DOM, the prototypical hallucinogen, cause changes in potency and activity. With respect to the terminal amine of DOM, a primary amine is more potent than a secondary amine, and homologation of the α-methyl group decreases potency.[18] However, opposite effects are observed at the chiral center as well as with

aromatic substitution, when compared with amphetamine.[18] Effect of substitution at the β-position is highly dependent upon configuration. Addition of a β-hydroxy group in the *R*-configuration has little effect on affinity versus DOB, however the same addition in the *S*-configuration decreases affinity by 50-fold from *R*(-)DOB.[58] Also, addition of a β-methoxy group has a 100-fold decrease in affinity in the *S*-configuration but maintains affinity in the *R*-configuration.[58] Increasing the length of the 4-methyl group enhances potency.[18] However, if this lengthening surpasses n-propyl then potency decreases.[18] Activity is maintained with substitution at the 4-position with electron-withdrawing groups such as bromine or iodine (i.e., DOB or DOI respectively).[18] The R(-)isomer has been shown to be more active than the S(+)isomer which in some cases has zero activity.[18] Also, reduction in activity and potency can be observed through N-monomethylation.[18] This comparison between amphetamine and DOM is summarized in Table 1.

Table 1. Comparative structure-activity relationships of amphetamine-like stimulants vs. DOM-like hallucinogens.[18]



Amphetamine (**2**)



DOM (**10**)

Position	**Amphetamine-like action**	**DOM-like action**
Terminal Amine	N-Methyl > NH_2 > NHR > NR_2	NH_2 > NHR > NR_2
Chiral Center	S(+) >(±) > R(-)	R(-) > (±) > S(+)
α- Methyl group	Homologation decreases potency Replacement by H decreases potency	Homologation decreases potency Replacement by H decreases potency
β-position	β-OH reduces potency β=O retains activity and potency	β-OH reduces potency β=O not well investigated
Aromatic substitution	Unsubstituted ring preferred	2,5-Dimethyl-substitution preferred 4-Substitution further modulates activity

The behavioral actions of arylalkylamines, in this case phenylalkylamines, can fall into one of three categories: classical hallucinogen (H), central stimulant (S), and PMMA-like (P) (Figure 4).[18,59,60] Each category contains a prototypical drug used to characterize other drugs in drug discrimination studies: DOM represents the hallucinogens, (+)amphetamine represents the stimulants, and PMMA, N-methyl-1-(4-methoxyphenyl)-2-aminopropane, represents PMMA-like actions.[18,59,60] Most phenylalkylamine drugs of abuse fall into one of these three categories, or behave similar to more than one category. For example, MDMA would fall under intersect 2 as it produces both amphetamine-like and PMMA-like activity.[18,59,60] R(-)MDA has both hallucinogenic-like activity as well as PMMA-like activity, placing it in intersect 3. However the (±)MDA, produces amphetaminergic, hallucinogenic, and PMMA-like activity, placing it in the center.[18,59,60] It is important to note that through this classification, there should be three different mechanisms of action as well as three separate structure-activity relationships.

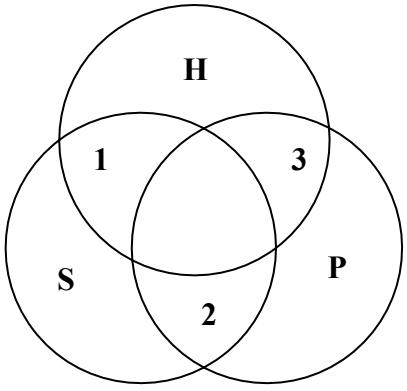


Figure 4. A modified Venn diagram of behavioral effects of arylalkylamines.[59,60]

C. Mechanism of Action of Stimulants

The behavioral pharmacology and addictive properties of the stimulants cocaine, amphetamine, and methamphetamine are related to the monoamines, serotonin, norepinephrine, and dopamine. Serotonin is involved with mood, fear, sleep, appetite, anxiety, reward, and aggression.[2] Serotonin is produced in the raphe nuclei of the brainstem and moves to the cortex, thalamus, basal ganglia, hippocampus, and amygdala.[2] Norepinephrine is involved with arousal, attention, memory, and mood, and is produced in the locus coeruleus.[2] It is also found in the hypothalamus, cortex, hippocampus, and striatal regions, along with several other parts of the brain.[2] Dopamine is involved in many processes such as movement, cognition, memory, and reward,[61] and plays a primary role in the reinforcing effects of cocaine, known as the dopamine hypothesis. Dopamine makes up approximately 80% of the catecholamine content in the brain.[62] All three of these neurons express transporter proteins that belong to the Na^+/Cl^- superfamily, (norepinephrine transporter, NET; dopamine transporter, DAT; and 5-HT transporter, SERT) which function to regulate monoaminergic activity in the brain.[63,64] Interestingly, psychostimulants affect all three transporters; cocaine binds with almost equal affinity to all three transporters, whereas (+)amphetamine and (+)methamphetamine bind with substantially higher affinity to DAT and NET than SERT as seen in Table 2.[2] However, addictive properties of psychostimulants are due to their effect on dopamine.[2]

Table 2. Stimulant drug affinities (K_i) at monoamine transporters (cocaine affinities at DAT and SERT are expressed as IC_{50} values).[2]

Drug	DAT	NET	SERT
(-)Cocaine	478 nM[65]	779 nM[7]	304 nM[65]
(+)Amphetamine	34 nM[7]	39 nM[7]	3830 nM[7]
(+)Methamphetamine	114 nM[66]	48 nM[66]	2137 nM[66]

Sometimes synonymous with addiction is withdrawal symptoms when the drug of abuse is no longer administered. Rothman *et al.* proposed a dual deficit model of stimulant addiction, stating withdrawal symptoms are a result of drug-induced dopamine and serotonin dysfunction as seen in Figure 5.[67] Dysfunction occurs when withdrawal from chronic stimulant use leads to a decrease in availability of dopamine and serotonin.[67] Dopamine deficit consists of decreased synaptic dopamine, altered dopamine transporter function, and/or postsynaptic receptor changes resulting in anhedonia and psychomotor retardation.[67] Serotonin deficit consists of decreased synaptic serotonin, decreased serotonin cell activity, and/or decreased synaptic dopamine, which results in depressed mood, obsessive compulsive thoughts and behaviors, and lack of impulse control.[67] Therefore, drugs that release dopamine or serotonin should effectively treat the withdrawal symptoms observed with addiction (i.e., such as *d*-amphetamine, a dopamine releasing agent, in the treatment of withdrawal symptoms from cocaine abuse).[67] Rothman *et al.* also stated that a drug that acts as both a dopamine and serotonin releasing agent could treat addiction with limited abuse liablility.[67]

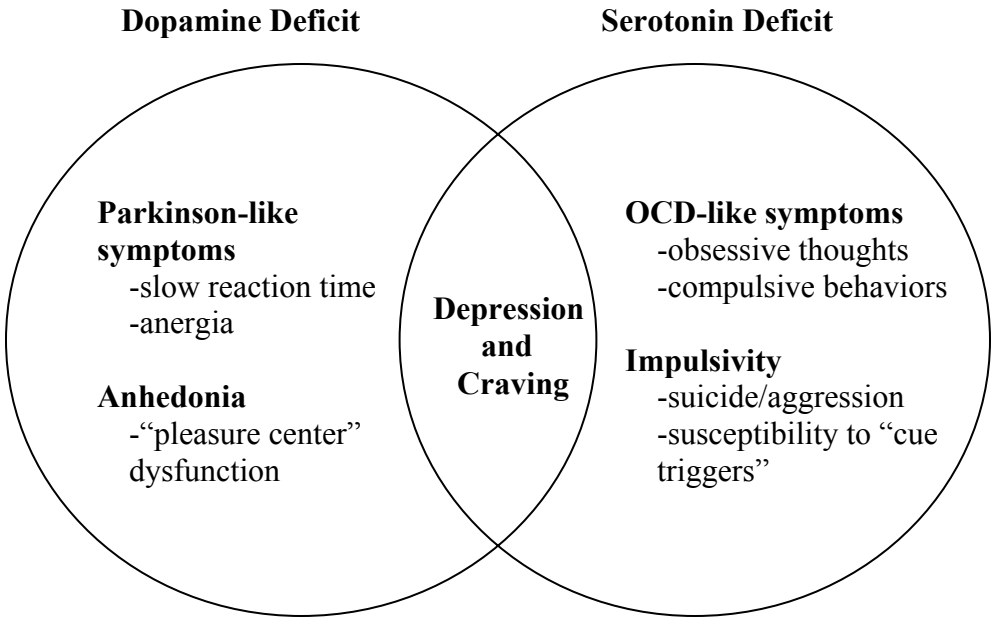
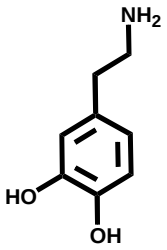


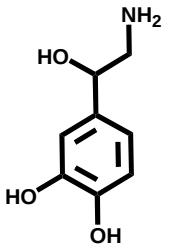
Figure 5. Dual-deficit model of psychostimulant addiction.[67]

1. Dopamine

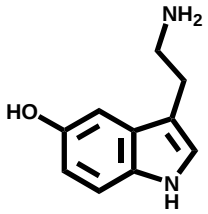
Dopamine (**12**; Figure 6) is a catecholamine neurotransmitter found both in the central and peripheral nervous system, that activates dopamine G-protein coupled receptors (GPCR).[2] In the peripheral nervous system it modulates cardiovascular and renal function, gastrointestinal motility, and the endocrine system.[68] In the central nervous system, dopamine effects cognition, emotion, locomotor activity, hunger, and regulation of the endocrine system.[68] Dopamine is produced in the substantia nigra (SN), the ventral tegmental area (VTA), and the hypothalamus.[2]



Dopmaine (**12**)



Norepinephrine (**13**)



Serotonin (**14**)

Figure 6. The structures of three important monoamine neurotransmitters.

The dopamine synapse consists both of presynaptic and postsynaptic nerve terminals. In the presynaptic terminal, dopamine is packaged into vesicles by VMAT-2 for storage, release, and protection from oxidation and reactive consequences.[69] When DA is released into the synapse it can bind to postsynaptic dopamine receptors D_1, D_2, D_3, D_4, and D_5.[68] Dopamine receptors can be divided into two groups containing the five different dopamine receptor subtypes. The D_1-like group consists of D_1 and D_5 receptors and is associated with stimulatory function and located postsynaptically, whereas the D_2-like group is located both pre- and postsynaptically, is associated with inhibitory function, and includes D_2, D_3, and D_4 receptor subtypes.[68,70,71] Agonists and/or antagonists can bind selectively to D_1-like receptors over D_2-like receptors and with selectivity within the D_2-like group.[68] However, currently no compounds can selectively differentiate between D_1 and D_5 receptors.[68]

The structure of the dopamine receptors varies slightly between each subtype, as some contain more amino acids than others.[68] However all have a few of the same key elements. For example, there is an NH_2-terminal stretch containing various numbers of

N-glycosylation sites between subtypes that stretches between seven transmembrane domains and ends with the carboxy terminus.[68] The carboxy terminus varies in length, but all contain serine, threonine, and a cysteine residue.[68,71] Two cysteine residues are present in the second and third extracellular loop which creates a disulfide bridge, providing structure stability.[71] As mentioned before, D_1-like receptors are involved with stimulatory function, due to their short third intracellular loop that interacts with G-stimulatory (G_s) proteins to stimulate cAMP production.[68,71] D_2-Like receptors are just opposite as their longer third intracellular loop interacts with G-inhibitory (G_i) proteins, to inhibit cAMP production.[68,71]

Activation of G-proteins affects adenylyl cyclase (AC) activity, which in turn affects cAMP accumulation modulating protein kinase A by phosphorylation or dephosphorylation (Figure 7).[62] Protein kinase A is responsible for regulating the synthesis of cytoplasmic and nuclear proteins, the function of membrane channels, and sensitization or desensitization of different G-protein coupled receptors.[62] Dopamine receptors are also involved with modulating the activity of phospholipase C, the release of arachidonic acid, the activity of calcium and potassium channels, the activity of Na/H exchangers, and Na-K ATPase.[68]

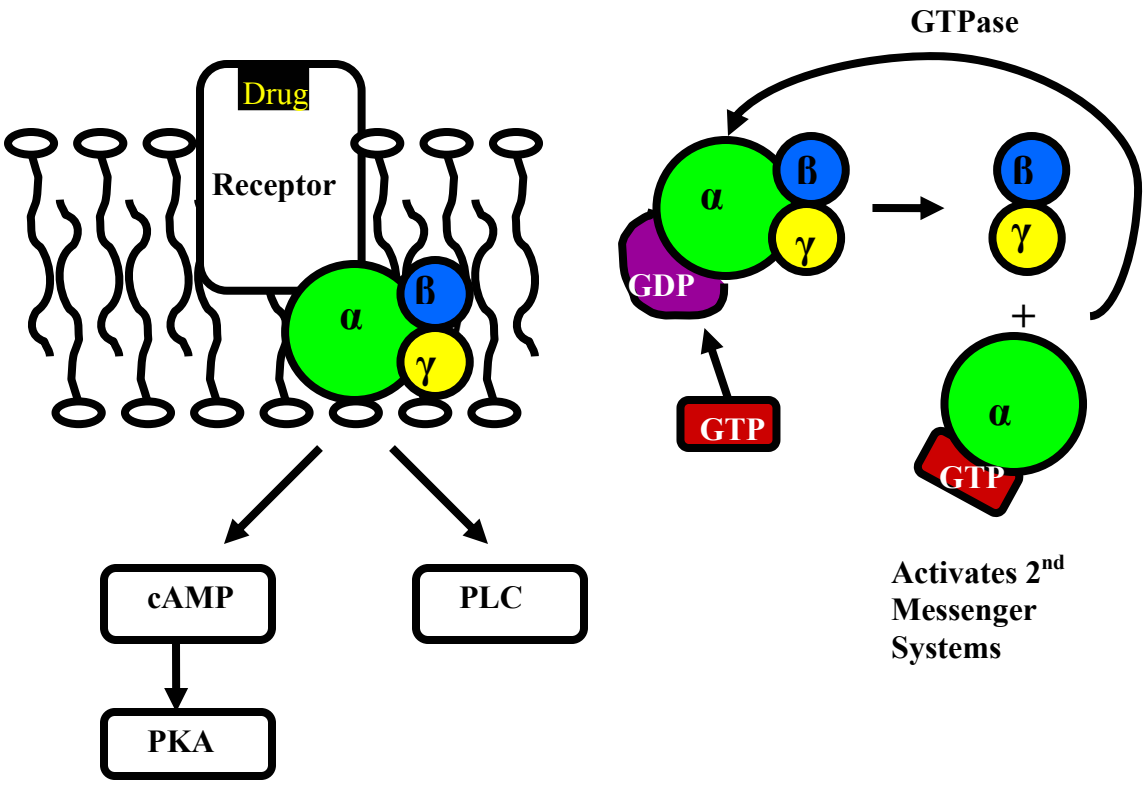


Figure 7. Activation of G-protein coupled receptors by a drug causes the dissociation of the αβγ complex. The α/GTP complex then activates second messenger systems such as cAMP or PLC.[72]

The euphoric feeling resulting from stimulant use is due to an increase in dopamine levels that can occur through several different routes.[73] The DAT and VMAT-2 regulate dopamine in both the synapse and cytosol; psychostimulants alter the function of both of these types of transporters.[73] Cocaine works primarily by blocking reuptake of dopamine into the presynaptic terminal by blocking the DAT (Figure 8).[30] Amphetamine and methamphetamine both can block the reuptake of dopamine, like cocaine, but also can cross the plasma membrane by lipophilic diffusion and act directly on vesicular

monoamine carriers by releasing transmitters from the vesicle.[63,74] Also, both amphetamine and methamphetamine promote efflux of transmitter (e.g. DA) by a transporter-mediated exchange.[63]

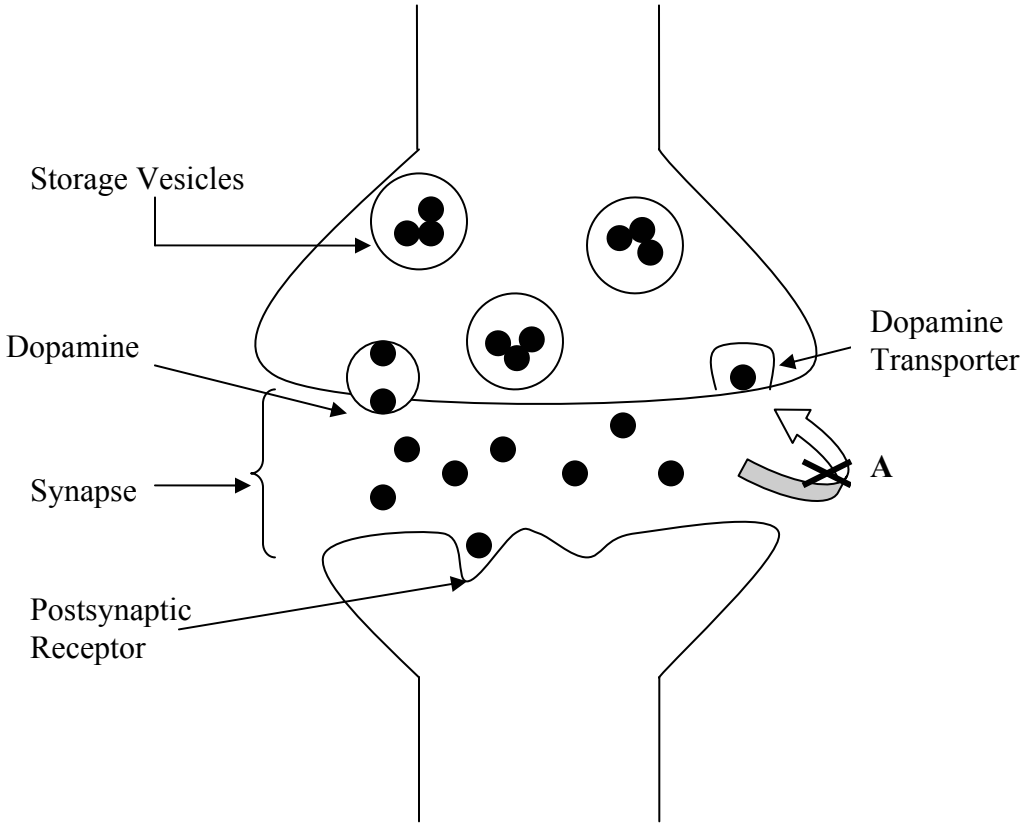


Figure 8. Schematic representation of a dopaminergic nerve terminal. Cocaine prevents reuptake ("A"), whereas amphetamine and methamphetamine cause release of dopamine from intracellular vesicles.[2,18]

2. Norepinephrine

Since repeated data support that dopamine plays a role in the reward/ reinforcement behavior observed in animal models,[7] the next question was to determine the effect of norepinephrine (**13**; Figure 6). Both amphetamine and methamphetamine increase NE concentrations by stimulation of release,[75] whereas cocaine increases NE by blocking reuptake.[76] However, blocking reuptake may not increase extracellular neurotransmitters as much as substrate-releasing agents, since it is nerve impulse-dependent.[7,77] Evidence shows that the release of NE may contribute to the positive subjective effects, such as the "high" produced by stimulants, coined the "noradrenergic hypothesis".[7] Since cocaine is unable to increase NE levels as much as substrate releasers like amphetamine, the subjective effects cannot be accounted for with this type of stimulant.[7]

3. Serotonin

As already discussed, central stimulants can increase synaptic levels of serotonin (e.g. see Table 2). This increase in serotonin levels can activate various populations of 5-HT receptors. The 5-HT receptor family will be described in the next section. Because emphasis of the present work is on 5-HT_3 receptors (and their ligands), these receptors will be discussed in detail.

D. Serotonin Receptors

1. Classification

Serotonin (5-HT) (**14**; Figure 6), is a neurotransmitter involved in anxiety, aggression, depression, schizophrenia, appetite control, drug abuse, and hallucinogenic activity.[78] Currently, there are seven families of serotonin receptors, 5-HT_1-5-HT_7, some of which are divided into subpopulations.[78] These families are characterized based on three components: drug binding characteristics, receptor-effector coupling, and structural sequences for the nucleotides and amino acids.[79] The majority of 5-HT receptors have been cloned as either human, mouse, rat, or guinea pig receptors leading to the generalization of their amino acid sequence homology.[78]

All of the serotonin receptors are G protein-coupled receptors except for 5-HT_3 receptors which are ligand-gated ion channel (LGIC) receptors.[78] G Protein-coupled receptors consist of seven transmembrane (TM) spanning helices, with an extracellular N-terminus, an intracellular C-terminus, and loops connecting the helices;[72] the intracellular loop between TM5 and TM6 is rather large and thought to be involved with second messenger system coupling.[78] Serotonin receptors can be coupled to two types of second messenger systems: adenylyl cyclase (AC), where coupling can occur positively or negatively, or phospholipase C.[78] All of the different subpopulations, with the currently accepted nomenclature, and their second messenger systems are summarized in Table 3.

Table 3. Classification of current populations of serotonin receptors.[78]

Currently Accepted Name	Second Messenger System
5-HT_1	
5-HT_{1A}	AC(-)
5-HT_{1B}	AC(-)
h5-HT_{1D}	AC(-)
h5-HT_{1B}	AC(-)
5-HT_{1E}	AC(-)
5-ht_{1F}	AC(-)
5-HT_2	
5-HT_{2A}	PI
5-HT_{2B}	PI
5-HT_{2C}	PI
5-HT_3	Ion Channel
5-HT_4	AC(+)
5-HT_5	
5-ht_{5A}	?
5-ht_{5A}	?
5-HT_6	AC(+)
5-HT_7	AC(+)

2. 5-HT_3 Receptors

a) Structure and Distribution

5-HT_3 receptors, formerly known as "M" receptors, due to inhibition of their response to morphine, are in the Cys-loop family of ligand–gated ion channel receptors, along with nicotinic acetylcholine (nACh) receptors, γ-aminobutyric acid type A ($GABA_A$) receptors, and glycine receptors.[8,80] LGIC receptors, of the 5-HT_3-type, consist of five homopentameric subunits, each made of four transmembrane-spanning amino acid chains (M_1-M_4), which form a pore; the M_2 chain faces the pore.[78] This pore rapidly opens when neurotransmitter binds to the receptor, and is permeable to sodium,

potassium, and calcium cations.[81] M_1 through M_4 are connected by extracellular and intracellular loops, with both a carboxy and amino terminus, as seen in Figure 9.[78] Ion selectivity and gating of the receptor is controlled by residues found between M1 and M3 (i.e., M2).[8]

Homology-based models of the extracellular domain were previously based on the crystal structure of acetylcholine-binding protein (AChBP), however recent discoveries have identified the crystal structure of the extracellular domain of the mouse nicotinic acetylcholine receptor (nAChR) α1 subunit.[8,82-85] These receptors are found both in the peripheral and central nervous system. There currently are five different subtypes of 5-HT_3 receptors; A, B, C, D, and E, but 5-HT_{3A} and 5-HT_{3B} are the only two shown to form functional receptors.[86]

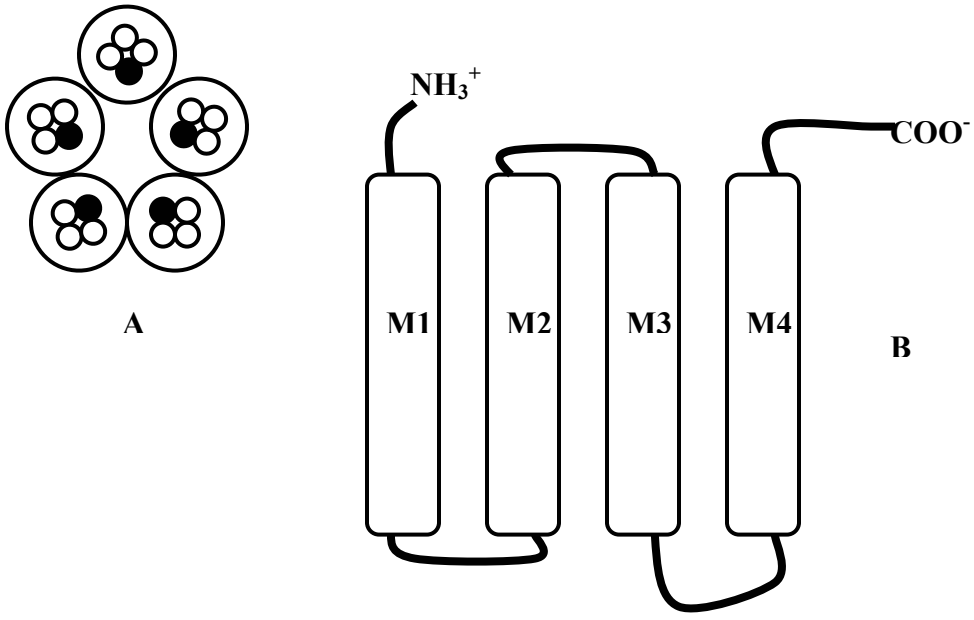


Figure 9. (A) Pore formed from five subunits, with M2 (shaded) facing the pore. (B) Four transmembrane-spanning amino acid chains.[78]

5-HT_3 receptors are found in both the peripheral nervous system, on the terminals of sympathetic, parasympathetic, and sensory neurons, and in the central nervous system.[9] In the central nervous system serotonin is produced in the raphe nuclei in the brainstem and then is able to project into the cortex, thalamus, basal ganglia, hippocampus, and amygdala.[87] Evidence suggests that 5-HT_3 receptors are presynaptic excitatory receptors allowing it to regulate the release of acetylcholine, dopamine, noradrenaline, cholecystokinin and serotonin.[9] Some studies have shown that 5-HT_3 receptor antagonists attenuate cocaine and amphetamine induced locomotor effects, suggesting that 5-HT_3 receptors modulate mesolimbic dopamine activity.[9] This modulation however is indirectly related to the increases in mesolimbic dopamine induced from psychomotor stimulants alone.[9] These effects are only observed with locomotor activity, and cannot be replicated with drug discrimination or self-administration studies, providing little information about abuse liability.[88-91] Evidence, however, does suggest that deficits in dopamine and serotonin neuronal function are observed with stimulant abuse withdrawal.[92] Since studies show there's an indirect correlation between 5-HT_3 receptor antagonists and mesolimbic dopamine activity, as well as evidence supporting that increased serotonin levels decrease withdrawal symptoms, stimulants could be working, at least in part, through a 5-HT_3 receptor mechanism.[9]

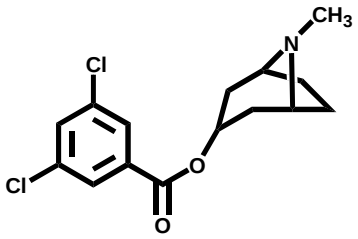
b) Function

5-HT_3 receptors themselves, as well as 5-HT_3 receptor antagonists, have a wide array of functions. Activation of 5-HT_3 receptors increases intracellular Ca^{2+}, modulates neurotransmitter release (dopamine and norepinephrine), excites central and peripheral neurons, and mediates emetic and inflammatory responses.[86,93] More specifically, the receptors are involved in dopamine and acetylcholine release, as well as control of the GABA-ergic system.[80,94] This ability to indirectly regulate dopamine is why 5-HT_3 receptors might be involved with drug dependence.[9] Not much is known about the function of 5-HT_3 receptor agonists, although emesis may be an occurring side effect, or partial agonists, which seem to portray an anxiolytic profile.[95] However, antagonists have been shown to relieve several types of ailments. Some 5-HT_3 receptor antagonists treat chemotherapy-induced or radiation-induced vomiting, and migraines.[96,97] Some are used in the treatment of anxiety, depression, pain, and dementia. Some 5-HT_3 receptor antagonists may suppress withdrawal symptoms in alcoholics, as well as nicotine-, cocaine-, and amphetamine-addicts.[94,96] Moreover, some are able to block the abuse-related effects of drugs.[9]

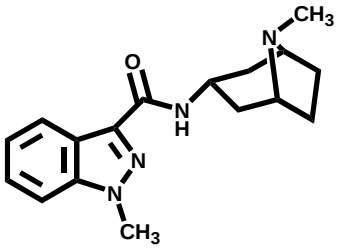
c) Antagonists

Hundreds of different 5-HT_3 receptor antagonists have been discovered since the initial selective 5-HT_3 receptor antagonist bemesetron (**15**; MDL 72222) was reported.[78] Bemesetron was formed by a slight structural modification to cocaine (a 5-HT-M receptor antagonist), by the removal of a carbomethoxy group, and addition of two

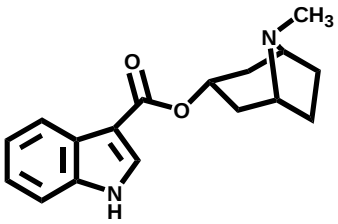
chlorine atoms.[78] Current antagonists fall into two groups, keto compounds and imidazoles. Some of the more common keto compounds include granisetron (**16**), tropisetron (**17**), and zacopride (**18**), as seen in Figure 10.[78] The imidazole-containing compounds (Figure 11) include ondansetron (**19**), alosetron (**20**), fabesetron (**21**), and ramosetron (**22**).[78] Granisetron (**16**), ondansetron (**19**), and tropisetron (**17**) are of special interest as they are highly selective and potent 5-HT_3 receptor antagonists used in the treatment of emesis associated with anticancer chemotherapy.[98] Several pharmacophore models have been reported for 5-HT_3 receptor antagonists.[99-104]



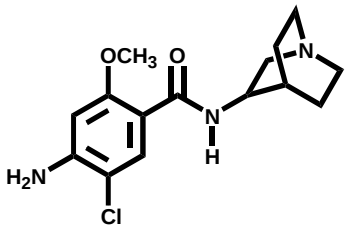
Bemesetron (**15**)



Granisetron (**16**)

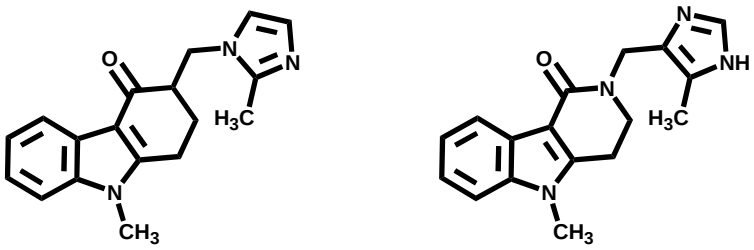


Tropisetron (**17**)



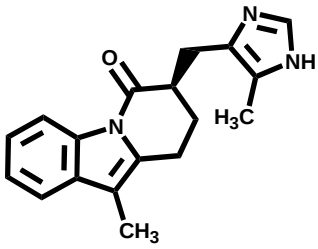
Zacopride (**18**)

Figure 10. Keto-group-containing 5-HT_3 receptor antagonists.[78]

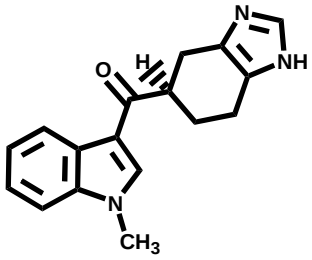


Ondansetron (**19**)

Alosetron (**20**)



Fabesetron (**21**)



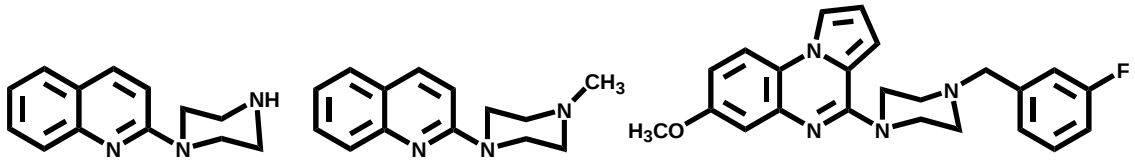
Ramosetron (**22**)

Figure 11. Imidazole-containing 5-HT_3 receptor antagonists.[78]

d) Agonists

Currently, there are not many known 5-HT_3 receptor agonists/partial agonists, however the few that exist fall into one of five categories: tryptamines, arylpiperazines, arylbiguanides, arylguanidines, and miscellaneous agents.[14] There is renewed interest in arylpiperazines which generally were initially considered non-selective for 5-HT_3 receptors, or could behave as 5-HT_3 receptor agonists, partial agonists, or antagonists.[78] Quipazaine (**23**; Figure 12) binds both at 5-HT_3 and 5-HT_{2A} receptors with a K_i value of around 1 nM.[105] However, it acts as an agonist in some assays and as an antagonist in others. Structure-activity studies have shown that appropriate structural modifications can result in more selective 5-HT_3 receptor agonists. For example, piperazine N_4-

methylation, creating N-methylquipazine (NMQ) (**24**; Figure 12) enhances the selectivity of the compound for 5-HT_3 receptors.[106] Also, ring-fusion creates the partial agonist MR 18445 (**25**; Figure 12).[78]

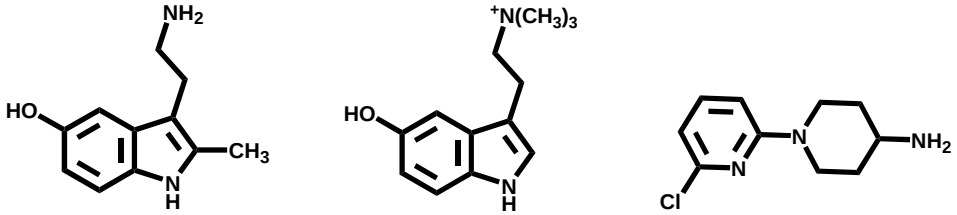


Quipazine (**23**) NMQ (**24**) MR 18445 (**25**)

Figure 12. A few examples of arylpiperazine 5-HT_3 receptor agonists and partial agonists.

Serotonin itself is a nonselective 5-HT_3 receptor agonist that does not bind with high affinity (K_i= ca 500-1,000 nM).[107] By methylating this structure to obtain 2-methyl-5-HT (**26**; Figure 13), a partial agonist, selectivity increases while binding affinity remains unchanged.[107] However, Glennon *et al.* showed that 2-methyl 5-HT (**26**) also binds to 5-HT_6 receptors with high affinity.[108] Structure-affinity studies identified a limited region of bulk tolerance at the terminal amine of serotonin for agonism at 5-HT_3 receptors. Subsequently, studies by our laboratory showed, using brain 5-HT_3 receptors, that N,N-dimethylated structure bufotenine (K_i= 280 nM) and the N,N,N-trimethylated structure 5-HTQ (**27**; Figure 13) (K_i= 75 nM), bind with higher affinity and selectivity than serotonin.[109] However, the quarternary structure of 5-HTQ (**27**) may prevent it from crossing the blood-brain barrier (BBB).[109] Previous structure-activity studies had shown

that di- and tri-methylation of serotonin resulted in potent serotonin agonists in a superior cervical ganglionic cell preparation.[110] Another potent and specific 5-HT_3 receptor agonist, that does not belong to the tryptamine-derived category is 4-amino-(6-chloro-2-pyridyl)-1piperidine hydrochloride, more commonly known as SR 57227A (**28**, Figure 13), which is a potent agonist that crosses the BBB.[111]



2-Methyl 5-HT (**26**) K_i ~ 1300 nM

5-HTQ (**27**) K_i ~ 75 nM

SR 57227A (**28**) K_i ~ 103-150 nM

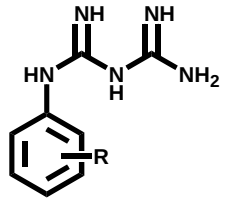
Figure 13. 5-HT_3 receptor agonists and partial agonists.[109,111]

Phenylbiguanide (**29**; Table 4) is a low affinity 5-HT_3 receptor agonist (K_i~1000 nM) that falls into the arylbiguanide classification.[112] Structure-affinity studies have shown that introduction of a chloro group at the 2-, 3-, or 4-position results in higher binding affinity, with *meta*-chlorophenylbiguanide (*m*CPBG) (**30**) having a K_i value of about 17 nM (Table 4).[11] Benz-fusion at the 3- and 4- positions of the phenyl ring as seen with the 2-naphthyl analog (i.e. **31**) mimics the effect of the 3-chloro group.[11,113] Improvements in affinity from the parent phenylbiguanide were observed with di- and tri-chloro substitution of the phenyl ring.[114] By adding an electron-withdrawing nitro group, a five-fold increase in affinity was observed versus the phenylbiguanide.[11,113] However,

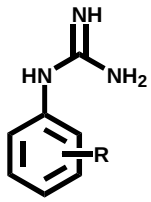
the addition of the electron-withdrawing 3-trifluoromethyl group resulted in lower affinity, around the 700 nM range; similar results were observed with the addition of a methyl substituent to phenylbiguanide.[11,113,114] The high affinity of the N-(2-phenylethyl)guanidine analog **52** supported the idea that the biguanide moiety was not essential for binding.[11] This led to a new series of structure-activity studies.

By shortening the biguanide chain, arylguanidines were created, with *m*-chlorophenylguanidine (*m*-CPG or MD-354) (**42**; Table 4) as a lead compound with a binding affinity at 5-HT_3 receptors of K_i = 35 nM.[11,113] The same types of structural modifications were made to the arylguanidines as in the arylbiguanides, which displayed similar results. For example, higher binding affinities were observed with di- and tri-chloro substituted phenyl rings (i.e. **48**-**50**; Table 4), and high binding affinity was observed upon benz-fusion as seen with the 2-napthyl analog (i.e. **43**; Table 4).[11,14,113] This information supports the concept that parallel structural changes result in parallel shifts in affinity. Also, affinity decreased with the addition of 3-methyl or 3-trifluromethyl substituents (i.e. **46**, **47**; Table 4).[11] The 3-trifluromethyl substituent is electron-withdrawing, which is seemingly favored, however it is much bulkier than the chloro-substituted analogs, which may result in its lack of binding affinity when added to phenylguanidine.[115] Lipophilic substituents are favored at the 4-position, but only up to a certain size, as wider lipophilic substituents decrease affinity.[115]

Table 4. Binding affinities of arylbiguanide and arylguanidine 5-HT_3 receptor agonist/partial agonist derivatives.[11,14,113-115]



29-39



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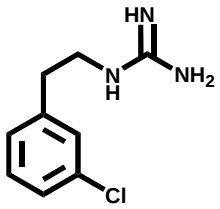


52

Aryl-biguanides	**Approximate K_i (nM)**	**R**	**Aryl-guanidines**	**Approximate K_i (nM)**
29	~1,000	H	**41**	2,340
30	17	3-Cl	**42**	35
31	12	3,4 fused phenyl	**43**	25
32	62	2-Cl	**44**	190
33	200	4-Cl	**45**	320
34	220	3-NO_2	-----	-----
35	780	3-CH_3	**46**	6,520
36	700	3-CF_3	**47**	5,700
37	0.4	2,3,5-Cl	-----	-----
38	12	3,4-Cl	**48**	3.1
39	1.8	3,5-Cl	**49**	5
40	2.7	3,4,5-Cl	**50**	0.7
-----	-----	3-CF_3, 4-Cl	**51**	36
-----	-----	-----	**52**	40

Few pharmacophore models have been proposed for 5-HT_3 receptor agonists/partial agonists, and none include all the different types of ligands. Glennon *et al*. proposed a pharmacophore model for ligands containing an indole moiety, stating that the distance from the aromatic centroid to the terminal amine is pertinent for binding.[116] Later, Yamada *et al.* proposed a 3-point pharmacophore model consisting of an aromatic region, an adjacent nitrogen atom, and a terminal amine.[117] However, a limitation to this model is that it only deals with 5-HT_3 receptor agonist binding at gut 5-HT_3 receptors.[117] Rault *et al.* proposed a more complex 5-point pharmacophore model using seven different structural classes of ligands.[95] This model consisted of two hydrogen bond acceptors, an aromatic moiety, a hydrophobic group, and an ionizable site that corresponds to the terminal amine.[95] However, none of these models accounts for the two major classes of 5-HT_3 receptor agonist ligands: arylguanidines and arylbiguanides.

A current working pharmacophore model (Figure 14) proposed by Dukat accounts for binding of arylbiguanides and arylguanidines. This model consists of N_1

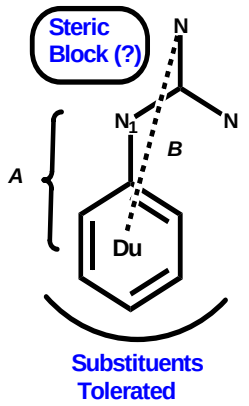


Figure 14. Current working pharmacophore model for 5-HT_3 receptor agonists and partial agonists.[14]

which is located 2.7Å from an aryl centroid (Du) (i.e. *A*), and a terminal amine located 4.5-4.9Å from Du (i.e. *B*).[14] All three guanidine nitrogen atoms are believed to be required, with limited substitution; the N_1 position contains a steric block.[14] Lastly, there is a region where affinity can be enhanced with substituents at the *meta* and *para* positions.[14] However, this model might not account for rotameric binding, since *meta*-substitution is preferred and two *meta* positions are present: the 3- and 5- position.[115] Further studies are required to resolve this problem.

E. Quantitative Structure-Activity Relationships

Pharmacophore models are extremely helpful in predicting the behavior of new molecules. Although, they are in fact, "just a model", information from a pharmacophore model can be helpful in the design of new ligands for particular receptors. The correlation between the biological activities of drugs with their physiochemical properties is known as quantitative structure-activity relationships (QSAR), which is employed to create pharmacophore models.[118] Methods used to describe structure-activity relationships include comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA).

In general, development of pharmacophore models usually separates agonists and antagonists, as agonists generally stabilize the active conformation of the receptor, whereas antagonists stabilize the inactive conformation.[118] Therefore, by placing both agonists and antagonists into the same receptor model, the predictability of the model might be low due to opposing biological factors.

In order to create a pharmacophore model using either CoMFA or CoMSIA, a "training set" of ligands with known biological activity is aligned within a fixed lattice.[119] This set should exemplify all types of ligand substituents equally (e.g. electron-withdrawing groups, electron-donating groups, bulky substituents), in all possible substitution locations. Once aligned, a partial least squares analysis is conducted to correlate the field values with biological data, usually binding affinity.[118] The "fit" of the binding affinity values is expressed by the squared correlation coefficient, r^2 which usually is high if the ligands are well aligned.[118] The predictability of the model is determined through cross-validation, and is expressed as q^2.[118] Predictability values of 0.6 and above exemplify a "good model", which can be used to predict the binding affinity of new receptor ligands.[119] The higher the q^2 value, the better the model should be at predicting the binding affinity of new ligands. The quality of this model can be verified using a test set of compounds not included in the training set, but for which biological data are known. If predicted binding affinities are similar to the actual binding affinities of test set compounds, then this further validates the predictability of the model.[118]

Other than predicting the binding affinity of new receptor ligands, QSAR models can assist in the design of new ligands. This is due to the fact that CoMFA and CoMSIA studies provide important information about the location of favorable and unfavorable substituents. For example, CoMFA studies provide information to where electrostatic fields are favorable or unfavorable, as well as to favorable and unfavorable steric bulk regions.[119] Once again, this information is based solely on ligands used in the training

set employed to create the model. CoMSIA studies also give information about the electrostatic and steric regions as well as hydrophobic, H-bond donor, and H-bond acceptor regions that are favorable or unfavorable.[120] With this information, the binding affinity of new compounds can be predicted, or novel ligands can be designed through analysis of favorable regions.

F. Behavioral Assays

There are several different rodent behavioral studies used to characterize stimulant drugs including drug discrimination, self-administration, and locomotor activity studies. Stimulants can act as discriminative stimuli in a drug discrimination (DD) paradigm from which information can be obtained on a drug's duration of action, time of onset, mechanism of action, potency, and structure-activity relationships.[121] Self-administration studies are used to determine the reinforcing efficacy of the drug.[122] Since it is already known that behavioral stimulants have a central stimulatory effect which results in changes in motor activity, locomotor activity assays are used to evaluate this change.

Locomotor activity assays are conducted in square transparent chambers surrounded by infared photo detectors; one bank of detectors measures activity at the plane of the floor, whereas another is positioned centimeters above the floor to measure vertical activities.[123] These measure the coordinates of an animal's location, as well as the type of motion.[123] Stimulants commonly increase motor activity such as the amount of movement. Some commonly analyzed parameters induced by stimulants include

movement time, distance, velocity, and episodes. Movement time is the sum of time of all movements in the floor plane as measured in seconds.[123] For example, if mouse movements were analyzed for 2700s, but the animal only moved a total of 1200s, the latter would be considered its movement time.[123] Movement distance is the sum of all vector coordinate changes in the floor plane, and movement episodes are total movements in the floor plane.[123] So, if a mouse continues to walk for one minute, then stops, this equates to one movement episode, whereas every centimeter the mouse walked in that one episode is counted in movement distance.[123] An average of the movement time and movement distance can be calculated as velocity (cm/min), which is considered the average speed of floor-plane coordinate-change defined movements.[123] Typical results for stimulants include increases in movement distance and movement time, but a decrease in movement episodes. These same parameters (time, distance, and episodes) can be applied to vertical entries, more commonly known as "rearing", which includes entries in the vertical plane that activate the upper infared photo-detectors.[123]

Some stimulant parameters that give insight into anxiolytic versus anxiogenic activity are margin distance, margin time, center distance, center time, and center entries. Margin distance is the same as movement distance, but is only calculated within a 2.5-beam-margin-of-space toward the interior walls.[123] Margin time is the amount of time spent within this same margin of space.[123] Mice that tend to stay near the margin display thigmotaxis, which can be related to either anxiety or agoraphobia.[124,125] Center distance and center time are the distance, in centimeters, and time, in seconds, spent in the center arena.[123] Center episodes include the number of times the mouse enters the center arena,

characterized by anything outside of the 2.5-beam-margin-of-space.[123] The more time spent near the margins is characterized as an anxiogenic-like effect as the mouse is thought to be more fearful.[123] When the opposite occurs, where the mouse spends more time in the center of the chamber, the animal is thought to be displaying anxiolytic-like character.[123]

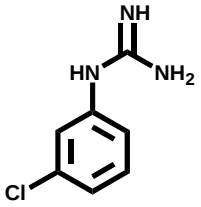
As in most behavioral assays, the actions of a test drug (i.e., stimulant) are compared to the actions induced by saline. All drug administration parameters remain the same such as duration of test, route of administration, pre-injection time, as well as habitat conditions. When the study is complete, the results are analyzed using a one-way analysis of variance (ANOVA) test on the mean response of each parameter, followed by a post-hoc test to determine statistical significance.[123] Results from many stimulant studies result in an inverted U-shaped function, due to the fact that locomotor activity increases as stimulant dose increases; however, after a certain dose, the effect either remains steady or decreases.[126] This type of behavioral assay should provide insight into the effects of stimulants as well as other drugs on mouse locomotor activity.

III. Specific Aims

Psychomotor stimulants such as (+)amphetamine (**2**), (+)methamphetamine (**3**), and cocaine (**1**) increase dopamine levels which, in turn, can increase locomotor activity when administered to rodents. However, recent studies have shown that activation of serotonin systems, more specifically 5-HT_3 receptors, may modulate dopamine release. 5-HT_3 receptor agonists have been shown to release dopamine in the striatum and nucleus accumbens.[127,128] 5-HT_3 receptor antagonists such as tropisetron (**17**; previously known as ICS 205-930), zacopride (**18**), and MDL 72222 (**15**) attenuate stimulant parameters when co-administered with acute treatments of cocaine in locomotor activity studies using rats.[129] The 5-HT_3 receptor antagonist ondansetron (**19**) was able to attenuate the effects of chronic treatment of cocaine and acute treatment of amphetamine on hyperlocomotion.[130,131] Similar results were observed using mice with acute treatments of cocaine in combination with tropisetron (**17**) and zacopride (**18**).[132] These data, as well as other studies, suggest that 5-HT_3 receptor agonists might indirectly increase dopamine levels, whereas 5-HT_3 receptor antagonists decrease dopamine levels, in different areas of the brain.[9]

MD-354 (**42**; Figure 15) is a 5-HT_3 receptor partial agonist that binds at 5-HT_3 receptors with rather high affinity (K_i = 35 nM).[11] MD-354 (**42**) behaves both as an

agonist and antagonist in different assays, with antagonist activity being more commonly associated with higher doses, such as in antagonizing cisplatin-induced emesis.[133]



MD-354 (**42**)

Figure 15. Structure of the arylguanidine *m*CPG, more commonly known as MD-354.

One purpose of the present studies was to determine the effects of MD-354 (**42**) on the locomotor actions of psychomotor stimulants; that is, might MD-354 potentiate (or antagonize) the locomotor effects of (+)amphetamine? Previous data have shown that MD-354, at doses of 1.0-10 mg/kg, does not produce a statistically significant difference on any locomotor parameter.[12] When administered alone, MD-354 behaves like saline. But, if in combination with psychomotor stimulants it potentiates the drug's locomotor effects, MD-354 might be used as a form of drug replacement therapy. Drug replacement therapy consists of combination treatment to wean a patient off the abused drug by decreasing the dose of the drug of abuse while maintaining its positive effects due to its combination with another non-stimulant drug.

Behavioral effects of MD-354 (**42**), as well as of several stimulants, will be analyzed using a mouse locomotor activity assay. (+)Amphetamine (**2**) and

(+)methamphetamine (**3**) are phenylalkylamine stimulants with similar mechanisms of action. Therefore, results should be similar, except that methamphetamine is a more potent stimulant. These data will be compared to those obtained with cocaine (**1**), a non-phenylalkylamine stimulant with a different mechanism of action (reuptake blocker, cocaine, versus substrate releaser). DOM (**10**) will be used as control, because it is structurally similar to amphetamine, but is a non-stimulant phenylalkylamine hallucinogen.

Dose response curves will be obtained for each drug to determine an effective dose, which is a dose that produces a statistically significant effect versus saline. An effective dose will be used in combination studies with varying doses of MD-354 (**42**). The preinjection times, as well as the recording-time of the assays, will be determined based on each drugs' known onset of action and duration of action determined from literature data. Statistical analysis will be performed on commonly used stimulant parameters such as movement distance, movement time, and movement episodes, as well as other informative parameters (center distance, center time, center entries, margin distance, margin time, and vertical entries). Potentiation of stimulant parameters (i.e., an increase in movement distance and movement time or a decrease in movement episodes) when MD-354 is administered in combination with an effective stimulant dose, would suggest that MD-354 (**42**) is working through an agonist mechanism. However, attenuation of stimulant parameters would indicate an antagonist mechanism of action.

To further support or refute the determined mode of action of MD-354 (**42**), a combination study of (+)amphetamine (**2**) and the 5-HT_3 receptor antagonist ondansetron

(**19**) will be performed. Since MD-354 is a partial agonist, it may behave as 5-HT_3 receptor agonist, which should increase dopamine levels (increasing locomotor activity), or as a 5-HT_3 receptor antagonist, which could have the opposite effect. If the results of a combination study of a 5-HT_3 receptor antagonist (e.g. ondansetron) with (+)amphetamine are similar to those obtained with MD-354 (**42**) in combination with (+)amphetamine (**2**), then MD-354 could be working through a 5-HT_3 receptor antagonist mode of action. However, if results differ from what is shown in the combination of MD-354 (**42**) with the stimulants, then MD-354 may be working through a 5-HT_3 receptor agonist mechanism.

This idea will further be evaluated by conducting a similar study using a 5-HT_3 receptor agonist, SR 57227A (**28**); a combination study of (+)amphetamine (**2**) and SR 57227A will be performed. If the results of the combination study are similar to those observed using (+)amphetamine in combination with MD-354 (**42**), then MD-354 may be working through a 5-HT_3 receptor agonist mechanism, which should further support or refute the data found in the combination study of ondansetron with (+)amphetamine.

Since the phenylalkylamine stimulants (+)amphetamine (**2**) and methamphetamine (**3**) work through a different mechanism of action than the non-phenylalkylamine stimulant cocaine (**1**), similar results from combination studies with MD-354 (**42**) may or may not be observed. This could be due to differences in mechanism of action, instead of the overall effect of increased synaptic dopamine levels. The data from these studies will be compared to the combination studies of cocaine with MD-354 to determine if MD-354 is working through a 5-HT_3 receptor agonist or 5-HT_3

receptor antagonist mechanism of action. This will be compared to the effects obtained in combination studies with amphetamine to determine if MD-354 behaves similarly with phenylalkylamine stimulants as with non-phenylalkylamine stimulants (which work through different mechanisms of action).

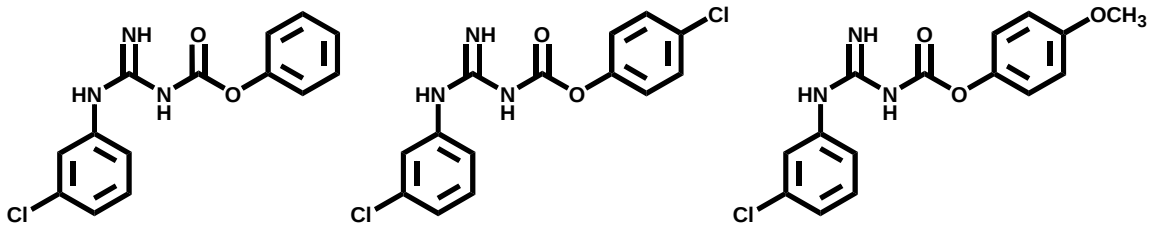
Combination studies will also be conducted using the 5-HT_3 receptor agonist SR 57227A (**28**) in combination with cocaine as well as the 5-HT_3 receptor antagonist ondansetron (**19**) in combination with cocaine to determine if MD-354 (**42**) is working through a 5-HT_3 receptor agonist or antagonist mechanism. This study will help determine whether MD-354 is behaving similarly in combination with cocaine as with (+)amphetamine. Differences in the two studies once again could be associated with the different mechanisms of action of the two psychomotor stimulants.

Since DOM (**10**) is a hallucinogen instead of a stimulant, locomotor activity parameters such as movement distance or movement time might not increase following DOM administration. However, consistent with what is known about hallucinogens,[3] DOM might increase vertical entries ("rearing").

MD-354 does not produce a locomotor stimulant effect by itself.[12] Either it is devoid of such action or MD-354 may not penetrate the BBB (i.e., it is assumed that a central action is responsible for locomotor stimulation). MD-354 has a Log P value of -0.64 which may prevent it from crossing the blood-brain barrier.[13] Compounds with low Log P values such as MD-354 may cross the BBB, however BBB penetration is more commonly observed with compounds having a Log P value between 1.5-2.5.[134] Therefore, a more lipophilic compound, the methyl carbamate analog of MD-354 (**42**),

will be synthesized and tested as a prodrug.[135] Data from pharmacological assays suggested that the methyl carbamate analog of MD-354 acted similarly to MD-354.[12] This could be a result of insufficient lipophilicity, causing difficulty in crossing the BBB, or that hydrolysis to MD-354 (**42**) occurred before it was able to cross the BBB.[135] Therefore, another purpose of the present study is to synthesize more lipophilic carbamate analogs of MD-354 that might act as prodrugs of MD-354 (Figure 16). These carbamate analogs include the phenyl carbamate **53**, the phenyl carbamate with an electron-withdrawing group, 4-chlorophenyl carbamate **54**, and the phenyl carbamate with an electron-donating group, 4-methoxyphenyl carbamate **55**. Although the Log P values of these three carbamates are currently unknown, because they have yet to be prepared, they can be predicted; using conversion ratios with the predicted Log P value of MD-354 versus its known Log P value (octanol/water), the predicted Log P values of the phenyl carbamates can be compared to MD-354. The phenyl carbamate was predicted to have a Log P value of 1.62 which is greater than that of MD-354 (**42**) and falls within the range of values which usually allows an agent to cross the BBB. Addition of the electron-withdrawing chloro-group to the phenyl carbamate **54** increases lipophilicity, with a predicted Log P value of 1.83. The addition of the electron-donating methoxy-group to the phenyl carbamate (i.e., **55**) decreases lipophilicity slightly (Log P = 1.56) from the phenyl carbamate **53**. Since all three of the carbamates had predicted Log P values in the range of 1.5-2.5, they should all cross the BBB, as they are predicted to be more lipophilic than MD-354. These three carbamates should also display differences in

rates of hydrolysis based on differences in hydrolytic stability due to the addition of electron-donating and electron-withdrawing groups to the phenyl carbamate **53**.



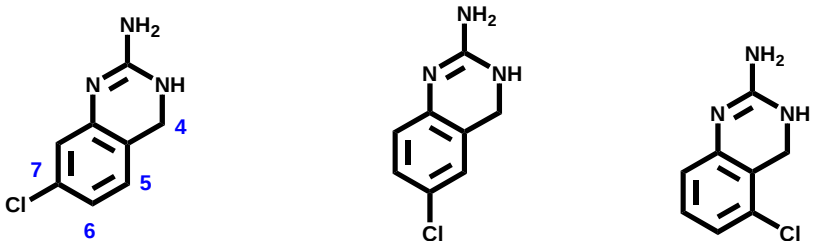
53 **54** **55**

Figure 16. Three proposed carbamate analogs of MD-354.

Once synthesized, the carbamates will be tested in rodent locomotor activity assays. These carbamates should be more lipophilic than MD-354 (*vide supra*), should more readily penetrate the BBB than MD-354 because of their increased lipophilicity, and should be hydrolyzed to MD-354 in vivo by brain tissue esterases. Thus, if MD-354 possesses latent stimulant properties but simply does not penetrate the BBB, then more lipophilic carbamates might produce locomotor stimulation.

In addition to the synthesis of the carbamate analogs, a conformationally constrained analog of MD-354 (**42**) will be synthesized. Previous literature reported the synthesis of 2-amino-7-chloro-3,4-dihydroquinazoline (**56**);[13] however, a review of available data suggested that the structure may have been misassigned and was actually

the 6-chloro analog (i.e., **57**). Therefore, 2-amino-6-chloro-3,4-dihydroquinazoline (**57**) will be synthesized and compared to the earlier sample for clarification of structure.



56 **57** **58**

Figure 17. Conformationally constrained analogs of MD-354.

Once the correct structure (i.e., **56** or **57**) is identified, this constrained analog as well as 2-amino-5-chloro-3,4-dihydroquinazoline (**58**)[135] will be used to further develop and test our current working pharmacophore model for 5-HT_3 receptor agonists and partial agonists (as shown in Figure 14). The use of conformationally-constrained analogs in the working pharmacophore model is necessary to account for rotameric binding. Some arylguanidine and arylbiguanide analogs contain substituents at the *meta*-position. However, it is not known at which *meta* position the substituents are located: the 3-position or the 5-position. This is due to the fact that rotameric binding might occur between the N_1-position of the guanidine moiety and the phenyl ring (Figure 14). By examining conformationally-constrained analogs, rotameric binding can be accounted for, indicating at which *meta*-position a chloro group is more favorable for binding.

A final goal of this work is to examine the quantitative structure-activity relationships (QSAR) for the binding of arylguanidines and arylbiguanides at 5-HT_3 receptors. As well as the conformationally-constrained analogs, about 40 other arylguanidine and arylbiguanide analogs will be employed as the training set. This training set will be aligned and analyzed using Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA). Results will be validated using a test set of five compounds not included in the training set, but for which binding data are available. These studies will attempt to identify regions favorable for steric, electrostatic, and hydrophobic interactions. This information will be used to predict the binding affinity of five new analogs that have been synthesized in our laboratories, binding data for which are not yet available.

The overall focus of these studies is to determine the effect of MD-354 (**42**) on psychomotor stimulants, to synthesize phenyl carbamate analogs of MD-354, and evaluate them in the mouse locomotor activity assay in order to determine whether or not MD-354 penetrates the BBB and lacks stimulant effect or possesses stimulant-like activity but does not cross the BBB. Another goal is to prepare a conformationally-constrained arylguanidine and conduct QSAR studies to further develop our current working pharmacophore model for 5-HT_3 receptor ligands.

IV. Results and Discussion

A. Behavioral Studies

1. Results

a) MD-354 (42)

Our laboratory has previously shown that, when administered alone, MD-354 (**42**) produces saline-like effects in the mouse locomotor activity assay.[12] In the present investigation, to more thoroughly document this effect, locomotor activity assays were conducted using i.p. injection doses of 1.0, 3.0, 6.0, and 10 mg/kg of MD-354 (**42**) with a 30-min pre-injection time, and a 45-min recording-time, as well as a 0-min pre-injection time and a 1-h recording-time. Current data are in agreement with, and extend, that in the literature; MD-354 (**42**) did not show a statistically significant difference versus saline in the measured stimulant parameters [e.g. movement episodes, movement time (Figure 18), movement distance, or vertical entries (data not shown)].

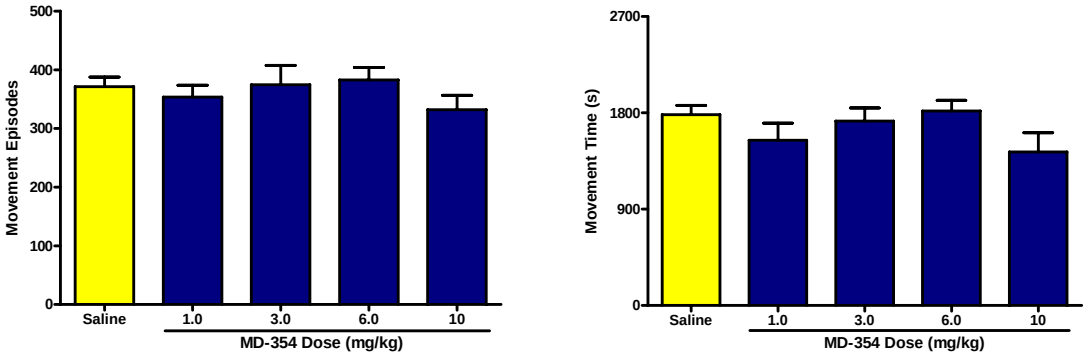


Figure 18. Effect (± S.E.M.) of MD-354 (**42**) (30-min pre-injection time) on total movement episodes and total movement time with a 45-min recording-time (n = 6 mice/treatment).

Other parameters analyzed, such as margin time (Figure 19) and center time (Figure 19), as well as, margin distance, center distance, or center entries (data not shown) also lacked a statistically significant difference versus saline. It is important to note that mice injected with MD-354 (**42**) or saline typically spent more time around the margin of the chamber than in the center as shown in Figure 19. These data support the notion that mice normally display as much anxiogenic-like activity in the presence of MD-354 as they do following administration of saline alone.

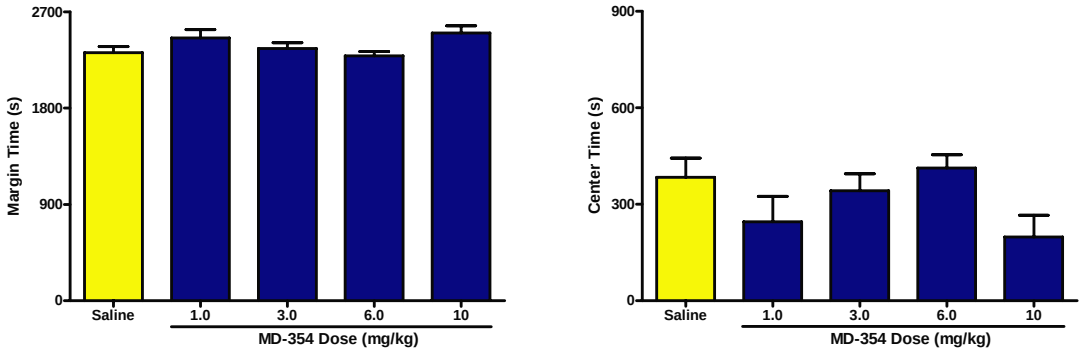


Figure 19. Effect (± S.E.M.) of MD-354 (**42**) (30-min pre-injection time) on total margin time and total center time with a 45-min recording-time (n = 6 mice/treatment).

b) Ondansetron (19)

MD-354 is a 5-HT_3 receptor partial agonist that can display both agonist and antagonist character (*vide supra*). The purpose of the current study was to determine the effect of MD-354 (**42**) on the locomotor effects of psychomotor stimulants. To further characterize the function of MD-354 (**42**), a 5-HT_3 receptor agonist and a 5-HT_3 receptor antagonist were used in combination with psychomotor stimulants. Comparison of the results from a combination of MD-354 with stimulants, to that of SR 57227A (**28**), a 5-HT_3 receptor agonist, and ondansetron (**19**), a 5-HT_3 receptor antagonist, with stimulants, should provide insight about the function of MD-354 (**42**). Therefore, dose-response studies were conducted with ondansetron (**19**) and SR 57227A (**28**), alone, and in combination with the stimulants amphetamine (**2**) and cocaine (**1**).

The present study analyzed the locomotor activity of the 5-HT_3 receptor antagonist ondansetron (**19**) at doses 0.1, 0.5, and 1.0 mg/kg. The protocol remained the same, with a 30-min pre-injection time followed by a 45-min recording-time. When administered alone, ondansetron (**19**) produced saline-like effects on stimulant parameters (movement episodes, movement time, movement distance, and vertical entries as shown in Figure 20) as well as on non-stimulant parameters (margin distance, margin time, center distance, center time, and center entries, as shown in Figure 21), which was consistent with literature data indicating that ondansetron lacks central stimulant action when administered via the i.p. route.[136]

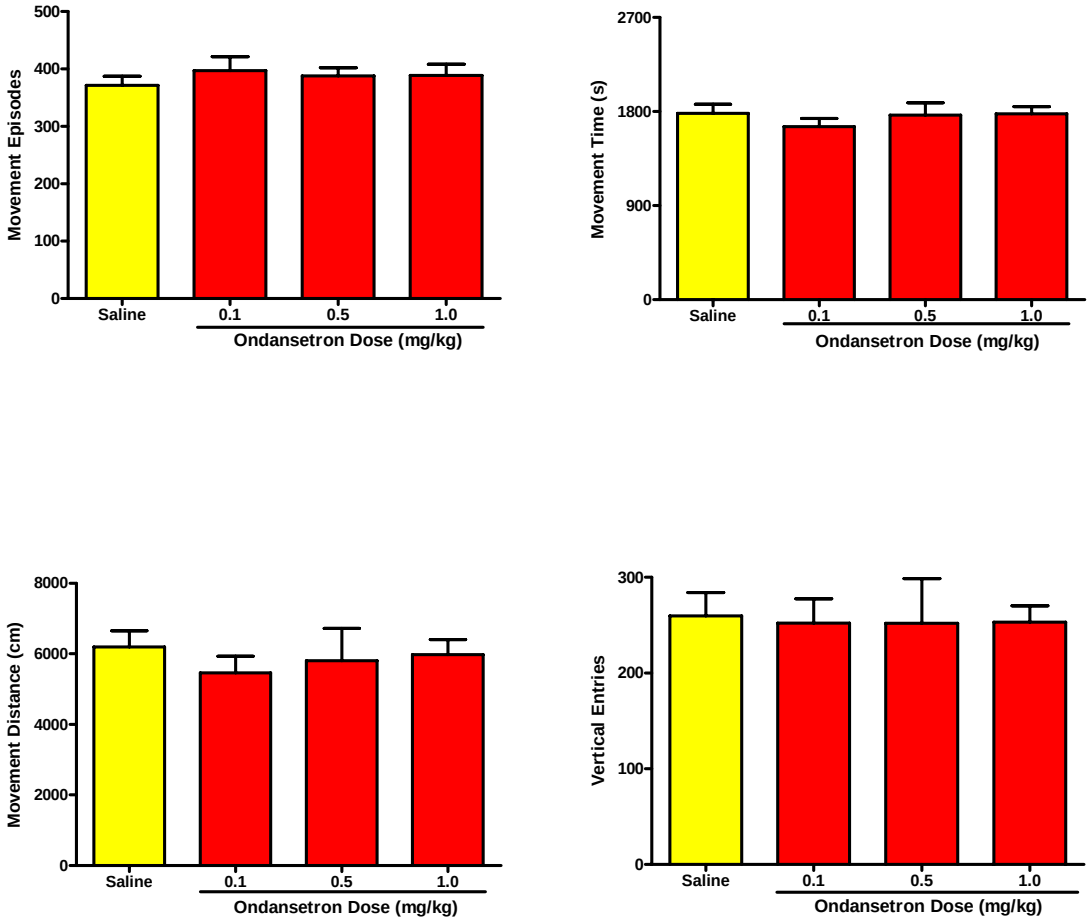


Figure 20. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) on total movement episodes, total movement time, total movement distance, and vertical entries versus saline with a 45-min recording-time (n = 6 mice/treatment).

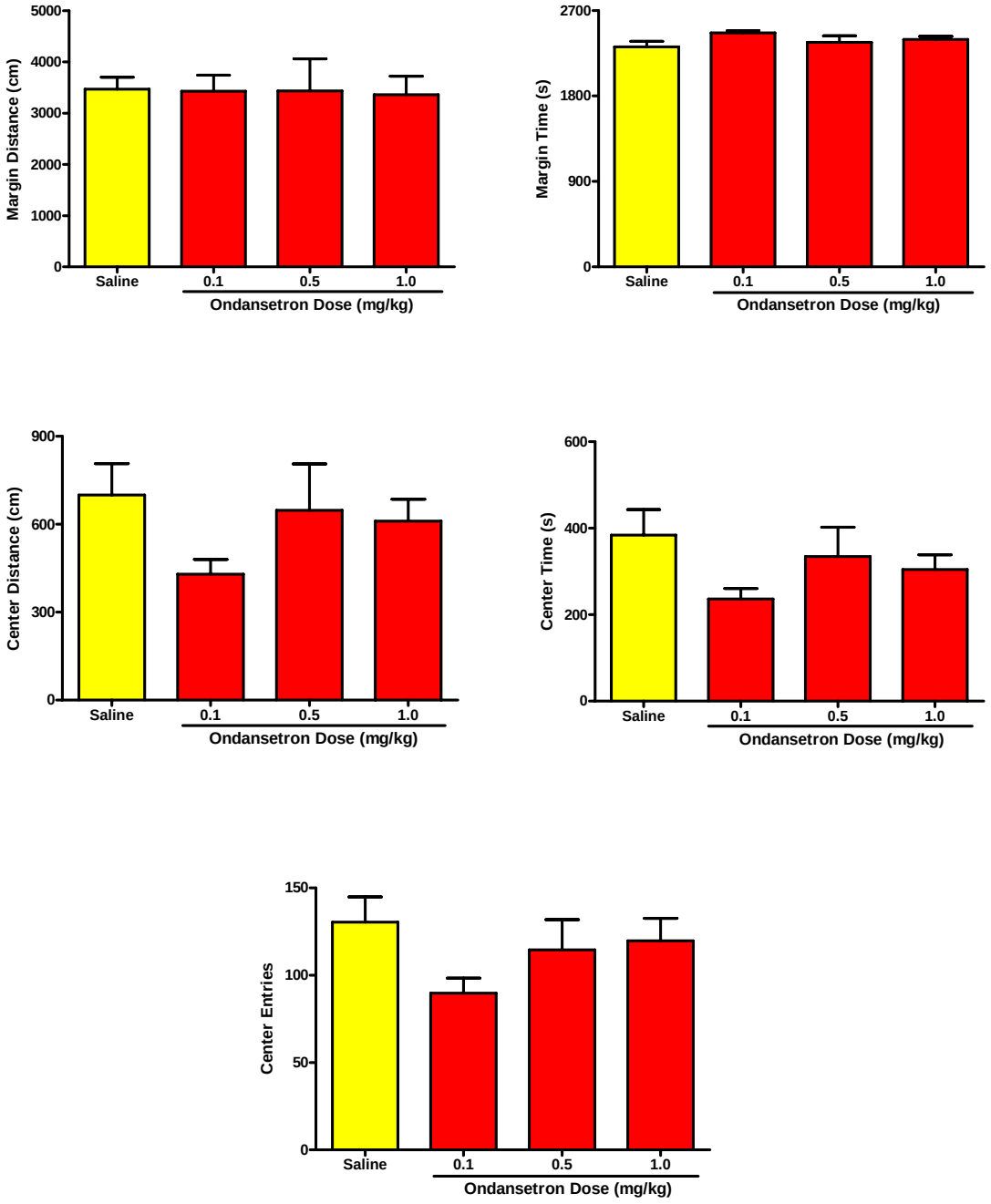


Figure 21. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) on total margin distance, total margin time, total center distance, total center time, and total center entries versus saline with a 45-min recording-time (n = 6 mice/treatment).

c) SR 57227A (28)

Locomotor activity assays were conducted with various doses of the agonist SR 57227A (**28**) (1.0, 3.0, and 10 mg/kg) which were injected i.p. 30 min prior to the test with a recording-time of 45 min. The same parameters were analyzed as with MD-354 (**42**) with similar results; SR 57227A (**28**) produced saline-like effects as shown in Figure 22. Analysis of center entries using a t-test instead of one-way ANOVA showed that SR 57227A (**28**) had a statistically significant effect versus saline at a 1.0 mg/kg dose, as SR 57227A suppressed center entries, suggesting that SR 57227A displayed anxiogenic-like behavior in mice (Figure 23). Even though SR 57227A doses were not statistically significant versus saline for either center distance or center time, the observed effect occurred in a dose-responsive manner, as a 1.0 mg/kg dose of SR 57227A was indicative of the upward slope of a dose-response curve.

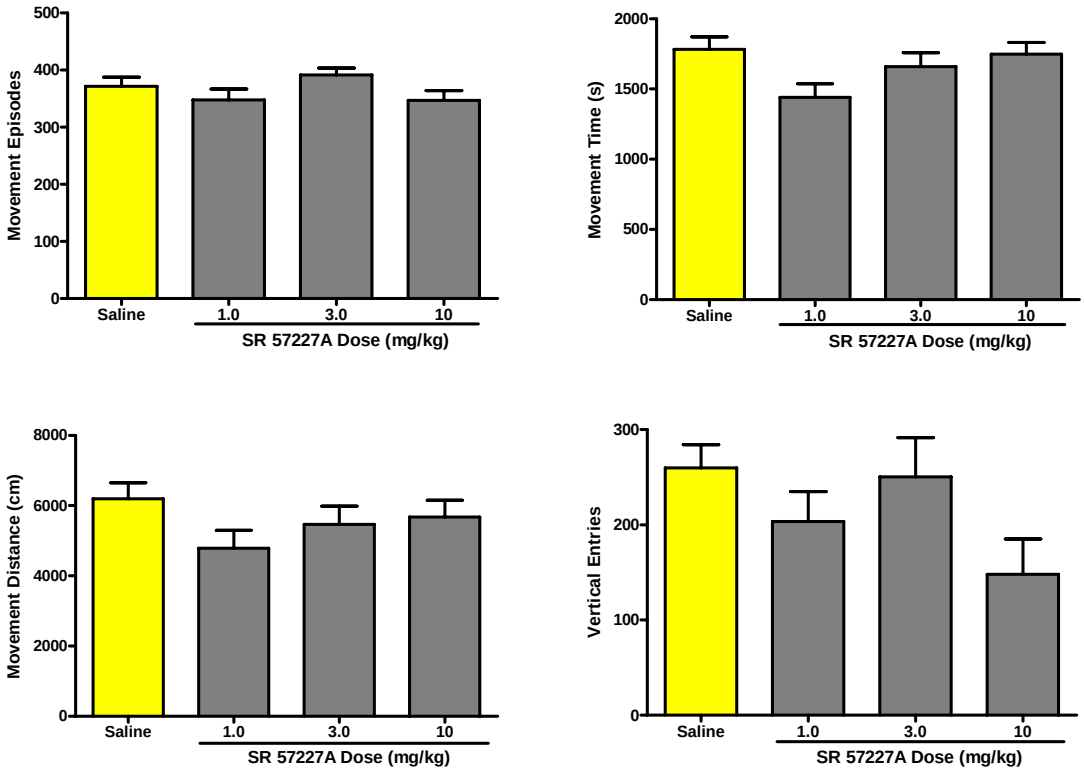


Figure 22. Effect (± S.E.M.) of varying doses of SR 57227A (**28**) (30-min pre-injection time) on total movement episodes, total movement time, total movement distance, and total vertical entries versus saline with a 45-min recording-time (n = 7 mice/treatment).

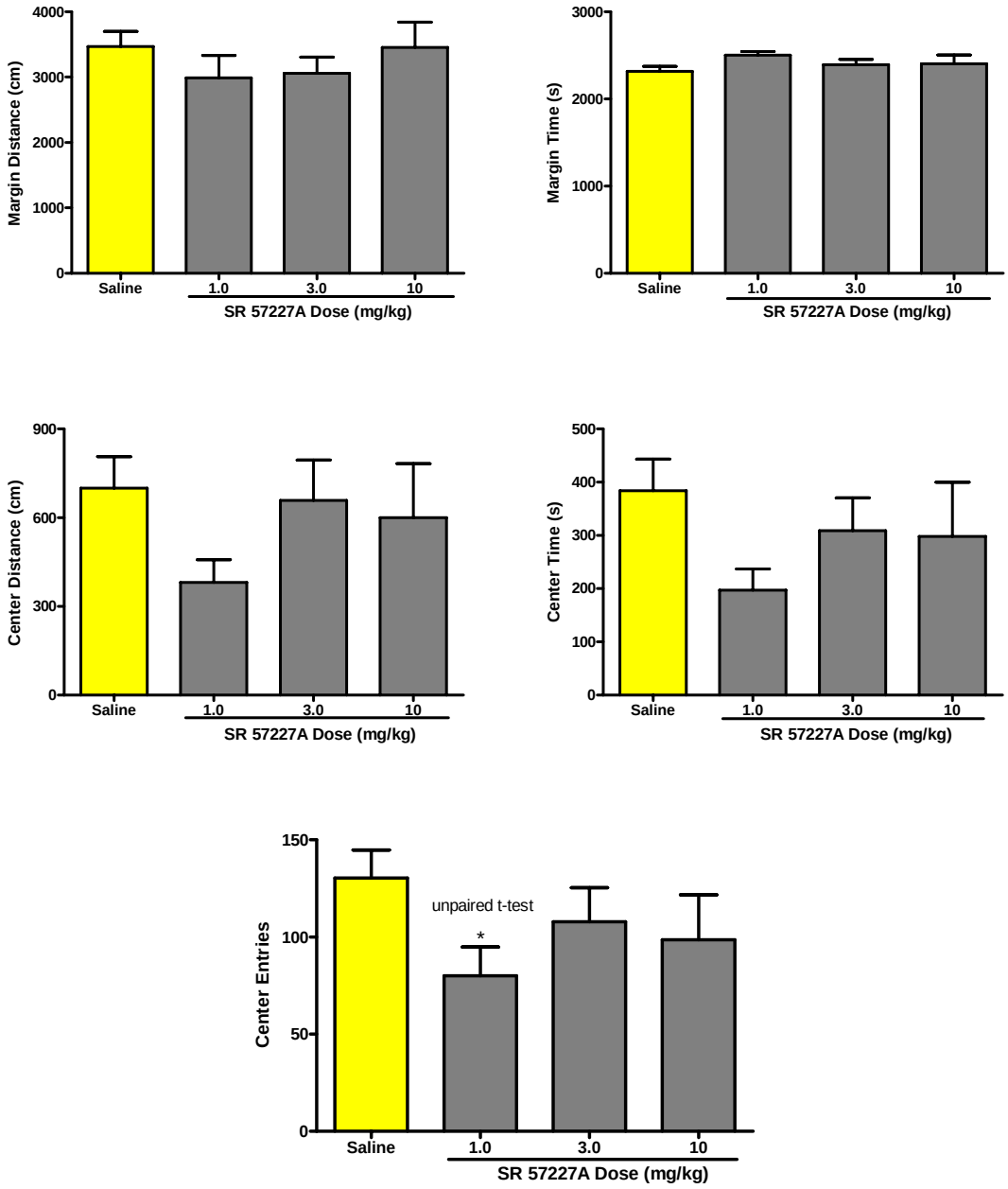


Figure 23. Effect (± S.E.M.) of varying doses of SR 57227A (**28**) (30-min pre-injection time) on total margin distance, total margin time, total center distance, total center time, and total center entries versus saline with a 45-min recording-time (n = 7 mice/treatment). Asterisk denotes statistical significance compared to the saline control group; *P<0.05; t-test.

d) (+)Amphetamine (2)

i. Dose Response

A locomotor activity assay was performed with varying doses of (+)amphetamine (**2**) to determine an effective dose (i.e., a dose that produces a statistically significant effect versus saline). In the present investigation, (+)amphetamine doses of 0.3, 1.0, 2.0, 3.0, and 6.0 mg/kg were injected to the mice, which were immediately placed into the chamber (0-min pre-injection time) with a recording-time of 45 min. After 15 min, (+)amphetamine (**2**) doses of 3.0 and 6.0 mg/kg produced a statistically significant stimulant effect on movement episodes, movement time, and movement distance (Figure 24). Similar effects were observed for the entire duration of the experiment.

Upon analyzing other parameters, it was observed that (+)amphetamine (**2**) produced no statistically significant difference versus saline on vertical entries, margin time, center distance, center time, and center episodes (Figure 25). However, doses of 3.0 and 6.0 mg/kg of (+)amphetamine statistically significantly increased margin distance during the entire recording-time as shown in Figure 24.

In order to visualize the stimulant effect of an effective dose of (+)amphetamine (**2**), a picture of the actual movements made by the animals following a 3.0 mg/kg dose of (+)amphetamine versus saline is shown in Figure 26.

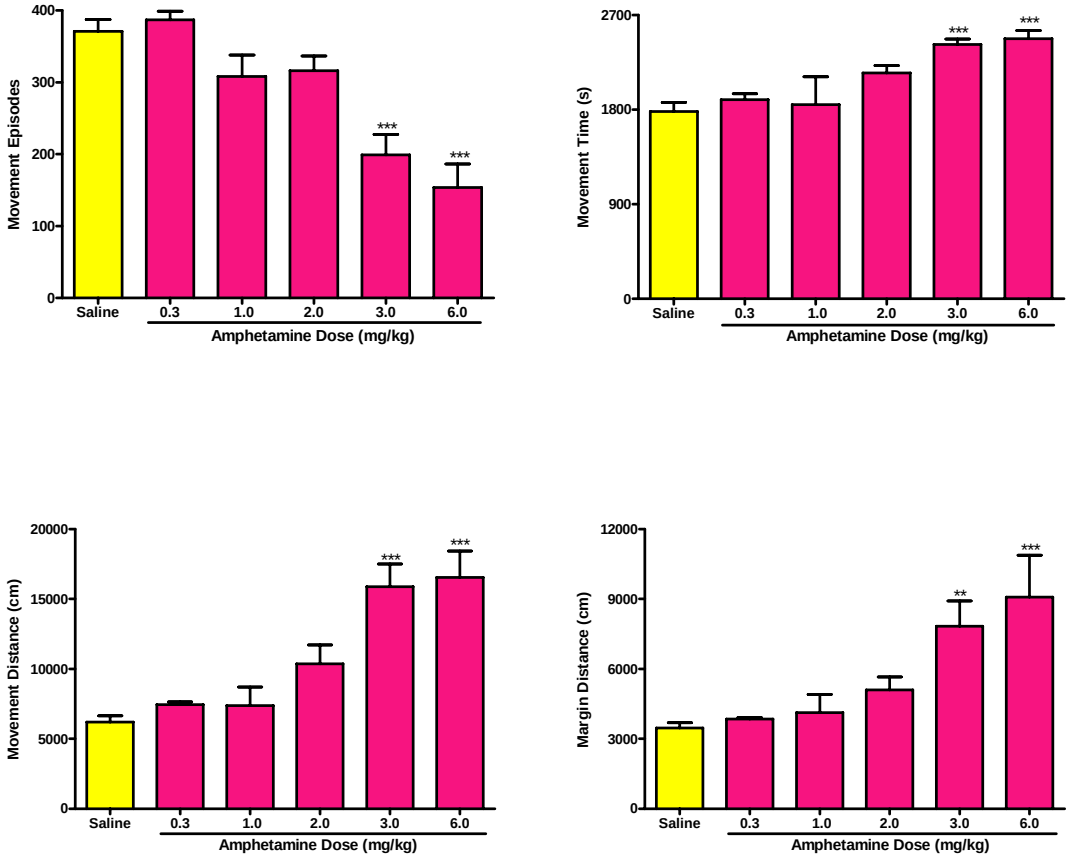


Figure 24. Effect (± S.E.M.) of (+)amphetamine (**2**) injected 0 min prior to examination on total movement episodes, total movement time, total movement distance, and total margin distance with a 45-min recording-time (n = 6-8 mice/treatment). Asterisk denotes a significant difference compared to the saline control group; $^{**}P<0.01$, $^{***}P<0.001$; one-way ANOVA ($F_{5,43}$ = 15.46 (movement episodes), $F_{5,43}$ = 7.44 (movement time), $F_{5,43}$ = 7.44 (movement distance), $F_{5,43}$ = 8.05 (margin distance)) followed by a Newman-Keuls post-hoc test.

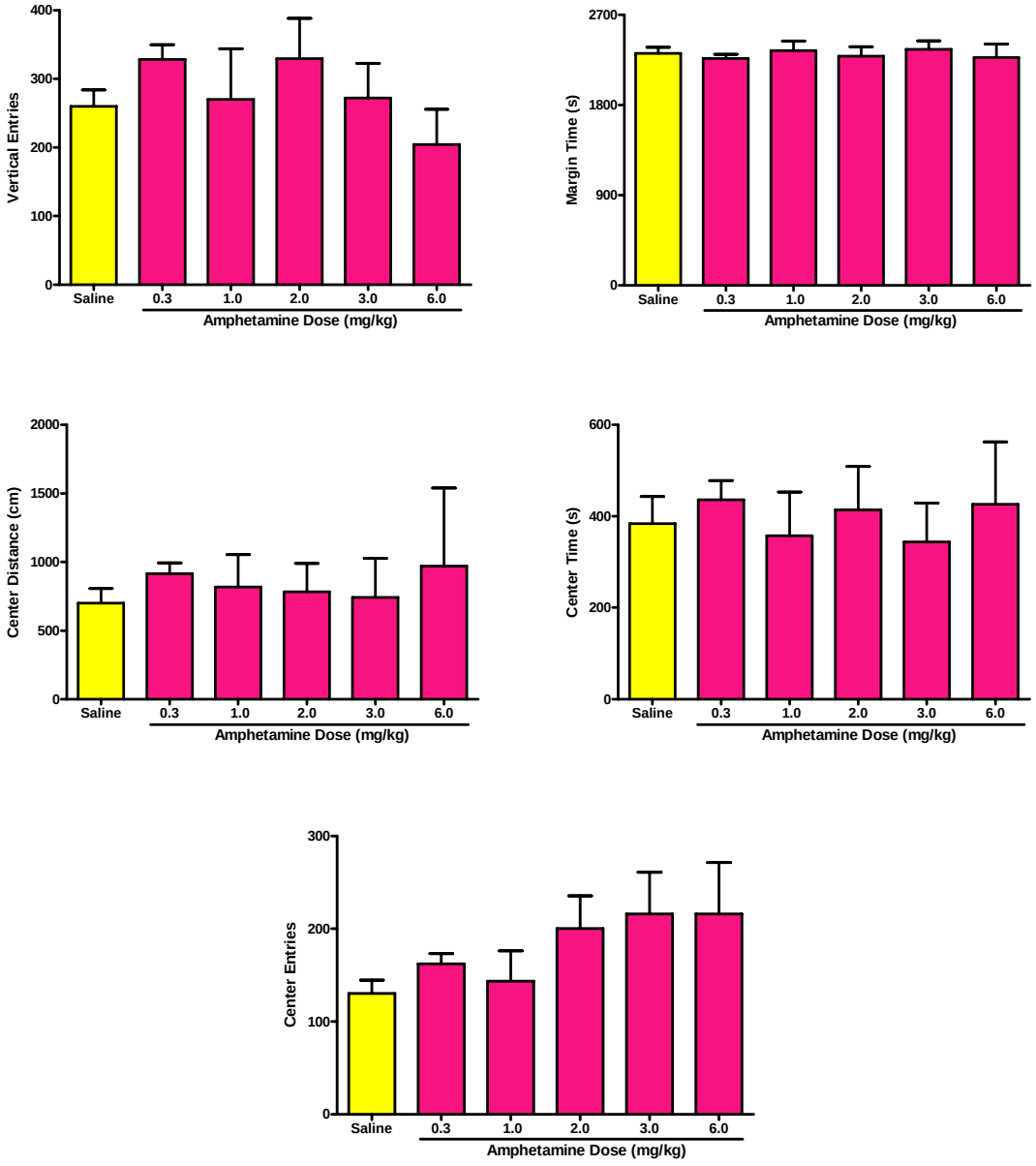


Figure 25. Effect (± S.E.M.) of (+)amphetamine (**2**) injected 0 min prior to examination on total vertical entries, total margin time, total center distance, total center time, and total center entries with a 45-min recording-time (n = 6-8 mice/treatment).

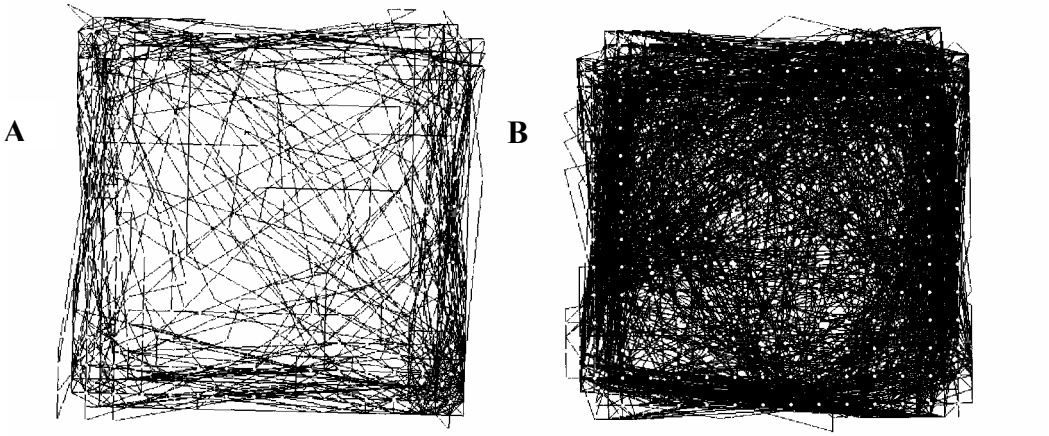


Figure 26. Graphical representation of the movement distance for the entire duration (45 min) of the experiment. A) The actual locomotion of a mouse when injected with saline. B) Stimulation by injection of an effective dose of (+)amphetamine (3.0 mg/kg).

ii. Combination of (+)Amphetamine (2) and MD-354 (42)

An effective dose of (+)amphetamine (3.0 mg/kg) was examined in combination with varying doses of MD-354 (**42**). The protocol entailed injecting varying doses of MD-354 (1.0, 3.0, and 10 mg/kg) i.p. 30 min prior to the test. (+)Amphetamine (**2**) was injected 0 min prior to the experiment and the mice were placed in the chamber with a 45-min recording-time. Combination of MD-354 with an effective dose of (+)amphetamine neither potentiated or modulated the stimulant effect of (+)amphetamine. For both stimulant and non-stimulant parameters (movement episodes, movement time, movement distance, vertical entries, margin distance, margin time, center distance, center time, and center entries), which were separated for convenience, results were essentially the same following administration of (+)amphetamine (**2**) alone, and following administration of (+)amphetamine in combination with doses of MD-354 (**42**) (Figure 27 and Figure 28). Although combinations of MD-354 with

(+)amphetamine were not statistically significant, the effects of MD-354 on (+)amphetamine were dose dependent for the parameters center distance and center entries.

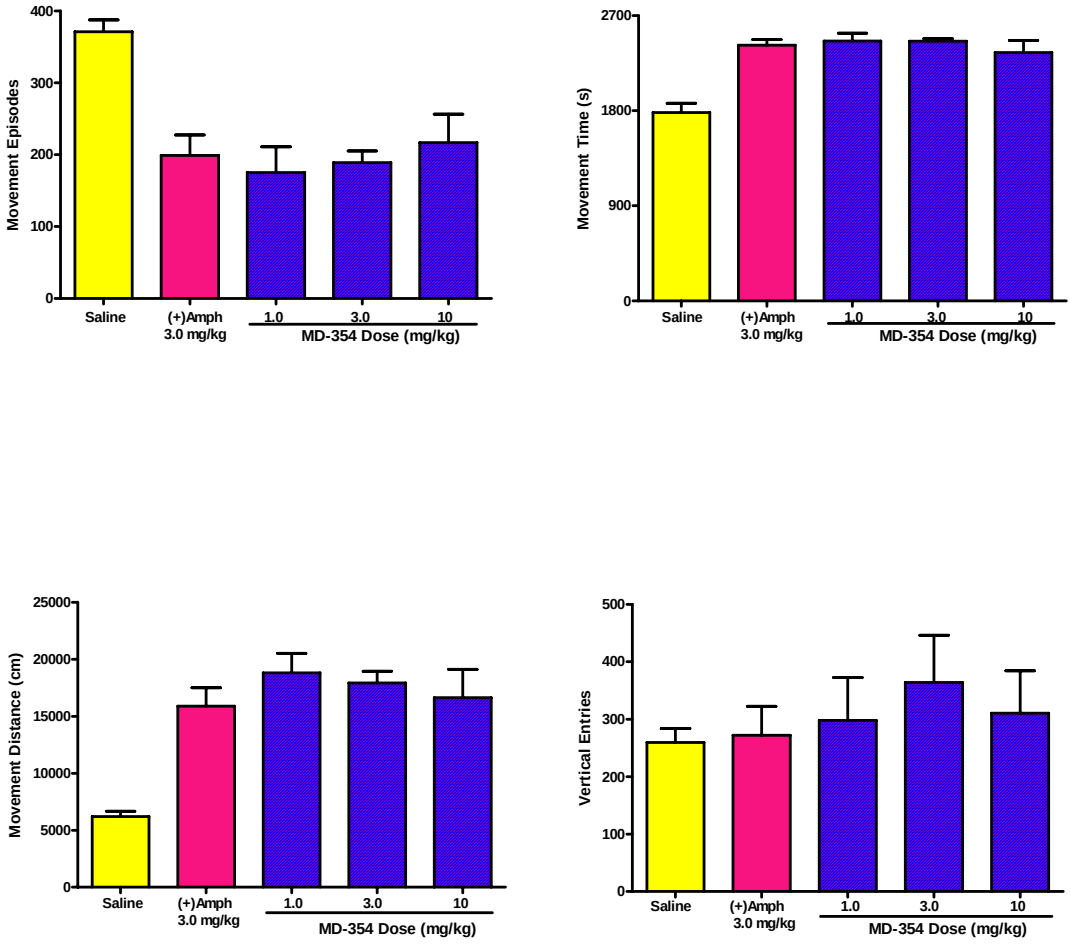


Figure 27. Effect (± S.E.M.) of combination of (+)amphetamine (**2**) (3.0 mg/kg) with varying doses of MD-354 (**42**) on total movement episodes, total movement time, total movement distance, and vertical entries following a 30-min pre-injection time of MD-354 and a 45-min recording-time (n = 7-8 mice/treatment).

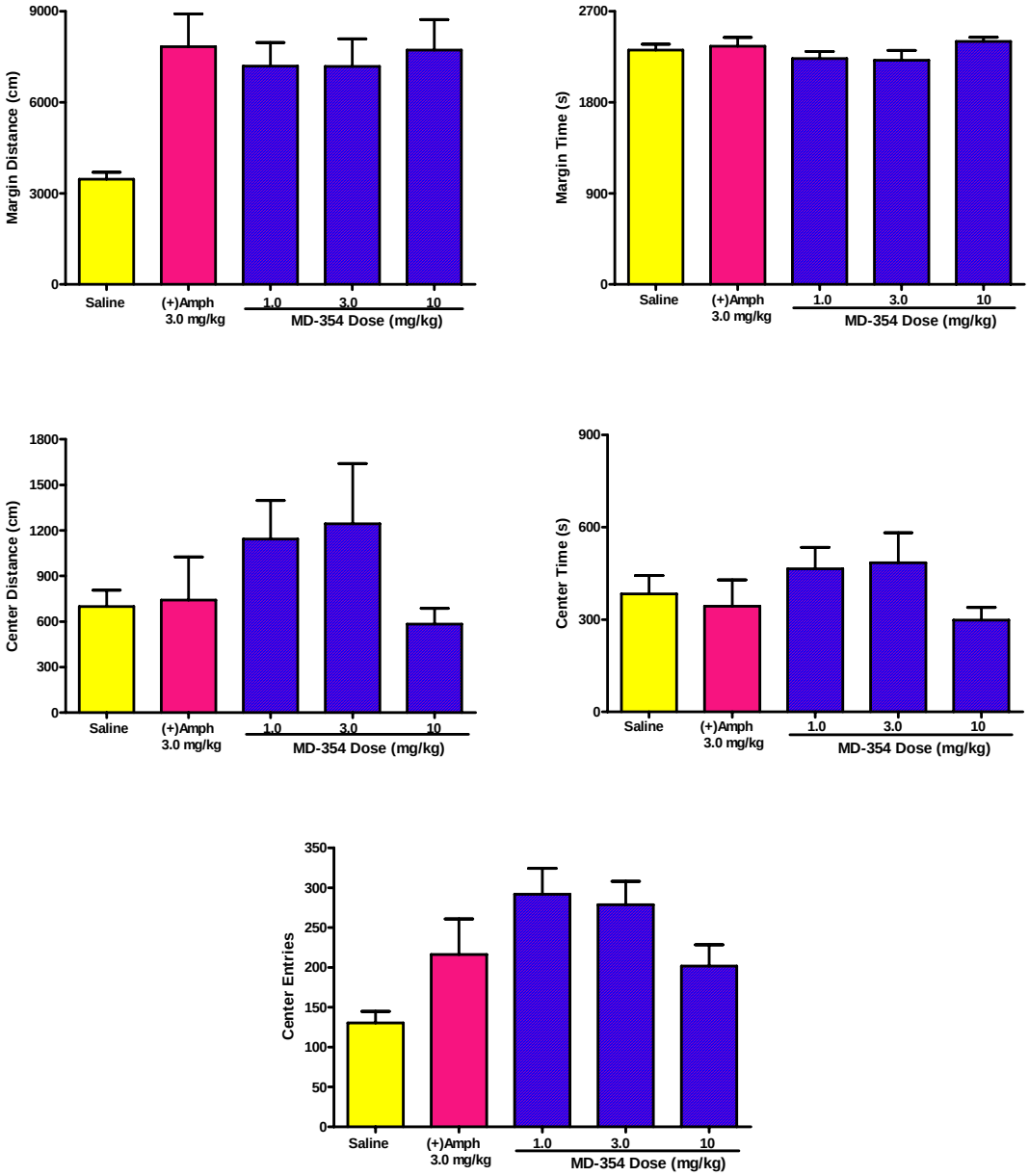


Figure 28. Effect (± S.E.M.) of combination of (+)amphetamine (**2**) (3.0 mg/kg; 0-min pre-injection time) with varying doses of MD-354 (**42**) (30-min pre-injection time) on total margin distance, total margin time, total center distance, total center time, and total center entries with a 45-min recording-time (n = 7-8 mice/treatment).

A similar study was conducted using doses of 1.0, 3.0, and 10 mg/kg of MD-354 (**42**) (30-min pre-injection time) in combination with a moderate dose of (+)amphetamine (**2**) (2.0 mg/kg; 0-min pre-injection time). The obtained results were similar to those observed with an effective dose of (+)amphetamine (3.0 mg/kg) alone, on all parameters observed (data not shown).

iii. Combination of (+)Amphetamine (2) and Ondansetron (19)

The effect of the 5-HT_3 receptor antagonist ondansetron (**19**) on the actions of an effective dose of (+)amphetamine (**2**) was examined. Doses of 0.1, 0.5, and 1.0 mg/kg of ondansetron were injected i.p., 30 min prior to the test. (+)Amphetamine (**2**; 3.0 mg/kg) was injected 0 min before the test and a 45-min recording-time was employed. In combination, ondansetron (**19**) neither potentiated nor modulated the action of an effective dose of (+)amphetamine. For parameters analyzed (movement episodes, movement distance, movement time, vertical entries, margin distance, margin time, center time, and center entries), the effect of ondansetron pre-treatment on the actions of (+)amphetamine were not different than that following administration of (+)amphetamine alone (Figure 29 and Figure 30), except at a 1.0 mg/kg dose of ondansetron, which potentiated the effect of (+)amphetamine on the parameter center distance (Figure 30). Since doses of ondansetron in combination with (+)amphetamine decreased margin distance and margin time, while increasing center measures, ondansetron in combination with (+)amphetamine was producing an anxiolytic-like effect. Even though this effect was not statistically significant in most cases, it was dose dependent.

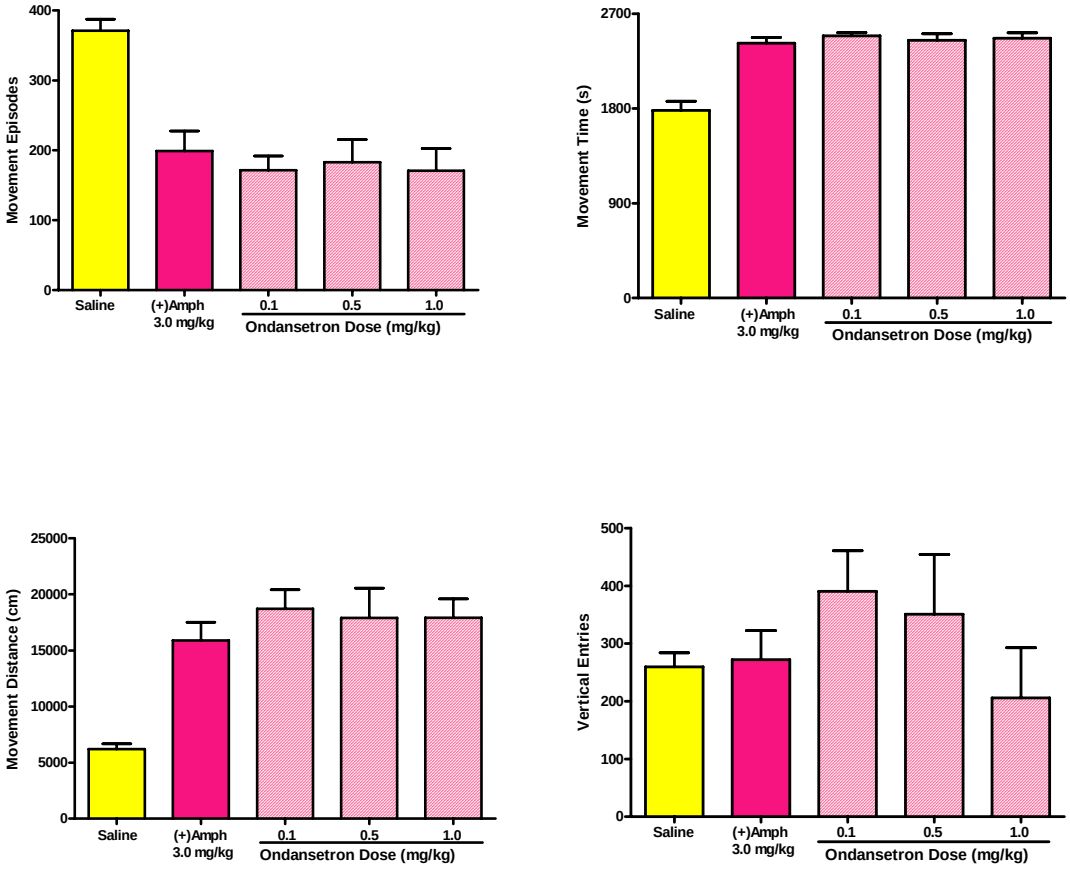


Figure 29. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) in combination with 3.0 mg/kg of (+)amphetamine (**2**) (0-min pre-injection time) on the stimulant parameters of total movement episodes, total movement time, total movement distance, and total vertical entries with a 45-min recording-time (n = 6-8 mice/treatment).

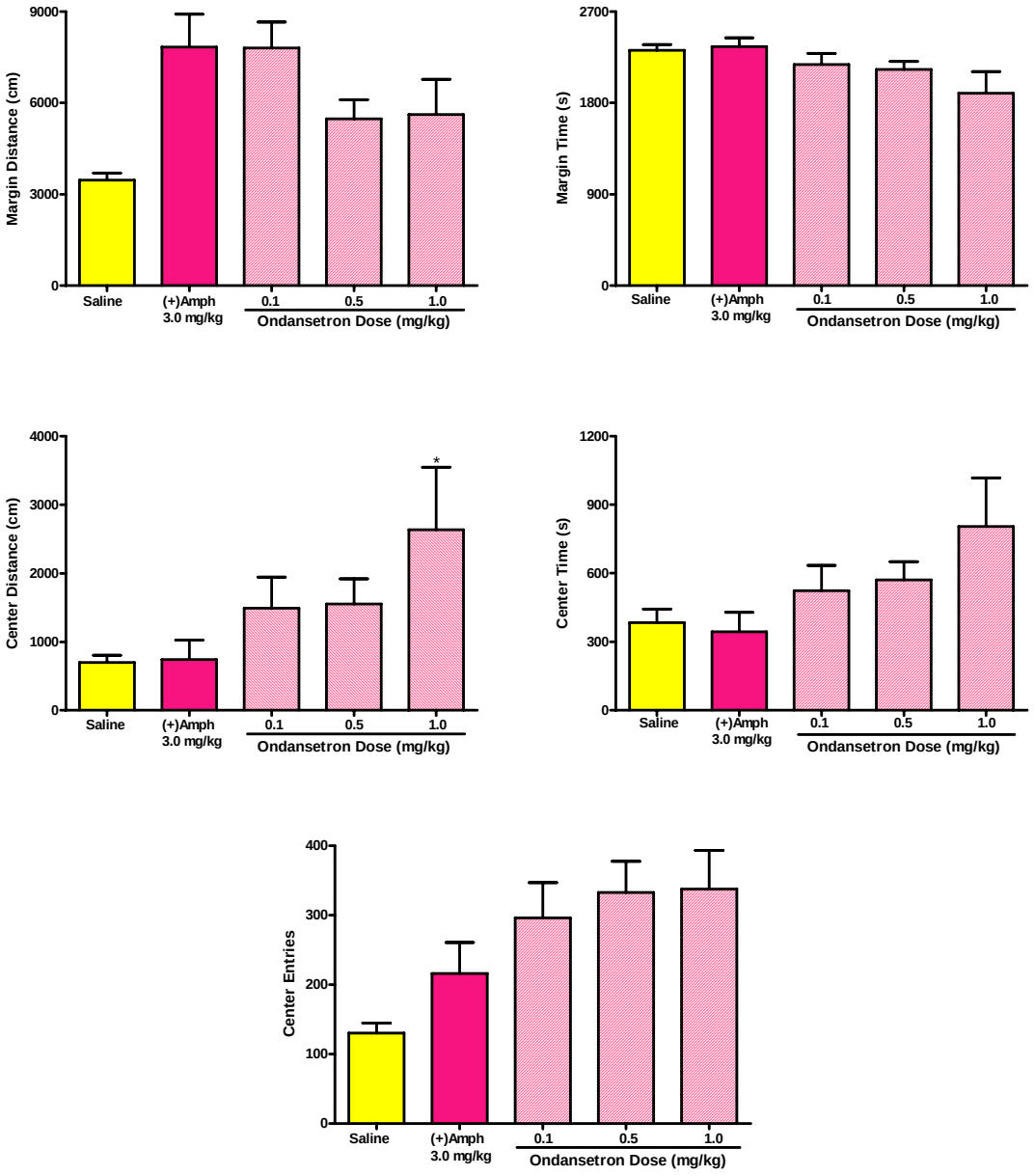


Figure 30. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) in combination with 3.0 mg/kg of (+)amphetamine (**2**) (0-min pre-injection time) on total margin distance, total margin time, total center distance, total center time, and total center entries with a 45-min recording-time (n = 6-8 mice/treatment).

iv. Combination of (+)Amphetamine (2) and SR 57227A (28)

To determine the effects of the 5-HT_3 receptor agonist SR 57227A (**28**) on the effects produced by a moderate dose of (+)amphetamine (**2**) (2.0 mg/kg), studies with combinations of both drugs were conducted. In the present investigation, SR 57227A (**28**) was administered 30 min prior to testing at i.p. doses of 1.0, 3.0, and 10 mg/kg. (+)Amphetamine (**2**) was administered 0 min prior to test. Within 15 min of the initiation of the experiment, potentiation of stimulant effects was observed; the effects lasted 30 min. The parameters affected by this combination included movement episodes, movement time, movement distance and margin distance for the first 15-min interval, and only movement episodes and movement distance for the second 15-min interval. For the parameters mentioned above, potentiation occurred at a 3.0 mg/kg dose of SR 57227A (**28**) with 2.0 mg/kg of (+)amphetamine (**2**), except movement episodes, where potentiation was observed with both 3.0 mg/kg and 10 mg/kg doses of SR 57227A (Figure 31). The other parameters (vertical entries, margin time, center distance, center time, and center entries) revealed amphetamine-like effects throughout the entire experiment (Figure 32). Comparison of the data indicated that SR 57227A (**28**) potentiated the stimulant effect of a moderate dose of (+)amphetamine (**2**) and that this potentiation occurred in a dose-dependent manner.

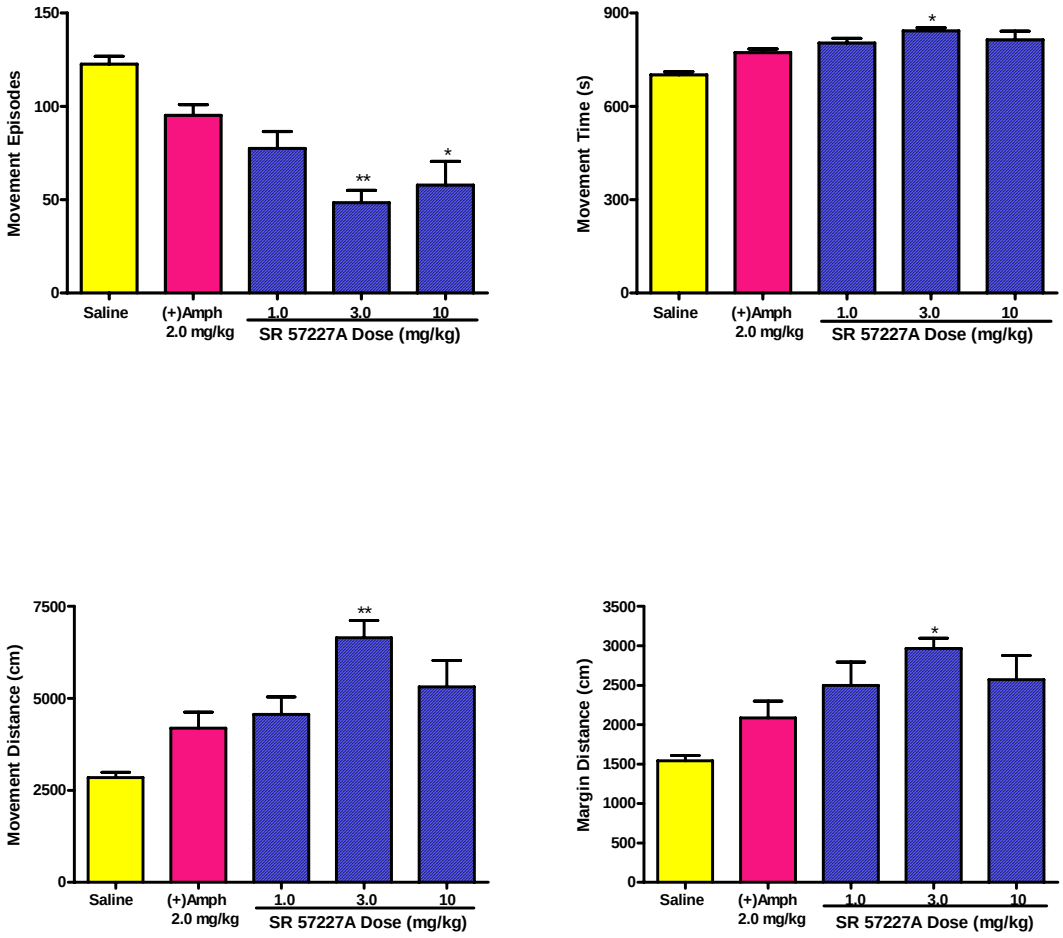


Figure 31. Effect (± S.E.M.) of varying doses of SR 57227A (**28**) (injected 30 min prior to examination) on a moderate dose of (+)amphetamine (**2**) (injected 0 min prior to examination) after the first 15-min interval of a 45-min protocol (n = 8 mice/treatment). Asterisk denotes a significant difference compared to a 2.0 mg/kg dose of (+)amphetamine; *P<0.05, **P<0.01, and ***P<0.001; one-way ANOVA ($F_{4,41}$ = 19.52 (movement episodes), $F_{4,41}$ = 16.15 (movement time), $F_{4,41}$ = 13.68 (movement distance), and $F_{4,41}$ = 10.36 (margin distance)) followed by a Newman-Keuls post-hoc test.

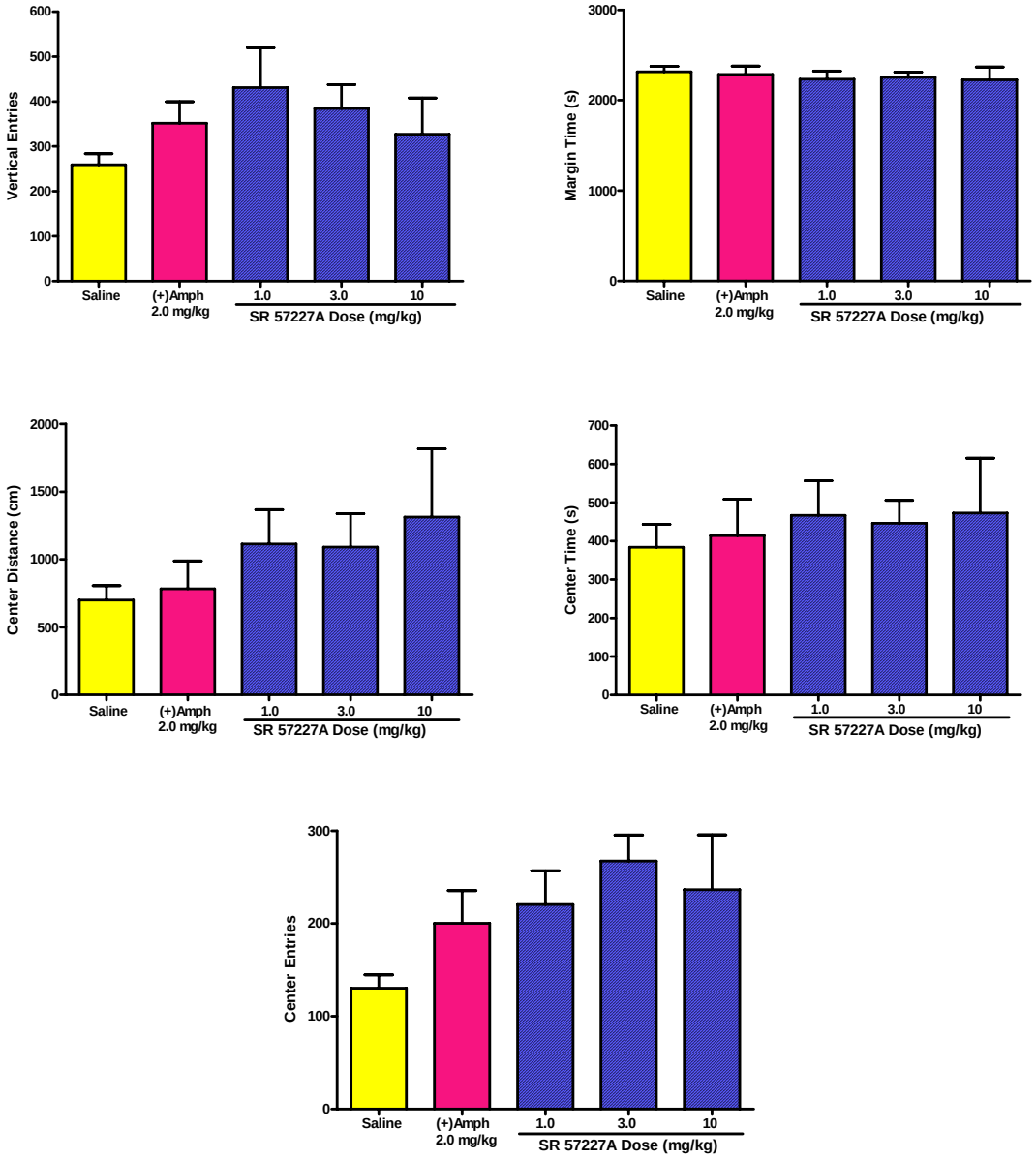


Figure 32. Effect (± S.E.M.) of varying doses of SR 57227A (**28**) (30-min pre-injection time) on a moderate dose of (+)amphetamine (**2**) (2.0 mg/kg, 0-min pre-injection time) on total vertical entries, total margin time, total center distance, total center time, and total center entries with a 45-min recording-time (n = 6-8 mice/treatment).

e) (+)Methamphetamine (3)

i. Dose Response

A mouse locomotor activity assay was conducted to determine an effective dose of (+)methamphetamine (**3**). Varying doses of (+)methamphetamine (0.3, 1.0, 1.5, 3.0 and 10 mg/kg) were injected i.p. 0 min before the mice were placed in the chamber with a recording-time of 60 min. Within the first 15-min interval, doses of (+)methamphetamine (**3**) displayed statistically significant stimulant effects versus saline. These effects varied between different doses and different parameters for the entire 60-min trial. Upon analysis of stimulant parameters, (+)methamphetamine (**3**) produced a statistically significant stimulant effect versus saline at doses of 1.5, 3.0 and 10 mg/kg on movement episodes, movement time, and movement distance within the first 15 min (Figure 33) and on various doses for the same three parameters for the entire duration of the experiment (Figure 34). These stimulant effects were observed at lower doses, than with those of (+)amphetamine, supporting the idea the (+)methamphetamine is the more potent stimulant. (+)Methamphetamine (**3**) also produced a statistically significant effect on vertical entries and margin distance from the first 15-min interval throughout the experiment (Figure 34). Center entries were statistically significantly increased within 30 min (data not shown) and continued so for the duration of the 60-min experiment (Figure 34). Parameters not affected by varying doses of (+)methamphetamine (**3**) include margin time, center distance, and center time (Figure 35).

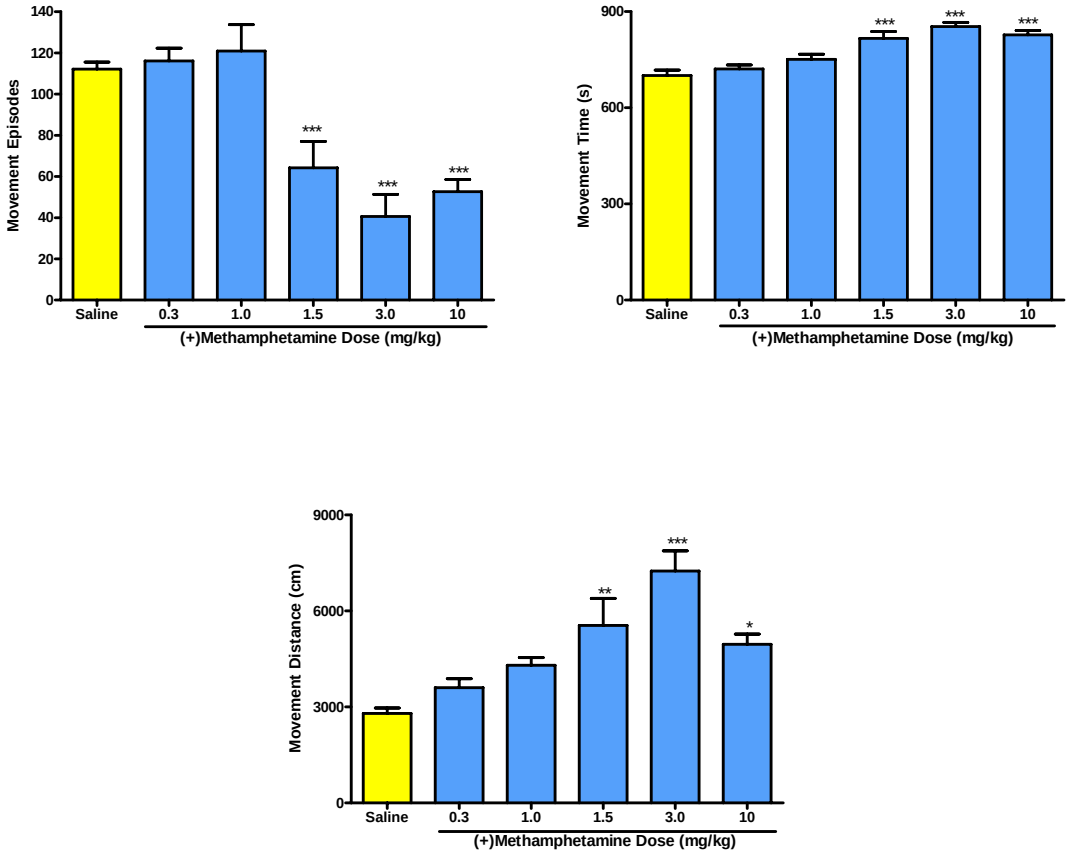


Figure 33. Effect (± S.E.M.) of varying doses of (+)methamphetamine (**3**) (0.3, 1.0, 1.5, 3.0, and 10 mg/kg; 0-min pre-injection) on the stimulant parameters of movement episodes, movement time, and movement distance within 15 min of a 60-min protocol (*n* = 6-8 mice/treatment). Asterisk denotes a significant difference compared to the saline control group; $^{**}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; one-way ANOVA ($F_{5,39}$ = 14.83 (movement episodes), $F_{5,39}$ = 15.49 (movement time), $F_{5,39}$ = 10.08 (movement distance)) followed by a Newman-Keuls post-hoc test.

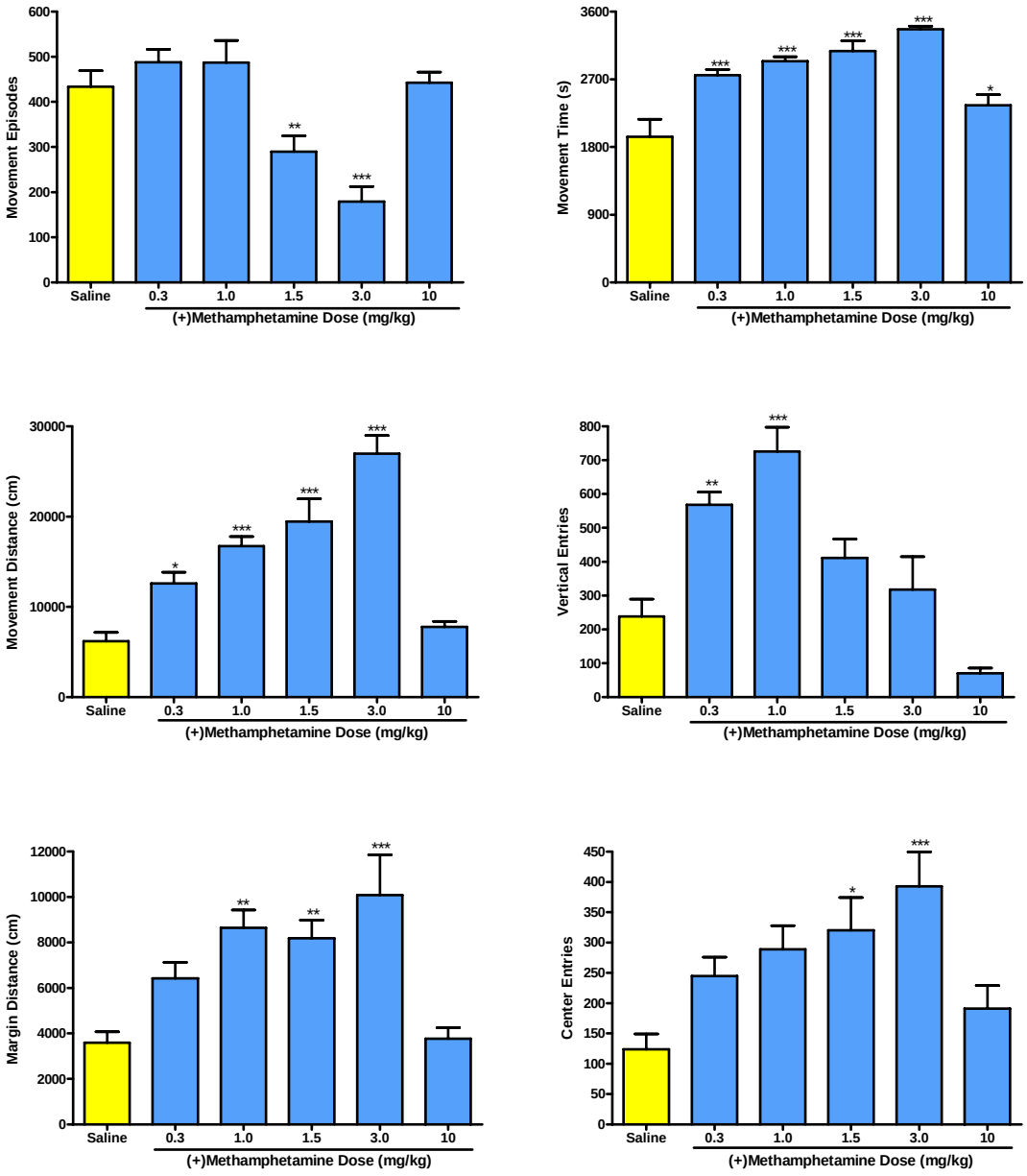


Figure 34. Effect (± S.E.M.) of varying doses of (+)methamphetamine (**3**) (0-min pre-injection) on total movement episodes, total movement time, total movement distance, total vertical entries, total margin distance, and total center entries with a 60-min protocol (n = 6-8 mice/treatment). Asterisk denotes a significant difference compared to the saline control group; $^{**}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; one-way ANOVA ($F_{5,39}$ = 13.58 (movement episodes), $F_{5,39}$ = 14.63 (movement time), $F_{5,39}$ = 25.01(movement distance), $F_{5,39}$ = 13.89 (vertical entries), $F_{5,39}$ = 7.89 (margin distance), $F_{5,39}$ = 5.16 (center entries)) followed by a Newman-Keuls post-hoc test.

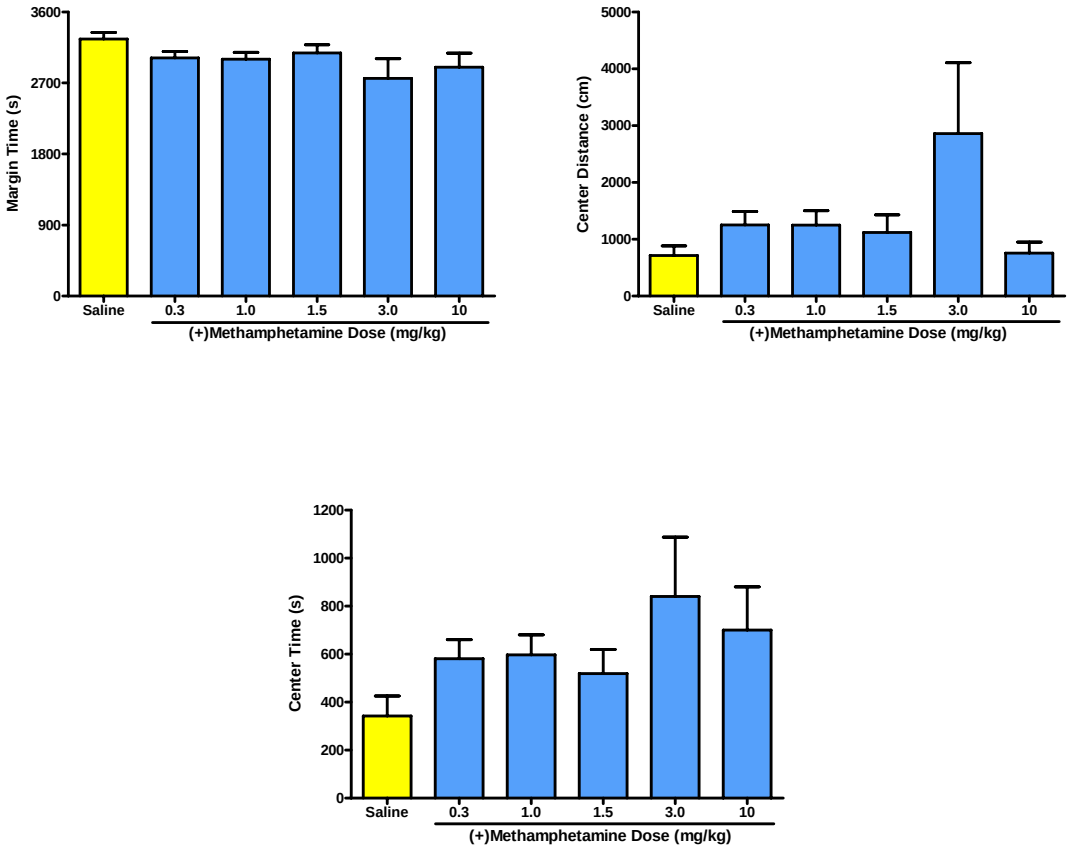


Figure 35. Effect (± S.E.M.) of varying doses of (+)methamphetamine (**3**) (0-min pre-injection time) on total margin time, total center distance, and total center time with a 60-min recording-time (n = 6-8 mice/treatment).

ii. Combination (+)Methamphetamine (3) and MD-354 (42)

The effect of varying doses of MD-354 (**42**) on an effective dose of (+)methamphetamine (**3**) was examined. MD-354 (**42**) doses of 0.3, 1.0, 3.0, and 6.0 mg/kg were administered i.p. 0 min prior to the experiment. (+)Methamphetamine (1.5 mg/kg) was administered 0 min prior to the experiment and the recording-time was 1 h. For all parameters analyzed, administration of MD-354 in combination with

(+)methamphetamine neither potentiated nor modulated the stimulant effect of (+)methamphetamine. A combination of MD-354 (**42**) and (+)methamphetamine (**3**) produced similar effects as (+)methamphetamine administered alone. The parameters analyzed included movement episodes, movement time, movement distance, vertical entries, margin distance, margin time, center distance, center time, and center entries as shown in Figure 36 and Figure 37.

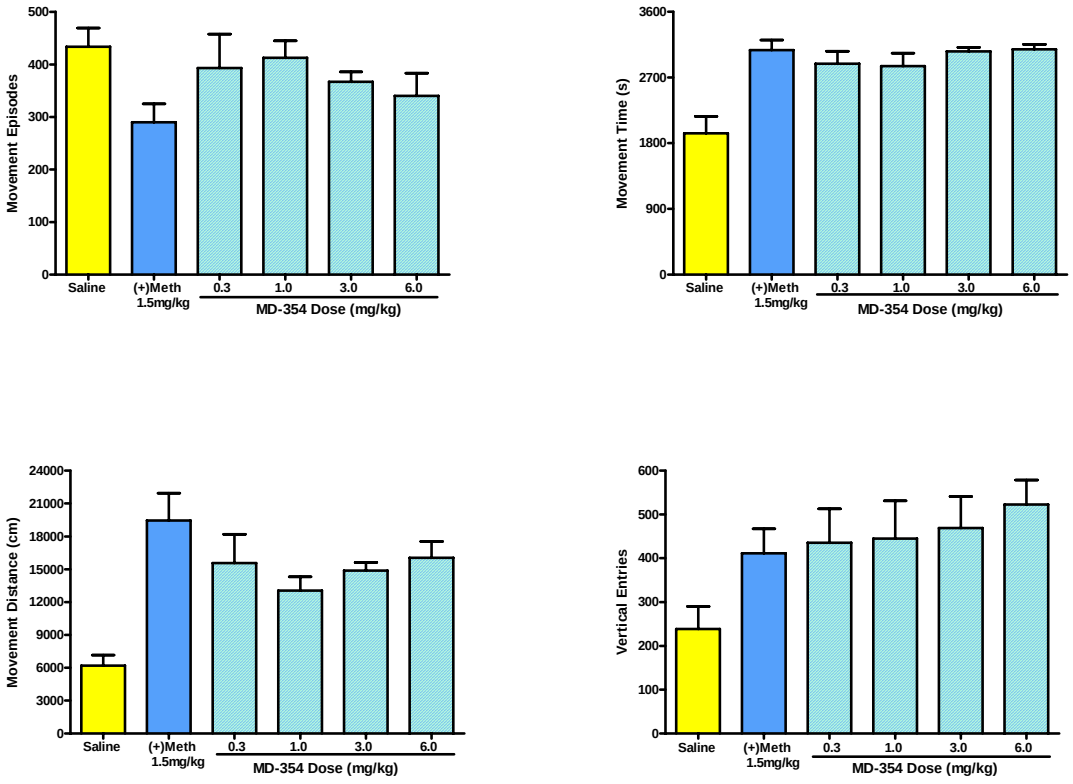


Figure 36. Effect (± S.E.M.) of varying doses of MD-354 (**42**) (0.3, 1.0, 3.0, and 6.0 mg/kg; 0-min pre-injection time) on an effective dose of (+)methamphetamine (**3**) (1.5 mg/kg; 0-min preinjection time) on total movement episodes, total movement time, total movement distance, and total vertical entries with a 60-min recording-time (n = 6-8 mice/treatment).

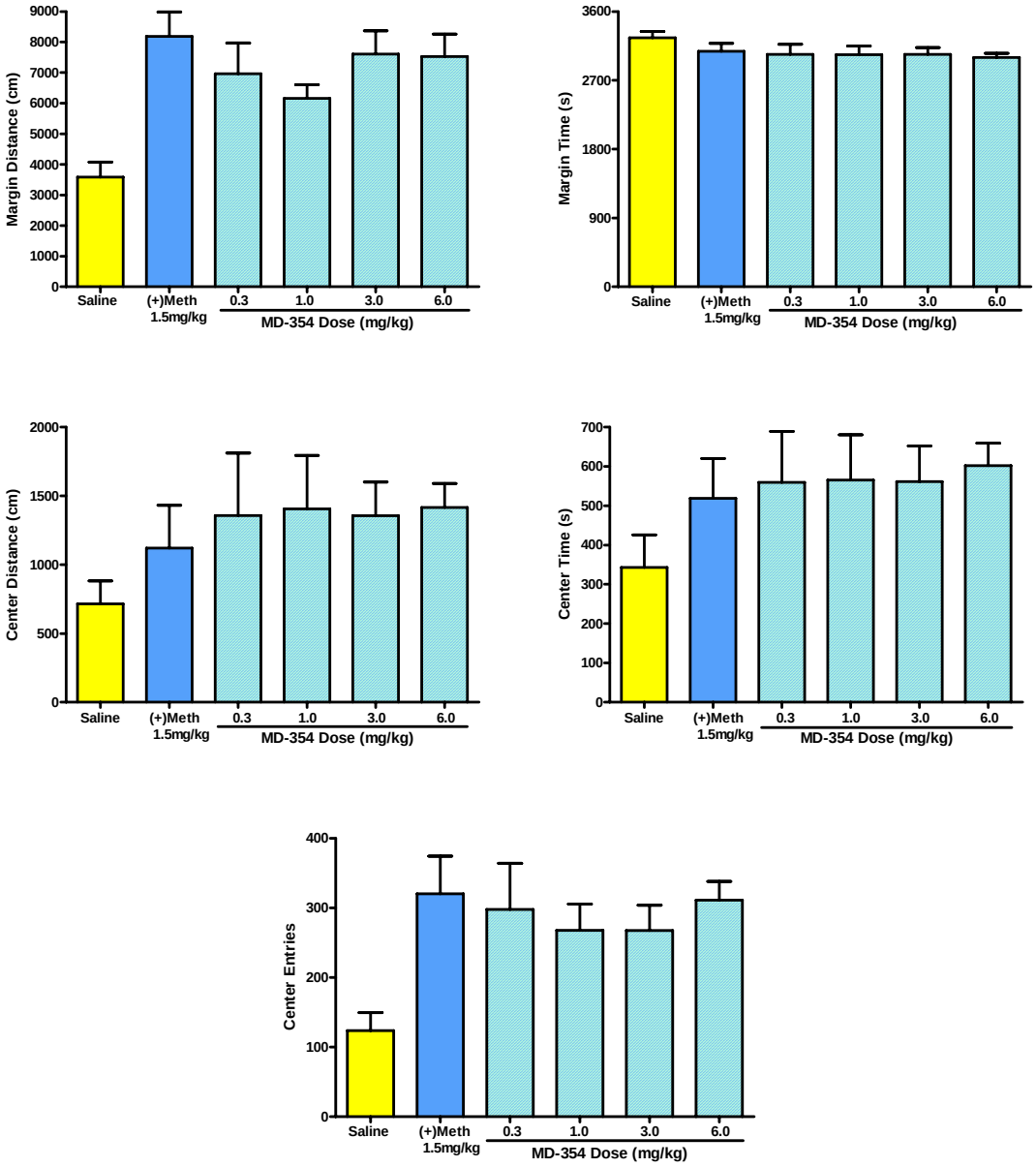


Figure 37. Effect (± S.E.M.) of varying doses of MD-354 (**42**) (0.3, 1.0, 3.0, and 6.0 mg/kg; 0-min pre-injection time) on an effective dose of (+)methamphetamine (**3**) (1.5 mg/kg; 0-min preinjection time) on total margin distance, total margin time, total center distance, total center time and total center entries with a 60-min recording-time (n = 6-8 mice/treatment).

A similar protocol was employed using a 3.0 mg/kg dose of (+)methamphetamine (**3**) in combination with 1.0, 3.0, and 6.0 mg/kg doses of MD-354. The pre-injection and recording-times remained the same, resulting in similar effects as observed with a combination of 1.5 mg/kg (+)methamphetamine (**3**) and varying doses of MD-354 (**42**): combination of MD-354 with (+)methamphetamine neither potentiated nor modulated the stimulant effects of (+)methamphetamine (data not shown).

f) DOM (10)

i. Dose Response

A locomotor activity assay was conducted to determine an effective dose of DOM (**10**) and to determine its effects on mouse locomotor actions. Varying doses of DOM (0.3, 1.0, and 3.0 mg/kg) were injected i.p. 0 min before the mice were placed in the chamber, with a recording-time of 60 min. After 15 min, DOM (**10**) behaved similarly to saline at all administered doses. However, within 30 min of the experiment, movement distance and margin distance were statistically significantly increased following all doses. Within 45 min, doses of 0.3 and 1.0 mg/kg of DOM (**10**) statistically significantly increased movement distance and margin distance. However, only a 0.3 mg/kg dose of DOM (**10**) increased vertical entries. Following 60 min post administration, DOM increased locomotor activity on the parameters of movement time, movement distance, vertical entries, and margin distance versus saline as shown in Figure 38. Throughout the entire study, DOM produced a saline-like effect on the parameters of movement episodes, margin time, center distance, center time, and center entries (Figure 39).

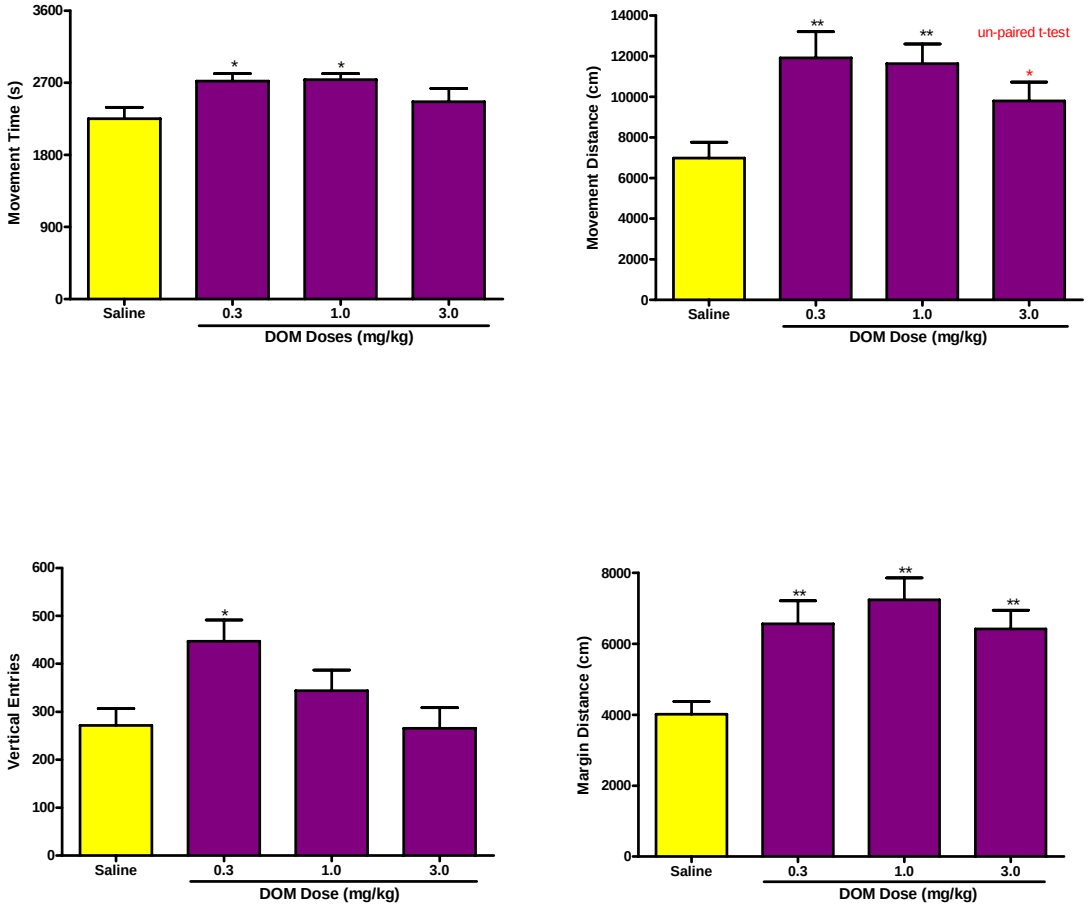


Figure 38. Effect (± S.E.M.) of varying doses of DOM (**10**) (0-min pre-injection time) on total movement time, total movement distance, vertical entries, and margin distance with a 60-min recording-time (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to the saline control group; *P<0.05, **P<0.01, ***P<0.001; one-way ANOVA ($F_{3,28}$ = 3.5 (movement time), $F_{3,28}$ = 5.03 (movement distance), $F_{3,28}$ = 4.15 (vertical entries), $F_{3,28}$ = 6.54 (margin distance)) followed by a Newman-Keuls post-hoc test.

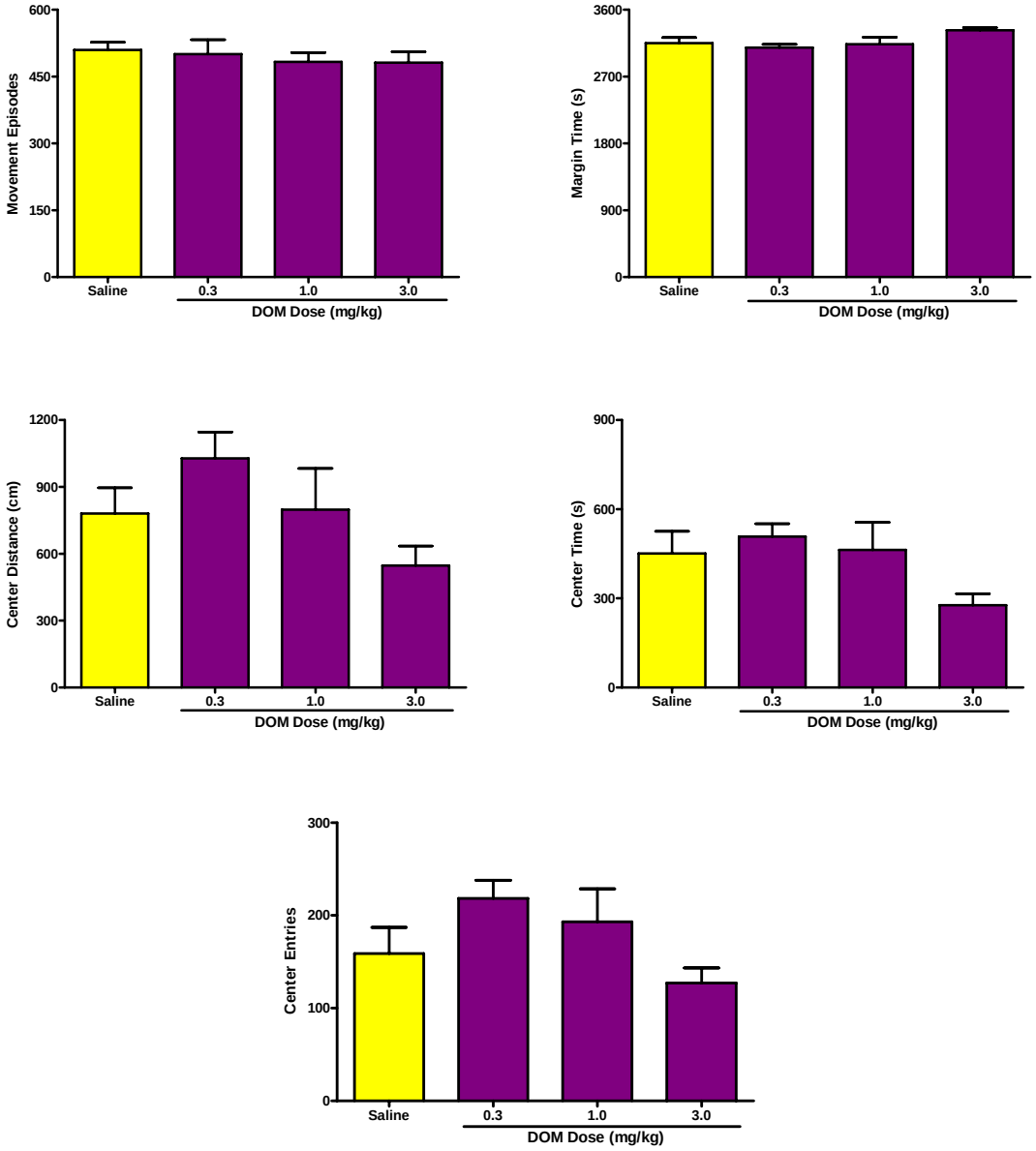


Figure 39. Effect (± S.E.M.) of varying doses of DOM (**10**) (0-min pre-injection time) on total movement episodes, total margin time, total center distance, total center time, and total center entries with a 60-min recording-time (n = 6-8 mice/treatment).

ii. Combination of DOM (10) and MD-354 (42)

The next step was to determine the effect of MD-354 (**42**) on an effective behavioral dose of DOM (**10**). A study was conducted using 0.1, 1.0, 3.0, and 6.0 mg/kg doses of MD-354 (**42**) (30-min pre-injection time) in combination with a 0.3 mg/kg dose of DOM (**10**) (0-min pre-injection time). The recording-time of the experiment was 60 min. Within 15 min of the initiation of the experiment, a low dose of MD-354 (0.1 mg/kg) antagonized the effect of DOM on movement distance, vertical entries, and center entries (a 1.0 mg/kg dose of MD-354 also antagonized the effect DOM displayed on vertical entries). As the experiment continued, MD-354 (**42**) antagonized various other parameters at various doses. Some parameters effected include movement time, movement distance, vertical entries, margin distance, center distance, and center entries as shown in Figure 40. The effect DOM (**10**) had on vertical entries alone and in combination with MD-354 (**42**) is shown in Figure 41. Combination of MD-354 with DOM produced similar results as DOM administered alone on movement episodes, margin time, and center time throughout the duration of the experiment (as shown in Figure 42).

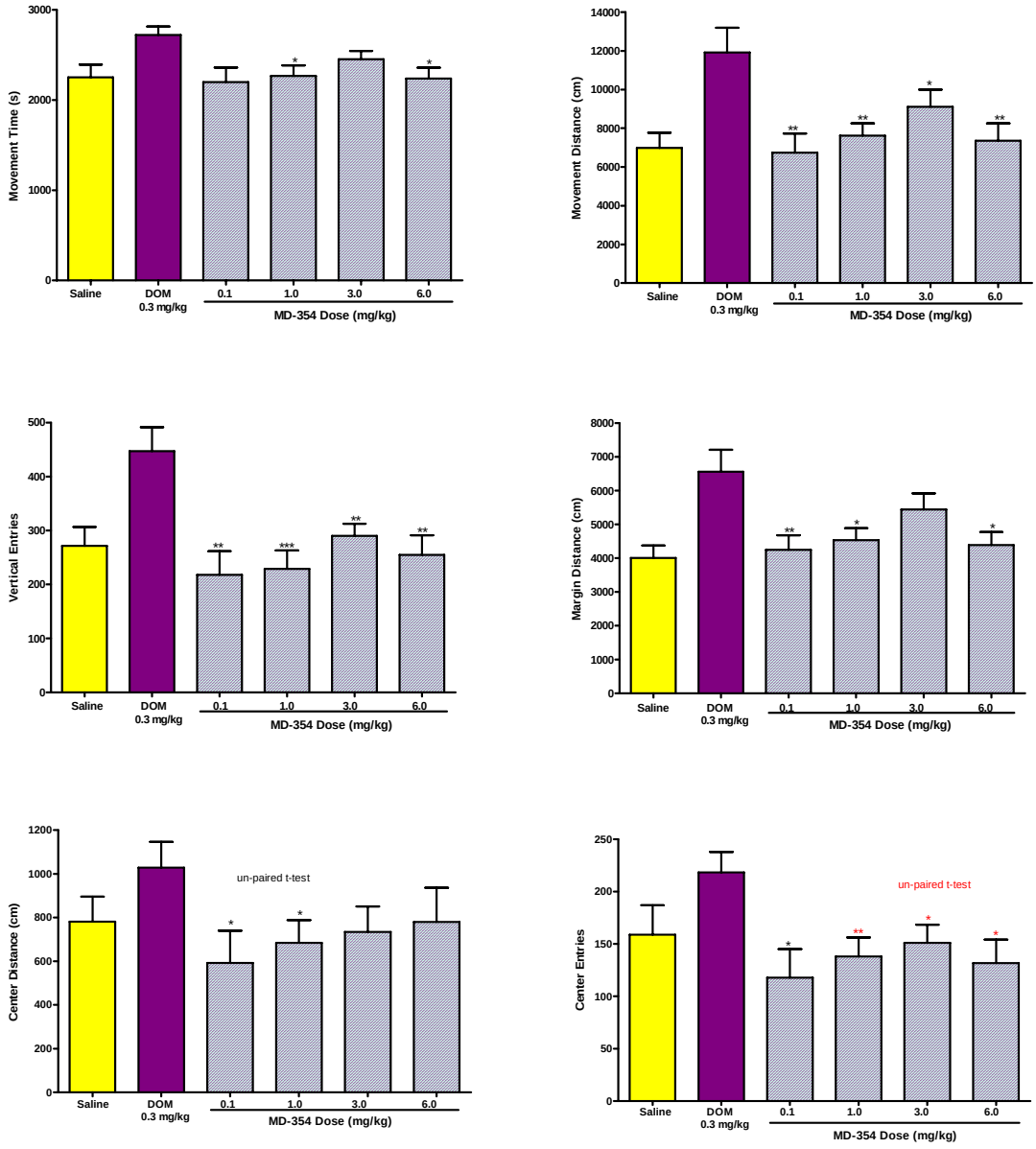


Figure 40. Effect (± S.E.M.) of varying doses of MD-354 (**42**) (30-min pre-injection time) on an effective dose of DOM (**10**) (0.3 mg/kg; 0-min pre-injection time) on total movement time, total movement distance, total vertical entries, total margin distance, total center distance, and total center entries with a 60-min recording-time (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to a 0.3 mg/kg dose of DOM; *P<0.05, **P<0.01, ***P<0.001; one-way ANOVA ($F_{5,41}$ = 2.74 (movement time), $F_{5,41}$ = 4.43 (movement distance), $F_{5,41}$ = 5.31 (vertical entries), $F_{5,41}$ = 4.49 (margin distance), $F_{5,41}$ = 1.30 (center distance) $F_{5,41}$ = 2.48 (center entries)) followed by a Newman-Keuls post-hoc test.

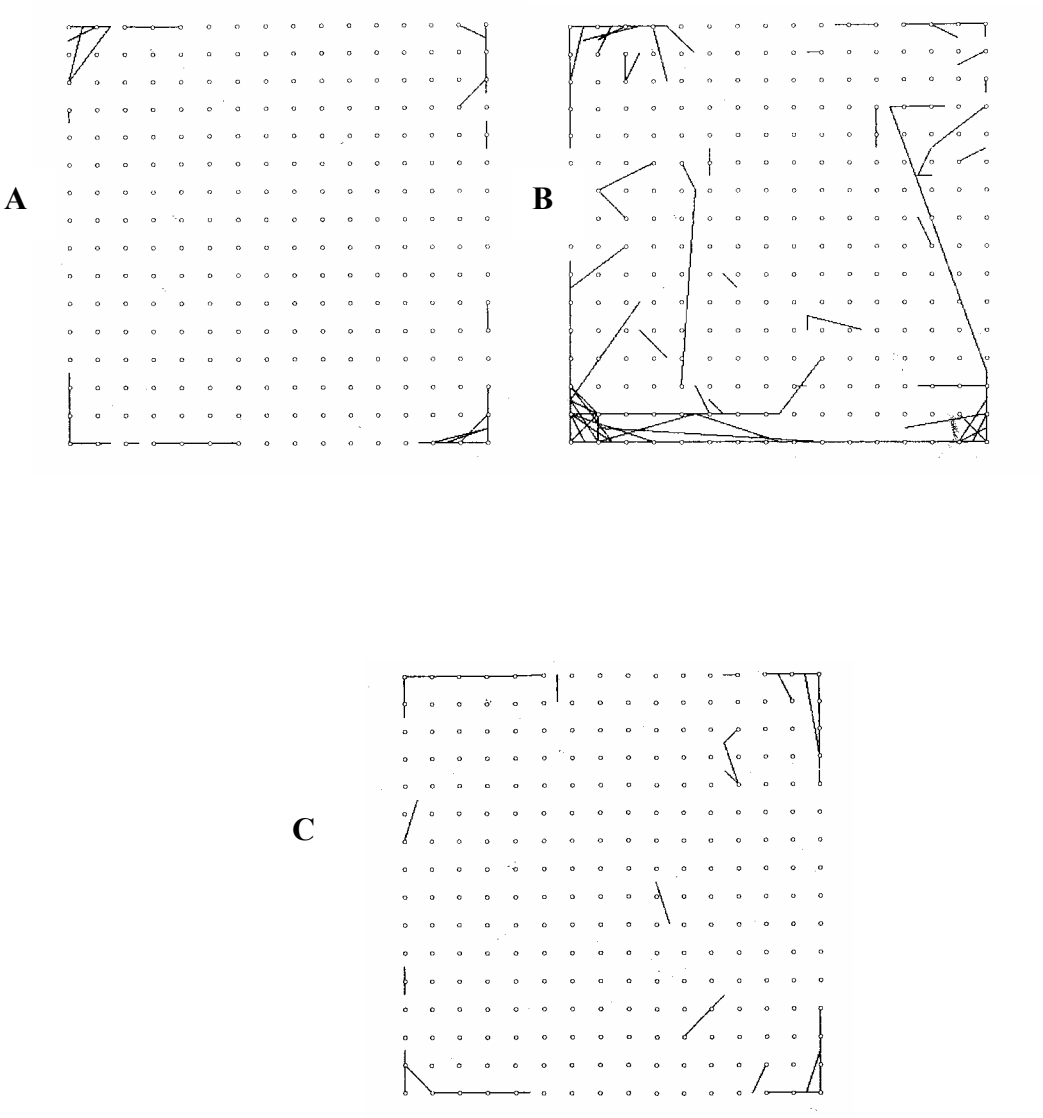


Figure 41. Graphical representation of vertical entries for the entire duration (60 min) of the experiment. A) Vertical entries of mice when administered saline. B) Vertical entries of mice when administered 0.3 mg/kg dose of DOM. C) Vertical entries of combination of 1.0 mg/kg dose of MD-354 and 0.3 mg/kg dose of DOM.

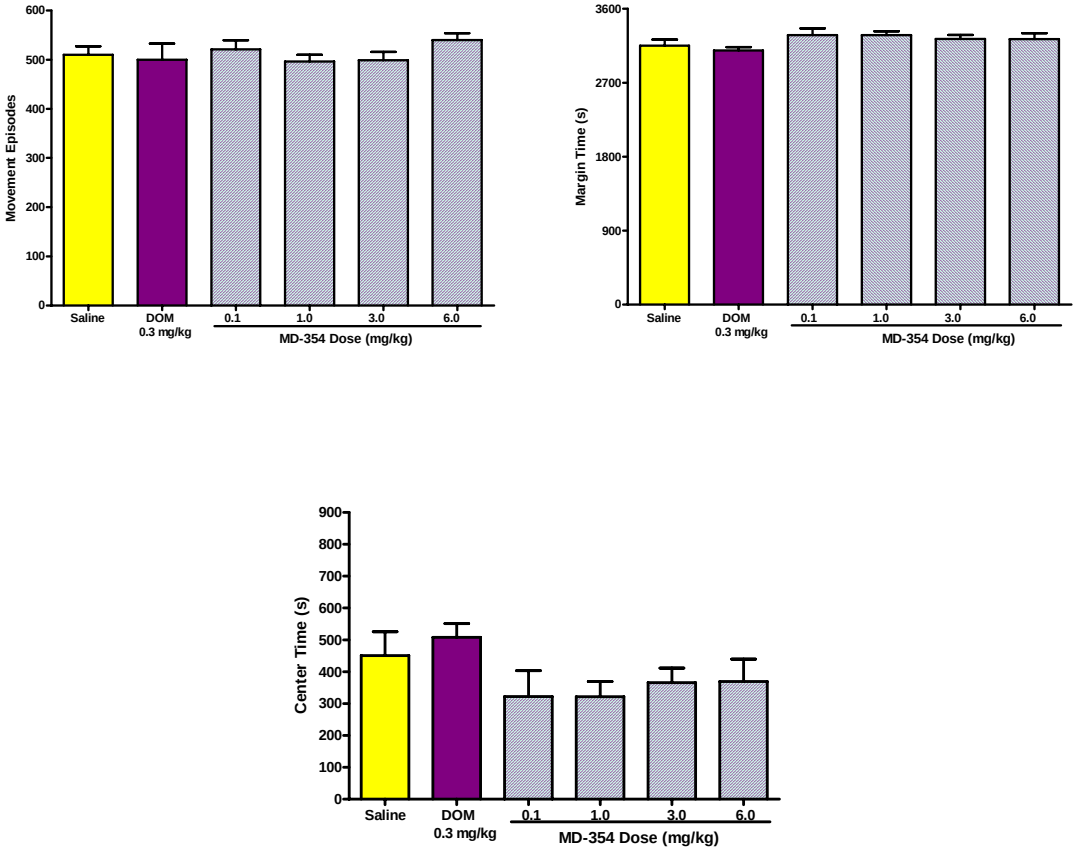


Figure 42. Effect (± S.E.M.) of varying doses of MD-354 (**42**) (30-min pre-injection time) on an effective dose of DOM (**10**) (0.3 mg/kg; 0-min pre-injection time) on total movement episodes, total margin time, and total center time with a 60-min recording-time (n = 6-8 mice/treatment).

g) Cocaine (1)

i. Dose Response

The present study was conducted to determine an effective dose of cocaine (**1**). Varying doses of cocaine (**1**) (1.0, 3.0, 10, and 30 mg/kg) were administered i.p. 0 min prior to the experiment. The mice were placed in the chamber and tested for 45 min. Within the first 15 min, locomotor activity was statistically significantly decreased versus saline on the stimulant parameter of movement episodes (30 mg/kg), whereas activity was significantly increased for movement time (30 mg/kg) and movement distance (10 and 30 mg/kg) as shown in Figure 43, as well as following 10 and 30 mg/kg doses of cocaine (**1**) on the non-stimulant parameters of margin distance and margin time (Figure 43). Statistically significant decreases in center distance and center time were observed versus saline at doses of 10 and 30 mg/kg of cocaine (Figure 44). Within 30 min, the only effects observed were statistically significant increases on the stimulant parameters of movement episodes, movement time, movement distance, and margin distance. By 45 min, movement time, movement distance, and margin distance were still increased following 10 and 30 mg/kg doses of cocaine, as well as statistically significantly increase in vertical entries versus saline (Figure 45). Overall, cocaine produced a saline-like effect at all doses administered on total vertical entries, total margin time, total center distance, total center time, and total center entries (Figure 46), however the effects on vertical entries, center distance and center time were dose-dependent.

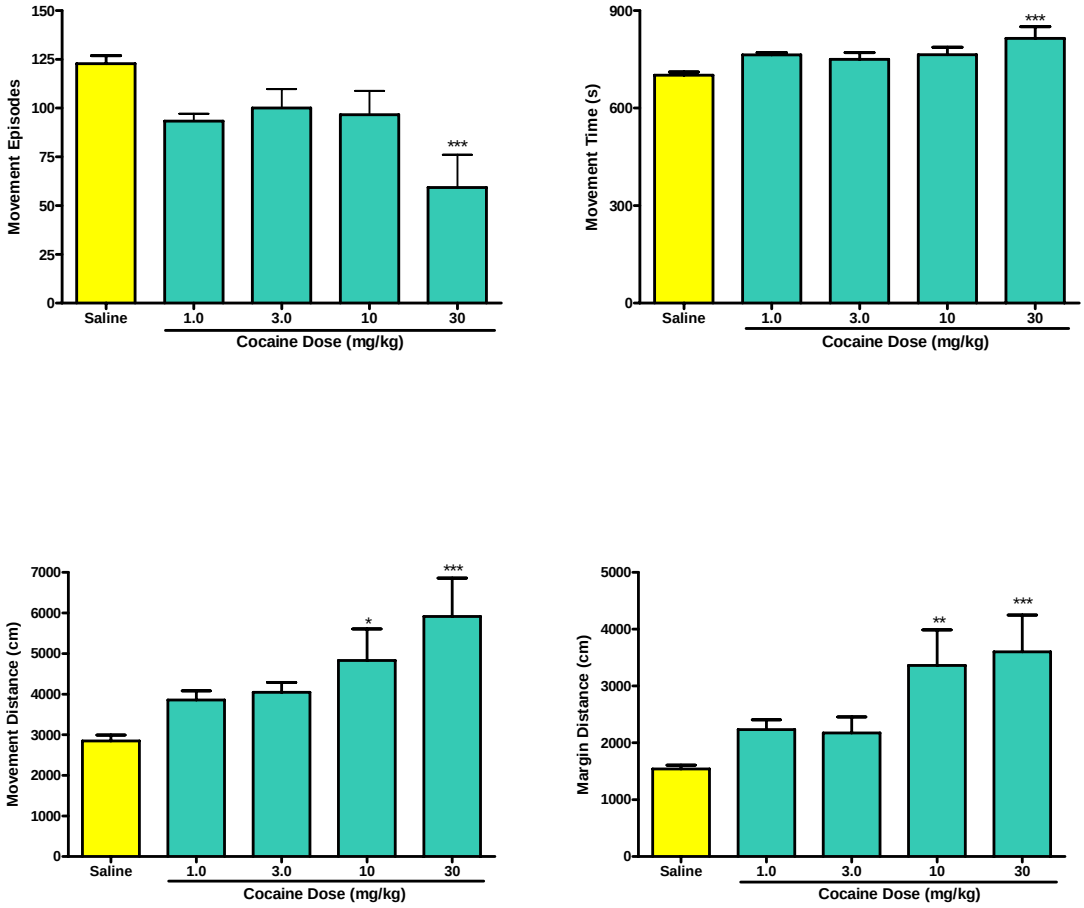


Figure 43. Effect (± S.E.M.) of varying doses of cocaine (**1**) (0-min pre-injection time; 45-min recording-time) on movement episodes, movement time, movement distance and margin distance within the first 15-min (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to the saline control group; *P<0.05, **P<0.01, ***P<0.001; one-way ANOVA ($F_{4,43}$ = 6.78 (movement episodes), $F_{4,43}$ = 5.37 (movement time), $F_{4,43}$ = 6.32 (movement distance), $F_{4,43}$ = 6.45 (margin distance))followed by a Newman-Keuls post-hoc test.

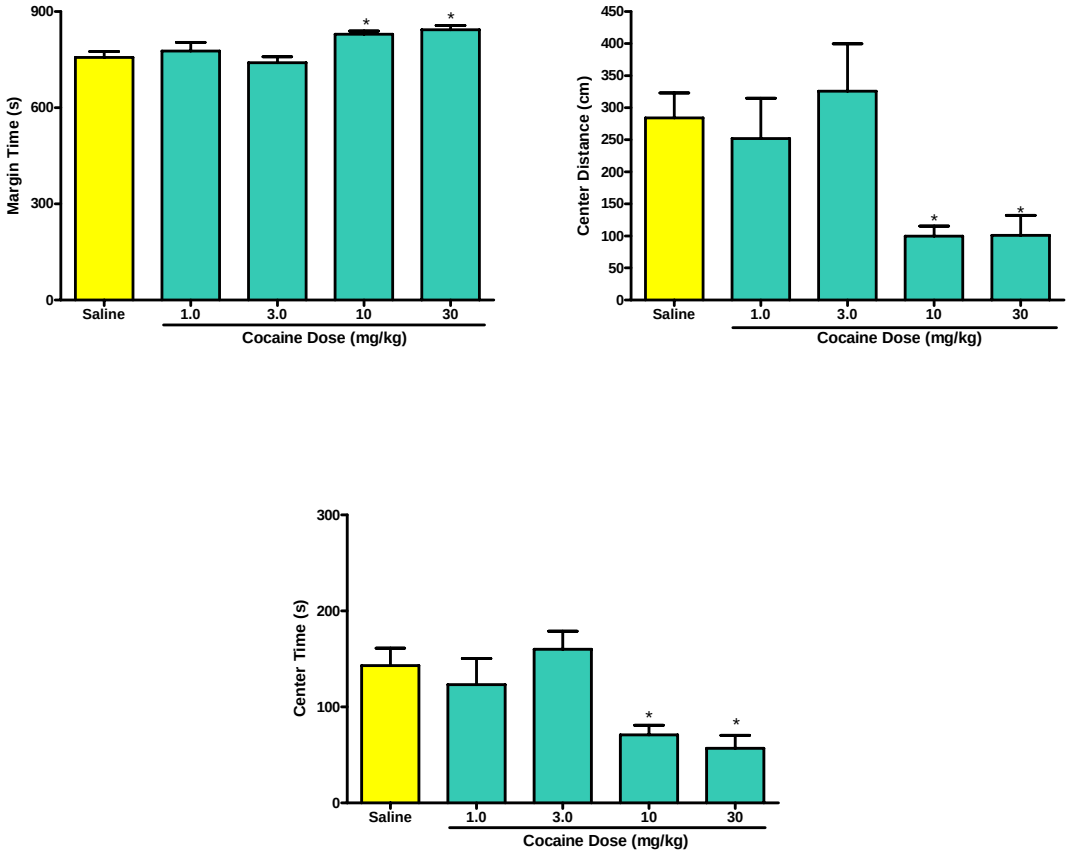


Figure 44. Effect (± S.E.M.) of varying doses of cocaine (**1**) (0-min pre-injection time; 45-min recording-time) on margin time, center distance and center time within the first 15-min (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to the saline control group; *P<0.05, **P<0.01, ***P<0.001; one-way ANOVA ($F_{4,43}$ = 4.91 (margin time) $F_{4,43}$ = 4.36 (center distance), $F_{4,43}$ = 4.91 (center time)) followed by a Newman-Keuls post-hoc test.

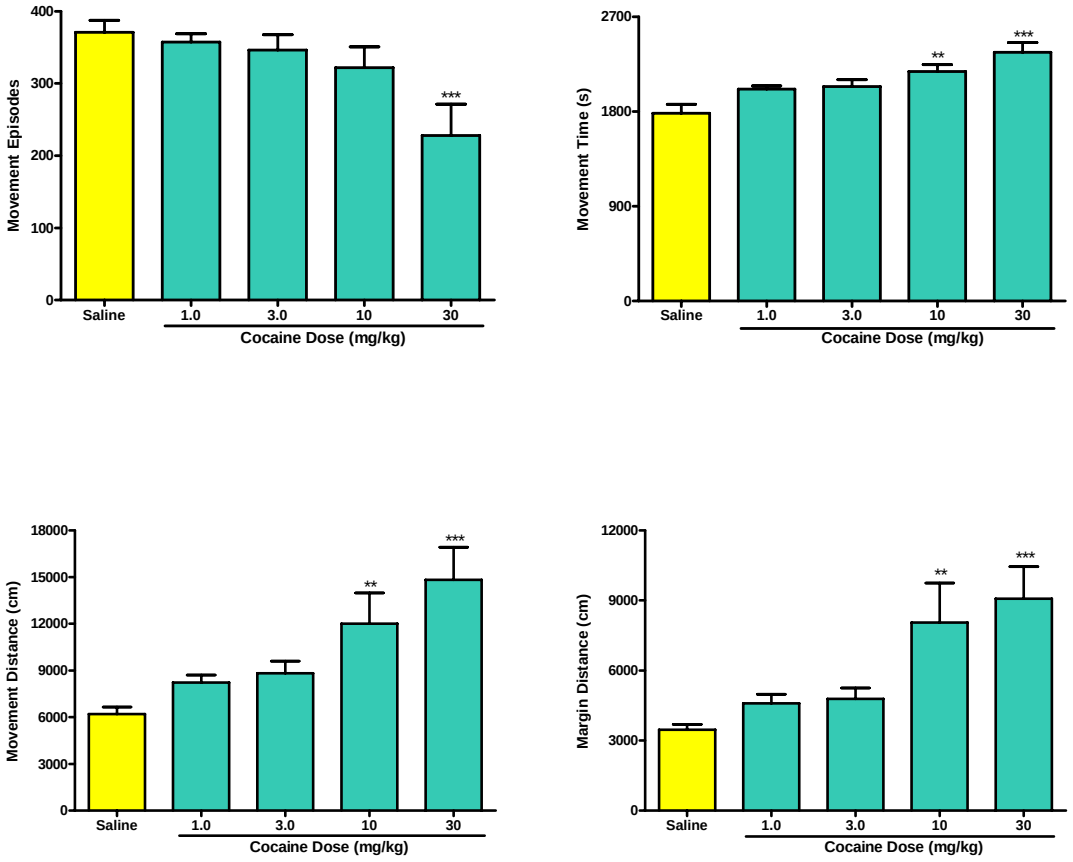


Figure 45. Effect (± S.E.M.) of varying doses of cocaine (**1**) (0-min pre-injection time; 45-min recording-time) on total movement episodes, total movement time, total movement distance, total margin distance (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to the saline control group; $^{**}P<0.01$ and $^{***}P<0.001$; one-way ANOVA ($F_{4,43} = 5.09$ (movement episodes), $F_{4,43} = 7.67$ (movement time), $F_{4,43} = 9.02$ (movement distance), $F_{4,43} = 8.31$ (margin distance)) followed by a Newman-Keuls post-hoc test.

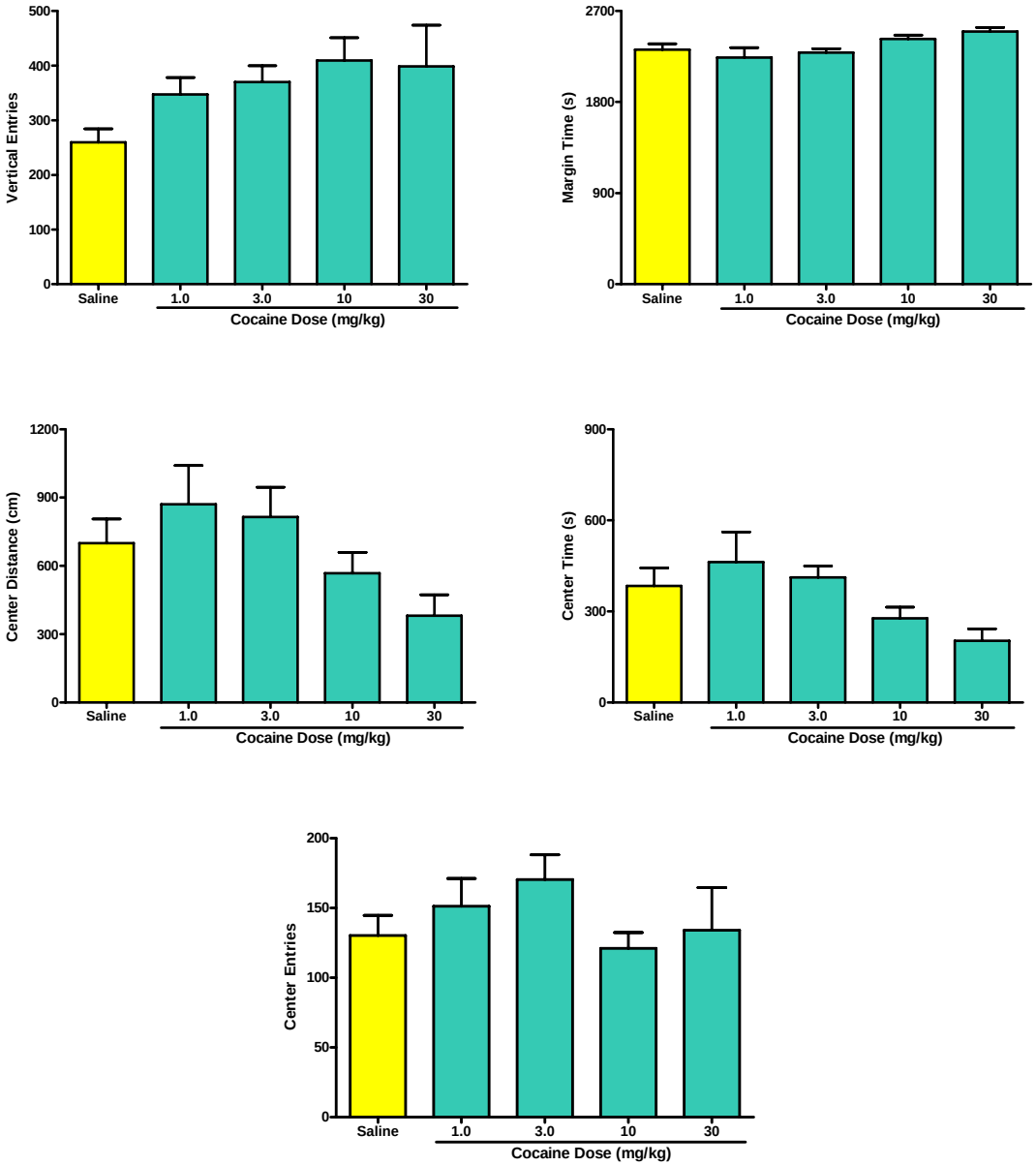


Figure 46. Effect (± S.E.M.) of varying doses of cocaine (**1**) (0-min pre-injection time; 45-min recording-time) on total vertical entries, total margin time, total center distance, total center time, and total center entries (n = 6-8 mice/treatment).

ii. Combination of Cocaine (1) and MD-354 (42)

The effect of varying doses of the 5-HT_3 receptor partial agonist, MD-354 (**42**) (1.0, 3.0, and 10 mg/kg) on an effective dose of cocaine (**1**) (10 mg/kg) was examined. MD-354 (**42**) was administered 30 min prior to examination, followed by cocaine (**1**) administered 0 min prior to examination. The recording-time for the present investigation was 45 min. Within the first 15 min, in combination with cocaine (**1**), MD-354 (**42**) potentiated the stimulant effect that cocaine displayed when administered alone. This potentiation was observed with a decrease in movement episodes and an increase in movement time as shown in Figure 47. Within this first 15 min all other parameters behaved similarly to cocaine when administered alone (data not shown).

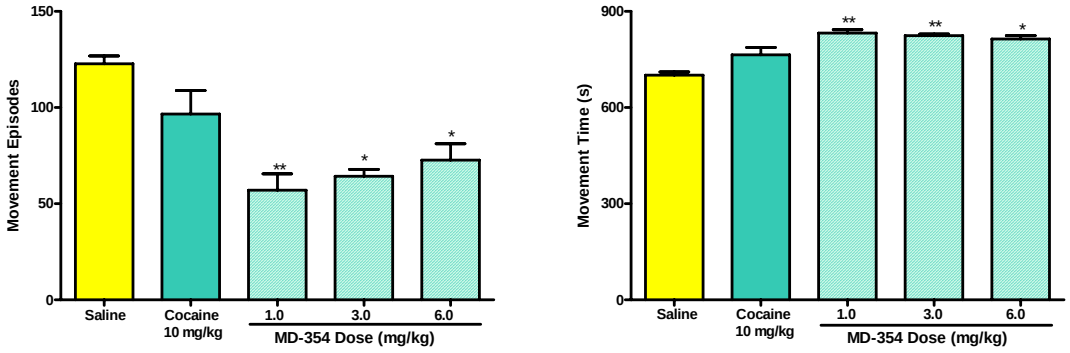


Figure 47. Effect (± S.E.M.) of varying doses of MD-354 (**42**) (30-min pre-injection time) on a effective dose of cocaine (**1**) (10 mg/kg; 0-min pre-injection time) within the first 15-min of the 45-min protocol (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to a 10 mg/kg dose of cocaine; *P<0.05 and **P<0.01; one-way ANOVA ($F_{4,43}$ = 16.90 (movement episodes) and $F_{4,43}$ = 23.73 (movement time)) followed by a Newman-Keuls post-hoc test.

Within 30 min of the experiment, a separate set of parameters were affected. Instead of stimulant parameters being potentiated as within the first 15 min, vertical entries, center distance, and center entries were potentiated as shown in Figure 48. Vertical entries were potentiated at all doses of MD-354 (**42**) versus cocaine (**1**), whereas 6.0 mg/kg dose of MD-354 in combination with cocaine potentiated the effect on the parameters center distance and center entries versus cocaine alone (Figure 48).

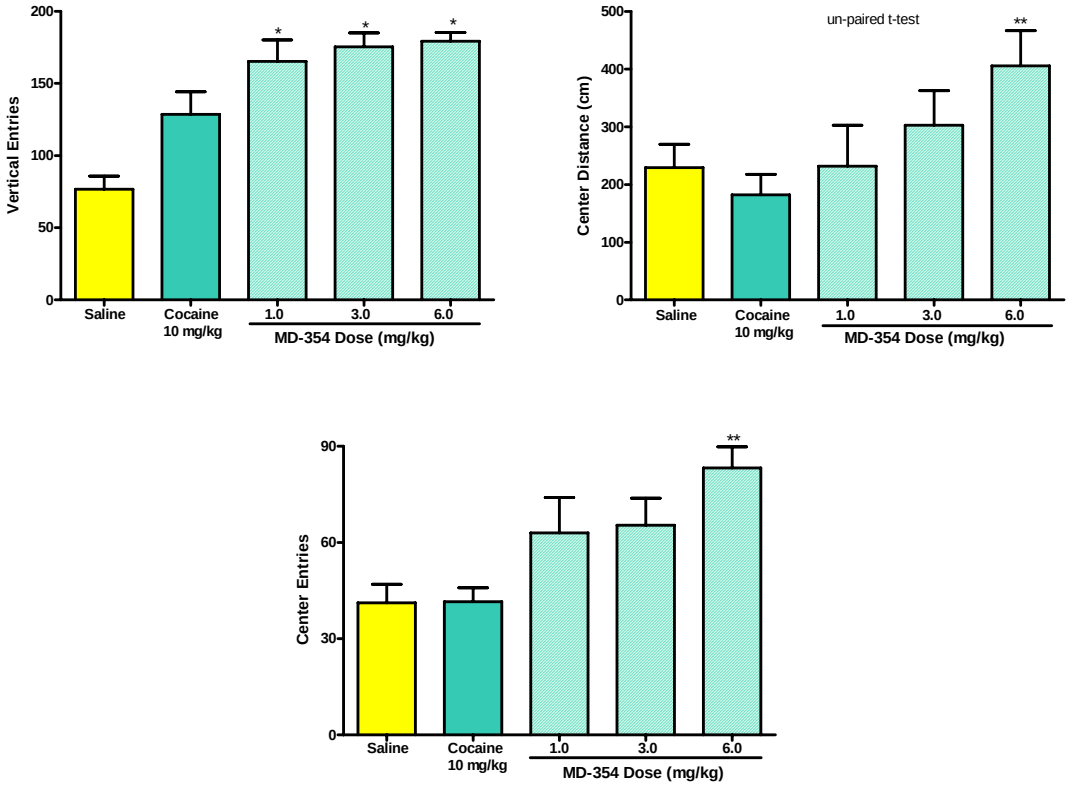


Figure 48. Effect (± S.E.M.) of varying doses of MD-354 (**42**) (30-min pre-injection time) on an effective dose of cocaine (**1**) (0-min pre-injection) within 30 min of the 45-min protocol (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to a 10 mg/kg dose of cocaine; *P<0.05 and **P<0.01; one-way ANOVA ($F_{4,43}$ = 17.92 (vertical entries), $F_{4,43}$ = 2.37 (center distance), $F_{4,43}$ = 5.88 (center entries)) followed by a Newman-Keuls post-hoc test.

Movement distance, margin distance, margin time, and center time were not affected by the combination of MD-354 (**42**) with cocaine (**1**) at any dose combinations administered. For these parameters, combination of MD-354 with an effective dose of cocaine (10 mg/kg) produced a cocaine-like effect (Figure 49).

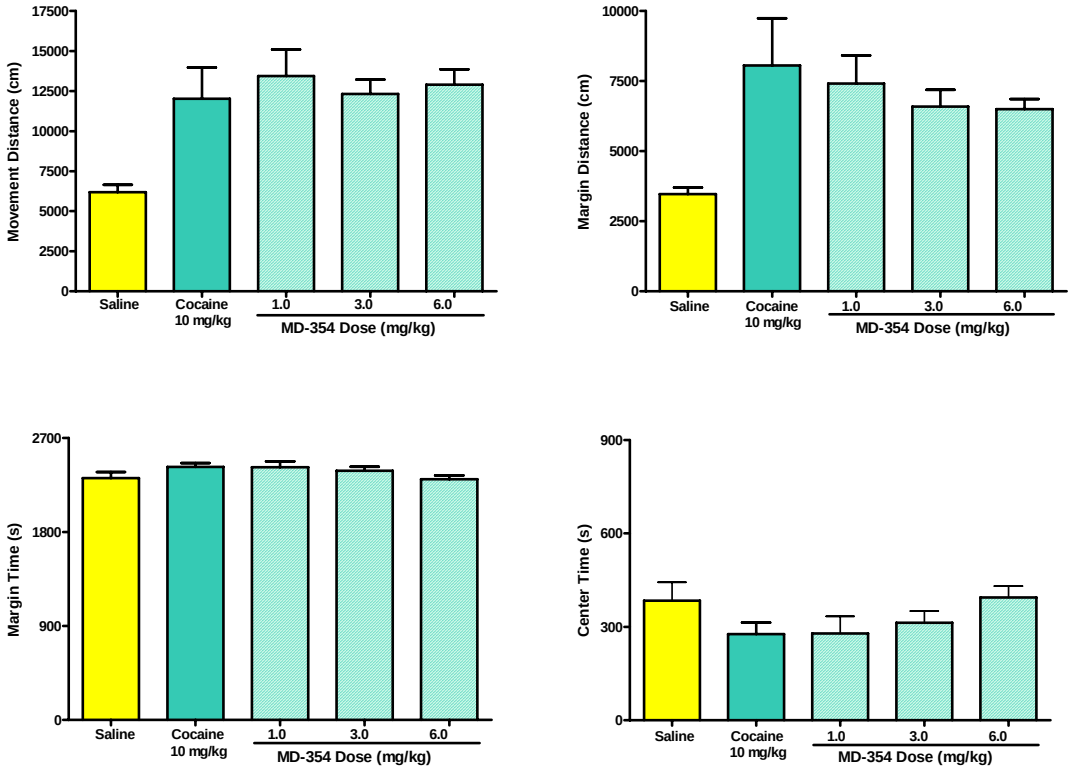


Figure 49. Effect (± S.E.M.) of varying doses of MD-354 (**42**) (30-min pre-injection time) on an effective dose of cocaine (**1**) (0-min pre-injection time) for total movement distance, total margin distance, total margin time, and total center time with a 45-min recording-time (n = 6-8 mice/treatment).

iii. Combination of Cocaine (1) and Ondansetron (19)

The effect of varying doses of the 5-HT_3 receptor antagonist ondansetron (**19**) was examined in combination with an effective dose of cocaine (**1**). Varying doses of ondansetron (0.1, 0.5, and 1.0 mg/kg) were injected i.p. 30 min prior to the experiment. A 10 mg/kg dose of cocaine was injected 0 min prior to the experiment; the recording-time of the experiment was 45 min. Within the first 15 min only one parameter was affected; movement episodes were suppressed by a 0.5 mg/kg dose of ondansetron (**19**) as shown in Figure 50. The stimulant effect of cocaine was potentiated by addition of the 5-HT_3 receptor antagonist ondansetron (**19**).

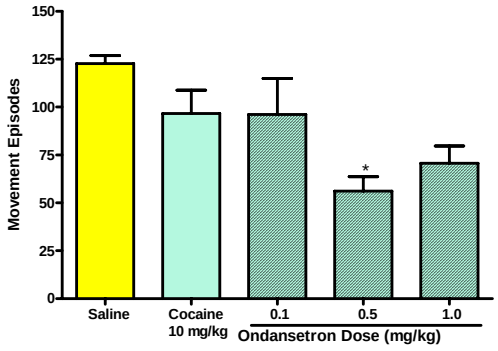


Figure 50. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) on an effective dose of cocaine (**1**) (10 mg/kg; 0-min pre-injection time) observed within the first 15-min of the 45-min protocol (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to a 10 mg/kg dose of cocaine; *P<0.05; one-way ANOVA ($F_{4,40}$ = 8.11 (movement episodes)) followed by a Newman-Keuls post-hoc test.

Within 30 min, only one parameter was affected by the combination of ondansetron (**19**) with cocaine (**1**); when administered together, ondansetron (0.5 and 1.0 mg/kg doses) potentiated the effect of cocaine on center entries as shown in Figure 51.

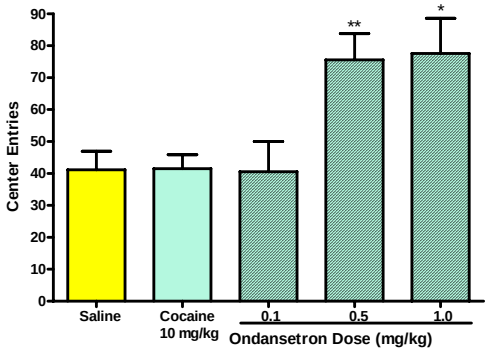


Figure 51. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) on a 10 mg/kg dose of cocaine (**1**) (0-min pre-injection time) observed within 30-min of the 45-min protocol (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to a 10 mg/kg dose of cocaine; *P<0.05 and **P<0.01; one-way ANOVA ($F_{4,40}$ = 5.81 (center entries)) followed by Newman-Keuls post-hoc test.

Within 45 min of initiation of the experiment, the effect of ondansetron (**29**) on cocaine (**1**) shifted to margin distance. The effect of cocaine was suppressed by 0.1 and 1.0 mg/kg of ondansetron in an inverted U-shape dose response manner as shown in Figure 52.

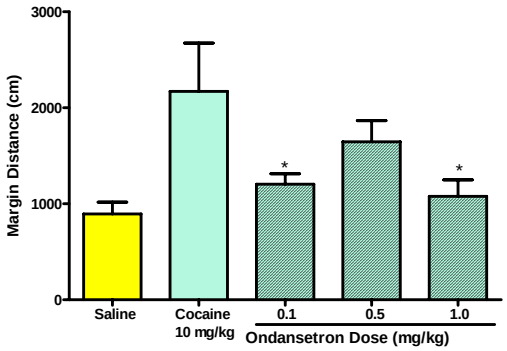


Figure 52. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) on a 10 mg/kg dose of cocaine (**1**) (0-min pre-injection time) observed within 45-min (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to a 10 mg/kg dose of cocaine; *P<0.05; one-way ANOVA ($F_{4,40}$ = 4.59 (margin distance)) followed by a Newman-Keuls post-hoc test.

Only one parameter was affected by the combination of ondansetron with cocaine in each 15-min interval. However, upon analysis of the entire study, combination of varying doses of ondansetron (**19**) with an effective dose of cocaine (**1**) neither potentiated nor antagonized the effect of cocaine when administered alone. This was observed for all parameters analyzed for total time of the experiment (movement episodes, movement time, movement distance, margin distance, margin time, center distance, and center time as shown in Figure 53 and Figure 54) except center entries which were potentiated at 0.5 and 1.0 mg/kg doses of ondansetron. Although statistically significant changes were not observed, the effects of ondansetron on cocaine were dose dependent.

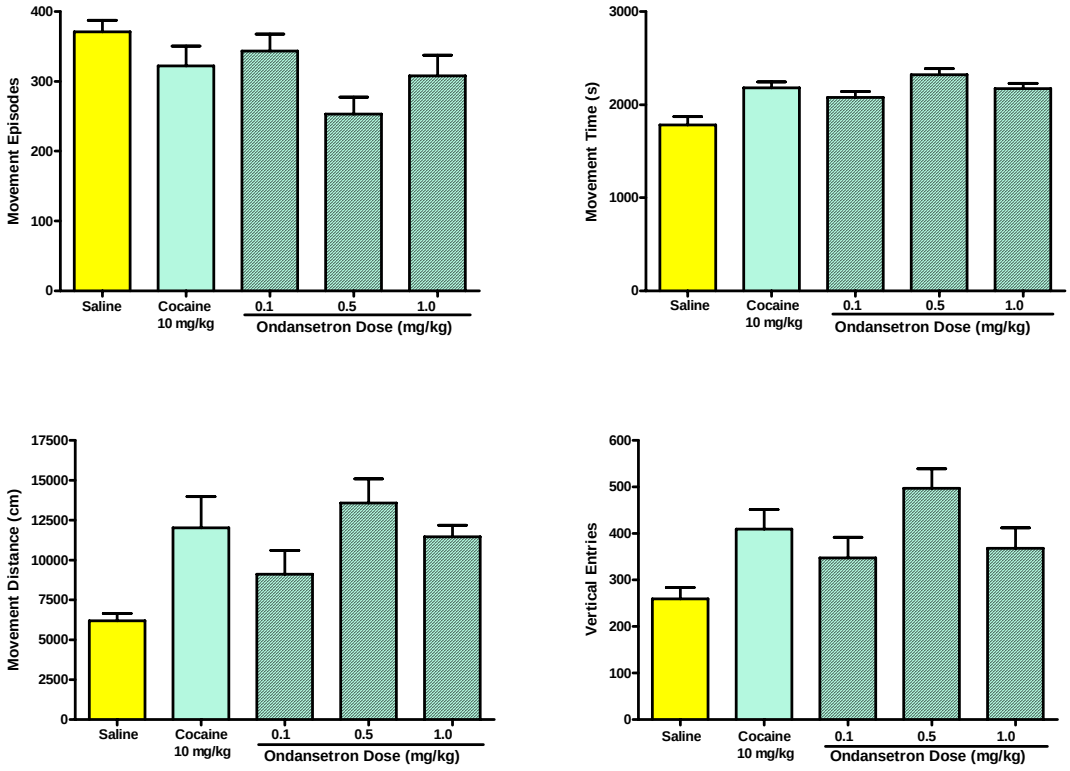


Figure 53. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) in combination with an effective dose of cocaine (**1**) (0-min pre-injection time) on total movement episodes, total movement distance, total movement time, total vertical entries with a 45-min recording-time (n = 6-8 mice/treatment).

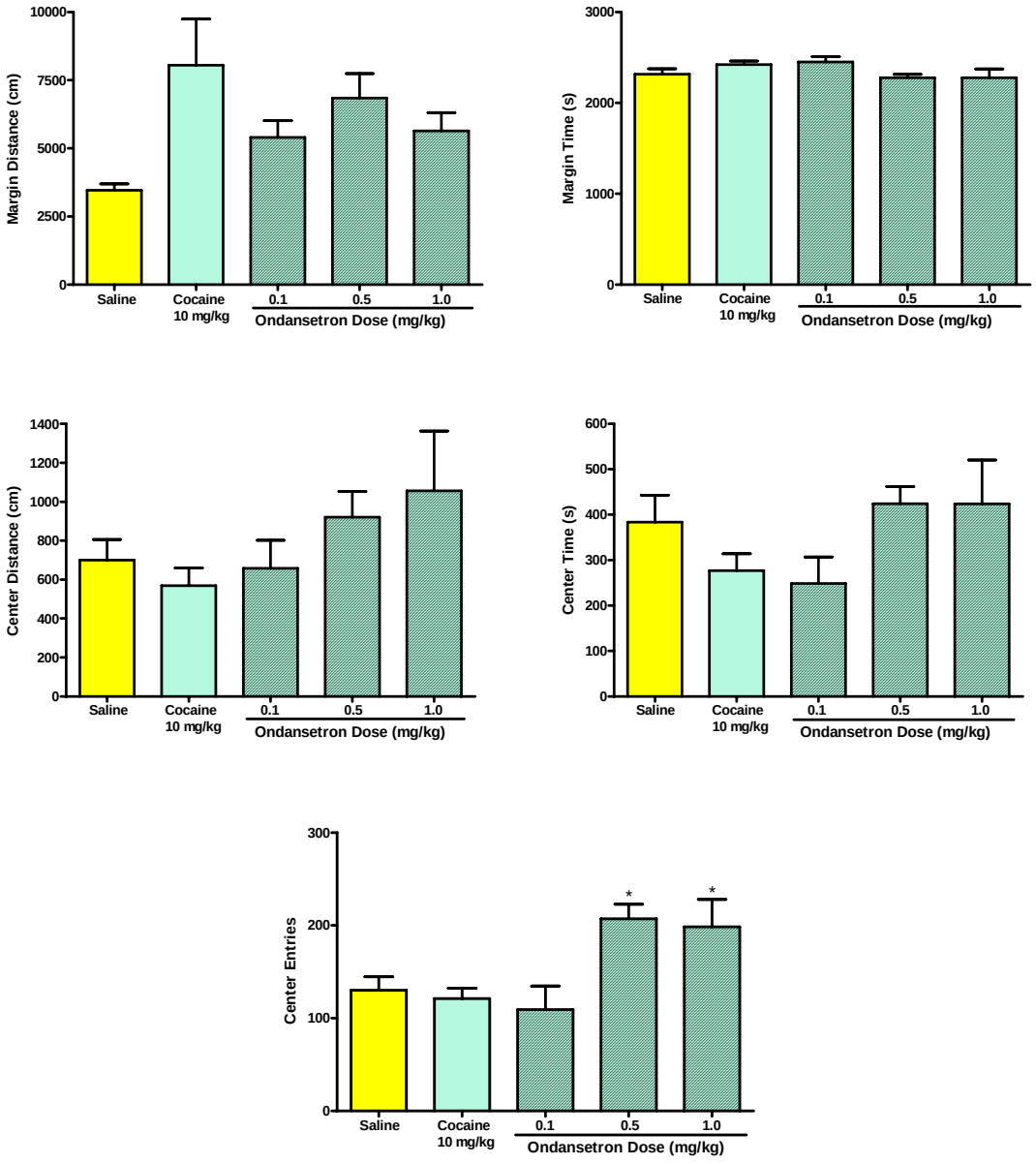


Figure 54. Effect (± S.E.M.) of varying doses of ondansetron (**19**) (30-min pre-injection time) in combination with an effective dose of cocaine (**1**) (0-min pre-injection time) on total margin distance, total margin time, total center distance, total center time, and total center entries with a 45-min recording-time (n = 6-8 mice/treatment).

iv. Combination of Cocaine (1) and SR 57227A (28)

Combination studies of the 5-HT_3 receptor agonist SR 57227A (**28**) with an effective dose of cocaine on locomotor activity were conducted. The present investigation employed 1.0, 3.0, and 10 mg/kg doses of SR 57227A (**28**) (30-min pre-injection time) in combination with a 10 mg/kg dose of cocaine (**1**) (0-min pre-injection time) administered i.p. The recording-time of the experiment was 45 min. Within the first 15 min, the stimulant effect of cocaine was potentiated as evidenced by increasing the parameters of movement episodes and movement time. This effect was observed following all three administered doses of SR 57227A (**28**) in combination with cocaine as opposed to the effect observed with cocaine (**1**) administered alone (Figure 55). Also, within the first 15-min interval, the effect of cocaine was antagonized by a 1.0 mg/kg dose of SR 57227A. When administered alone, cocaine (**1**) suppressed center entries, however in combination with SR 57227A (**28**), the number of center entries was increased (Figure 55).

These were the only parameters influenced by the combination of varying doses of SR 57227A with an effective dose of cocaine. For the duration of the experiment, the combination of SR 57227A (**28**) and cocaine (**1**) behaved similarly to cocaine administered alone for all parameters analyzed (movement episodes, movement time, movement distance, vertical entries, margin distance, margin time, center distance, center time and center entries as shown in Figure 56 and Figure 57).

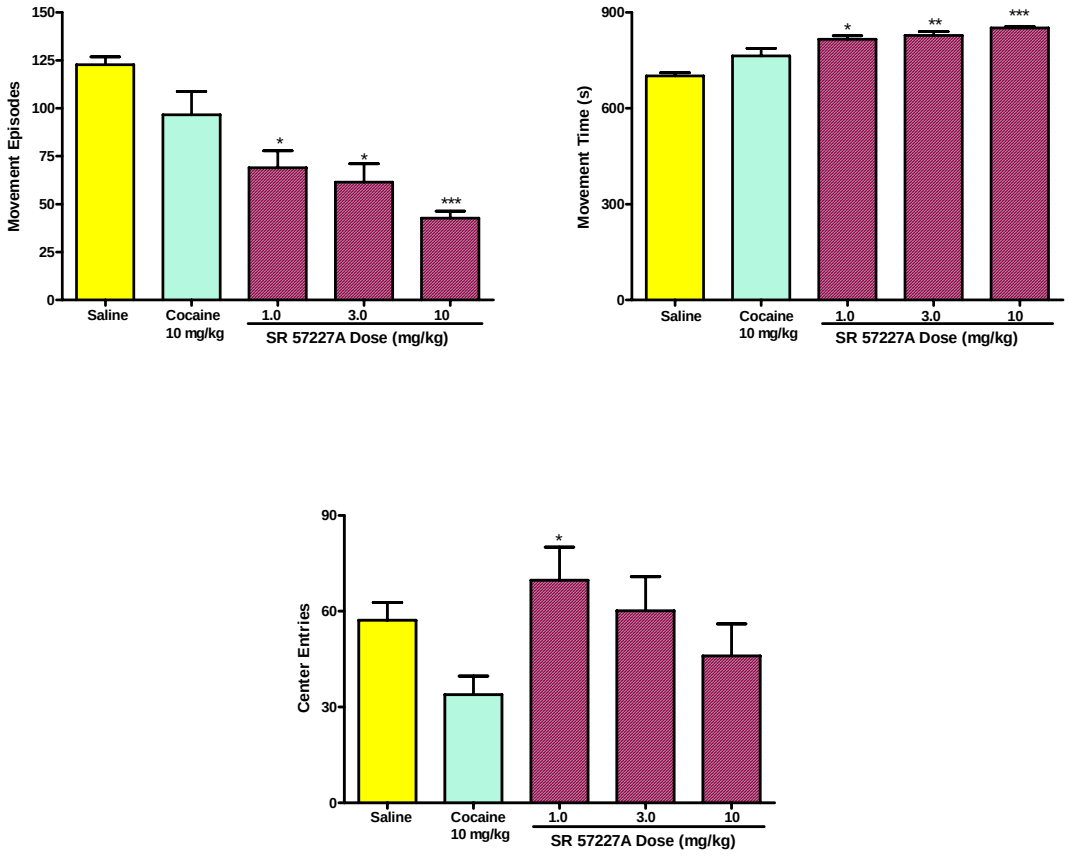


Figure 55. Effect (± S.E.M.) of SR 57227A (**28**) (30-min pre-injection time) on an effective dose of cocaine (**1**) (0-min pre-injection time) within the first 15-min of the experiment of a 45-min protocol (n = 6-8 mice/treatment). Asterisk denotes statistical significance compared to a 10 mg/kg dose of cocaine; *P<0.05, **P<0.01, ***P<0.001; one-way ANOVA ($F_{4,40}$ = 20.38 (movement episodes), $F_{4,40}$ = 25.01 (movement time), $F_{4,40}$ = 2.55 (center entries)) followed by Newman-Keuls post-hoc test.

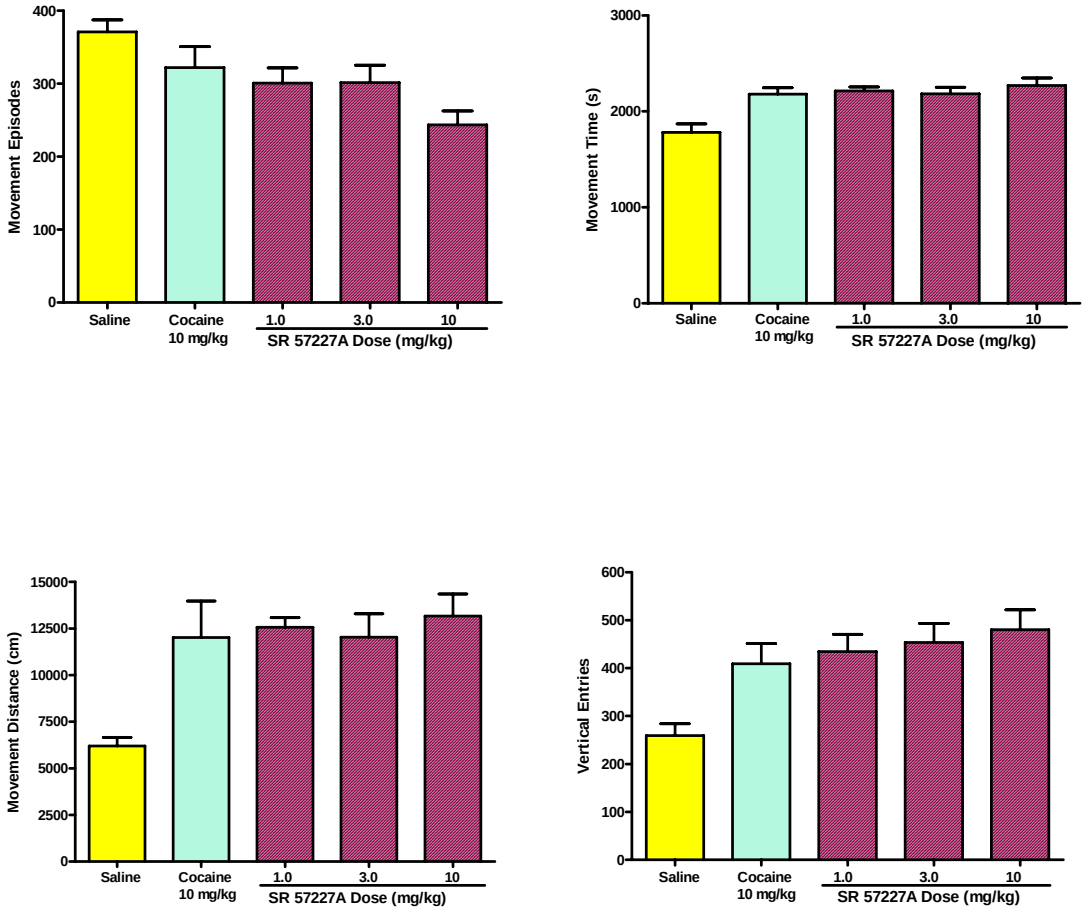


Figure 56. Effect (± S.E.M.) of SR 57227A (**28**) (30-min pre-injection time) in combination with cocaine (**1**) (0-min pre-injection time) on total movement episodes, total movement distance, total movement time, and vertical entries with a 45-min recording-time (n = 6-8 mice/treatment).

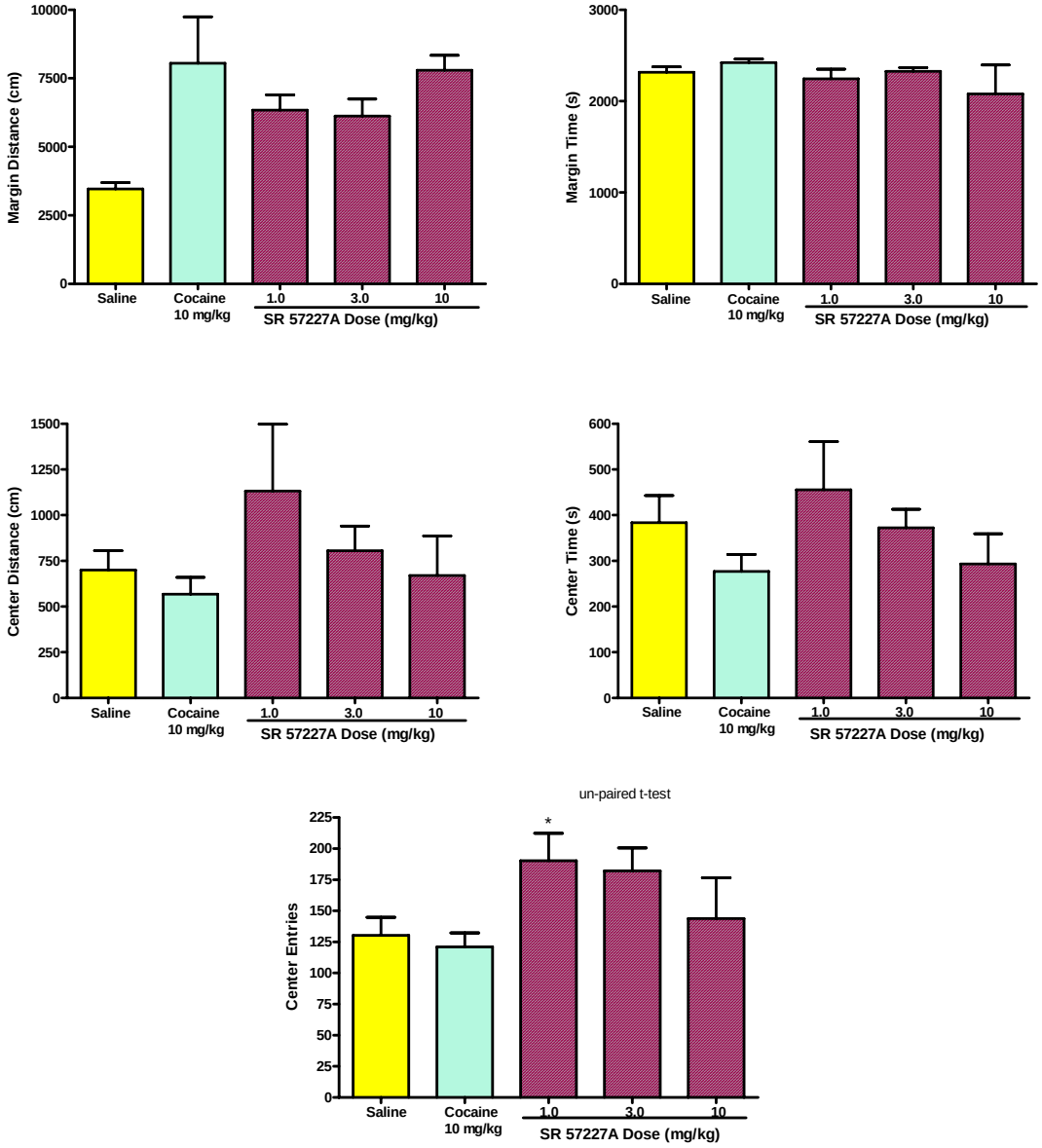


Figure 57. Effect (± S.E.M.) of SR 57227A (**28**) (30-min pre-injection time) in combination with cocaine (**1**) (0-min pre-injection time) on total margin distance, total margin time, total center distance, total center time, and total center entries with a 45-min recording-time (n = 6-8 mice/treatment).

2. Discussion

The purpose of the present study was to determine the influence of the 5-HT_3 receptor partial agonist MD-354 on the locomotor actions of psychomotor stimulants (as 5-HT_3 receptor ligands have been shown to indirectly affect dopamine levels) and if this effect could be characterized as acting through a 5-HT_3 receptor agonist or 5-HT_3 receptor antagonist mode of action. The present investigation was conducted in order to determine the locomotor effects of the phenylalkylamine stimulant (+)amphetamine (**2**) in combination with MD-354 (**42**), ondansetron (**19**), and SR 57227A (**28**) as compared to similar tests using cocaine (**1**) (a non-phenylalkylamine stimulant) with the same 5-HT_3 receptor ligands. (+)Methamphetamine (**3**) (a structurally similar amphetamine phenylalkylamine stimulant) and DOM (**10**) (a phenylalkylamine non-stimulant used as a control) were also administered in combination with MD-354 to determine the effects of MD-354 in combination with these drugs of abuse, on locomotor activity. The overall effects of these agents and drug combinations are shown in Table 5.

Varying doses of the 5-HT_3 receptor ligands were administered alone to determine whether or not they bestowed an effect versus saline. When administered alone, MD-354 had no effect on mouse locomotor activity (Table 5). These data are consistent with and further extend earlier findings. Dukat *et al.* investigated the effect of MD-354 (**42**) on mouse locomotor activity at doses ranging from 1.0 to 10 mg/kg; results suggested that MD-354 produces saline-like effects on locomotor activity.[12] Ondansetron was found to behave similarly to MD-354; the saline-like effect of ondansetron was consistent with

Table 5. Summary of the effects of all drugs tested in the mouse locomotor activity assay.[a]

Measure:	Movement Episodes	Movement Time	Movement Distance	Vertical Entries	Margin Distance	Margin Time	Center Distance	Center Time	Center Entries
MD-354	-	-	-	-	-	-	-	-	-
Ondansetron	-	-	-	-	-	-	-	-	-
SR 57227A	-	-	-	-	-	-	-	-	↑
(+)Amph	↓	↑	↑	-	↑	-	-	-	-
+ *MD-354*	-	-	-	-	-	-	-	-	-
+ *Ondan*	-	-	-	-	↓	↓	↑	-	-
+ *SR 57227A*	↓	↑	↑	-	↑	-	-	-	-
(+)Meth	↓	↑	↑	↑	↑	-	-	-	↑
+ *MD-354*	-	-	-	-	-	-	-	-	-
DOM	-	↑	↑	↑	↑	-	↓	-	-
+ *MD-354*	-	↓	↓	↓	↓	-	↓	-	↓
Cocaine	↓	↑	↑	↑	↑	↑	↓	↓	-
+ *MD-354*	↓	↑	-	↑	-	-	↑	-	↑
+ *Ondan*	↓	-	-	-	↓	-	-	-	↑
+ *SR 57227A*	↓	↑	-	-	-	-	-	-	↑

[a.] The "-" symbol indicates no change in effect compared to control, the "↑" symbol indicates an increase in effect versus control, and the "↓" indicates a decrease in effect versus control. See text for a more detailed description and discussion of the effects.

that which was found in the literature. Ramamoorthy *et al.* published a paper discussing the antidepressant potential of the 5-HT_3 receptor antagonist ondansetron (**19**).[137] In this study, locomotor activity was characterized as "locomotor scores", analyzing various doses of ondansetron (0.005-1000 μg/kg).[137] For the entire dose range, ondansetron produced saline-like effects in mice.[137] Analysis of SR 57227A, when administered alone, also resulted in saline-like effects, with the exception of center entries (Table 5); this effect was only statistically significant when analyzed using the t-test instead of a one-way ANOVA which was employed for testing the significance of other parameters. The saline-like effect of SR 57227A is consistent with literature findings. Yoo *et al.* analyzed the effect of SR 57227A (**28**) on locomotor activity and showed that SR 57227A did not produce locomotor stimulation when administered alone on the stimulant parameter of distance traveled (movement distance).[138] For all three 5-HT_3 receptor ligands, the results observed in the present investigation are consistent with literature findings, even though not exactly the same types of parameters were measured. The 5-HT_3 receptor ligands are considered to not display any effect on mouse locomotor activity when administered alone.

For each drug of abuse, varying doses were administered in order to determine an effective dose of each drug. An effective dose of (+)amphetamine (**2**) was determined to be 3.0 mg/kg as this dose (as well as a 6.0 mg/kg dose) increased the effect on the common stimulant parameters of movement time and movement distance, while decreasing movement episodes. Movement episodes are counted as one movement episode being equivalent to the entirety of the movement between the initial start until the

rodent stops. Therefore, if the mouse is continuously walking, movement time and distance would increase, whereas movement episodes would decrease. The effect on margin distance was also potentiated by 3.0 and 6.0 mg/kg doses of (+)amphetamine (**2**), suggesting that the activity of the rodent was not only stimulated by (+)amphetamine, but the rodent also began to display anxiogenic-like behavior. Mcgeehan *et al.* showed that the locomotor activity parameter average distance traveled was significantly increased following administration of 3.0 and 5.0 mg/kg doses of (+)amphetamine versus saline.[139] Data from Glennon *et al.* further support this idea as locomotor activity (measured as interruptions of photocell beam breaks) at even lower doses (1.0 mg/kg doses) of racemic amphetamine injected i.p. increased locomotion in mice.[140] However, doses of 0.3 mg/kg produced saline-like effects.[140] It is important to note, although Glennon's study was conducted using racemic amphetamine instead of one isomer, such as (+)amphetamine (**2**) used in the current study, the use of a different type of locomotor activity apparatus might account for the lower effective dose observed in the Glennon studies.[140] Bushnell also reported on the dose-dependent stimulant response of (+)amphetamine (0.3 to 10 mg/kg i.p.) on locomotor activity in mice;[141] the findings of the present investigation were consistent with the literature findings. It is important to note that an immense amount of research has been published (thousands of papers) on dose response curves of all four drugs ((+)amphetamine, (+)methamphetamine, DOM, and cocaine) in locomotor activity assays using both mice and rats. Since this literature is so exhaustive, only a few references have been cited. But, in general where literature data are available, they are not inconsistent with the present findings.

With many drugs of abuse, dose-response studies can be conducted to demonstrate how increasing doses have an effect on particular parameters, such as increasing movement distance with increasing doses of (+)amphetamine (**2**). Eventually, however, a threshold is reached, where the dose of administered drug results in stereotypy. In dose response curves this is often observed as either a plateau or even a decrease in effect, creating an inverted U-shaped curve.[126]

Since MD-354 (**42**) is a 5-HT_3 receptor partial agonist, it has the potential to behave as either an agonist or an antagonist, and could possibly potentiate or antagonize the effect of (+)amphetamine (**2**) when administered in combination by indirectly influencing dopamine levels. Therefore, both an effective dose (3.0 mg/kg) and moderate dose (2.0 mg/kg) of (+)amphetamine (**2**) were used in combination with MD-354 (**42**), in case MD-354 was to potentiate the effect of (+)amphetamine to the point of stereotypy. Drug discrimination studies in rats have been conducted using similar dose combinations of (+)amphetamine and MD-354; data showed MD-354 enhances the stimulus effects of moderate doses of (+)ampethamine.[142] Although there is a difference in species and type of study conducted, the results support the notion that MD-354 can potentiate a behavioral action of (+)amphetamine. However, in the present investigation, neither dose combination resulted in potentiation or antagonism of the (+)amphetamine (**2**) effect when compared to the effect of the stimulant administered alone. These results were observed both with stimulant and non-stimulant parameters throughout the entirety of the experiment.

For comparison, an effective dose of (+)amphetamine (**2**) (3.0 mg/kg) was administered in combination with the 5-HT_3 receptor antagonist ondansetron (**19**). Costall *et al.* indicated that the 5-HT_3 receptor antagonist GR38032F (known as ondansetron) inhibited hyperlocomotion (measured as counts) induced by intra-accumbens injections in rats.[131] However in the present investigation, the 5-HT_3 receptor antagonist ondansetron did not behave in a similar manner when administered in combination with (+)amphetamine. Similar to the results observed with a combination of MD-354 (**42**) and (+)amphetamine, ondansetron (**19**) neither potentiated nor antagonized the stimulant effect of (+)amphetamine (**2**) compared to (+)amphetamine administered alone. This effect was observed throughout the entirety of the experiment for all stimulant parameters. However, a 1.0 mg/kg dose of ondansetron was able to potentiate the effect of (+)amphetamine on the parameter center distance; that is, ondansetron in combination with (+)amphetamine displayed an anxiolytic-like effect. The difference in effect between the present investigation and that observed by Costall *et al.*[131] could be due to the difference in species, route of administration, and method of determining locomotor activity. With the exception of this non-stimulant parameter (center distance), it was concluded that in combination with (+)amphetamine (**2**), MD-354 (**42**) is either behaving similar to a 5-HT_3 receptor antagonist, or MD-354 is devoid of action. Since the effects of the combination of MD-354 and (+)amphetamine on locomotor activity parameters were similar to those observed with the combination of ondansetron and (+)amphetamine, the conclusion that MD-354 is behaving in a similar

manner to a 5-HT_3 receptor antagonist is supported. However, the lack of potentiation or antagonism of effect could also suggest MD-354 is devoid of action.

Combination of an effective dose of (+)amphetamine (**2**) with the 5-HT_3 receptor agonist SR 57227A (**28**) was examined. Whereas neither MD-354 (**42**) nor ondansetron (**19**) altered the effect of (+)amphetamine (**2**) when administered in combination, SR 57227A (**28**) influenced the effect of a moderate dose of (+)amphetamine (2.0 mg/kg). A moderate dose of (+)amphetamine was used instead of the effective dose in case the 5-HT_3 receptor agonist potentiated the stimulant effect of (+)amphetamine (**2**) to the point of stereotypy. The four parameters that were affected by administration of (+)amphetamine (**2**) alone at a 3.0 mg/kg dose were also affected when (+)amphetamine was administered in combination with SR 57227A (**28**). The combination of SR 57227A (3.0 and 10 mg/kg doses) with (+)amphetamine potentiated the effect (+)amphetamine on movement episodes (i.e., the number of movement episodes was decreased). This potentiation was more significant at the 3.0 mg/kg SR 57227A dose than the 10 mg/kg dose. The combination of SR 57227A (**28**) with (+)amphetamine (**2**) also potentiated the effect of (+)amphetamine on the stimulant parameters of movement time and movement distance, as well as the non-stimulant parameter margin distance. This potentiation was observed as an inverted U-shaped curve as the 3.0 mg/kg SR 57227A dose was the only one that potentiated (+)amphetamine's actions on all three parameters. For the other parameters, vertical entries, margin time, center distance, center time, and center entries, the combination of (+)amphetamine (**2**) with SR 57227A (**28**) behaved similarly to

(+)amphetamine when administered alone. Analysis of these results showed that (+)amphetamine when administered alone produced a saline-like effect.

Results from combinations of (+)amphetamine (**2**) (3.0 mg/kg) with varying doses of the 5-HT_3 receptor antagonist ondansetron (**19**) indicated that the combination behaved similarly to the results observed with (+)amphetamine and MD-354. The 5-HT_3 receptor agonist SR 57227A (**28**) behaved differently than either MD-354 (**42**) or ondansetron (**19**) when administered in combination with (+)amphetamine (**2**). That is, SR 57227A potentiated certain aspects of (+)amphetamine-induced locomotor stimulation, whereas both MD-354 and ondansetron in combination with (+)amphetamine produced (+)amphetamine-like effects. Hence, two conclusions are possible. Either MD-354 lacks sufficient agonist potential to mimic the actions of SR-57227A, or too little MD-354 is penetrating the BBB to reach its central sites of action, as drug discrimination studies suggest that MD-354 is centrally acting;[142] however, peripheral effects cannot be excluded.

(+)Methamphetamine (**3**), a psychomotor stimulant structurally similar to (+)amphetamine, was also tested in locomotor activity assays. Literature states that (+)methamphetamine is a more potent central stimulant than (+)amphetamine (**2**);[16] similar central stimulant effects should be evident at lower doses. This idea was supported as stimulant measures were increased at a 1.5 mg/kg dose of (+)methamphetamine as compared to 3.0 mg/kg dose of (+)amphetamine. Doses of (+)methamphetamine (**3**) increased movement time and movement distance while suppressing movement episodes; this was similar to the results observed following

administration of an effective dose of (+)amphetamine (**2**) alone. These effects also created an inverted U-shaped curve as doses of 1.5 mg/kg increased stimulant parameters, where a dose of 10 mg/kg had less of an effect; in some cases, no effect was observed. Not only did (+)methamphetamine (**3**) potentiate the effect on stimulant parameters, but lower doses (as low as 0.3 mg/kg) somewhat potentiated the three key stimulant parameters (movement episodes, movement distance, and movement time) as well as vertical entries, margin distance, and center entries after the first 15 min of the experiment. Glennon *et al.* analyzed racemic methamphetamine in similar male ICR mice and also observed an increase in locomotion (measured as interruptions of photocell beams) at 1.0 mg/kg and above, whereas a 0.3 mg/kg dose of racemic methamphetamine produced a saline-like effect.[140] Once again, variances in effective dosage amounts could be due to the use of racemic methamphetamine (**3**) as compared to its more potent isomer.

In the present investigation, center entries was the only parameter increased by (+)methamphetamine (**3**) that was not affected by (+)amphetamine (**2**), suggesting that (+)methamphetamine is behaving more in an anxiolytic nature than (+)amphetamine. However, both (+)methamphetamine and (+)amphetamine, at all doses administered, produced saline-like effects on margin time, center distance, and center time.

Studies with 1.5 and 3.0 mg/kg doses of (+)methamphetamine (**3**) in combination with varying doses of MD-354 (**42**) were conducted to determine if MD-354 was behaving similarly to (+)amphetamine in combination with a structurally similar psychomotor stimulant. For all combinations, MD-354 (**42**) plus (+)methamphetamine

(**3**) produced results similar to those observed following administration of (+)methamphetamine alone; this was observed for all nine parameters analyzed for the entire duration of the experiment. Data from the combination of MD-354 (**42**) and (+)methamphetamine (**3**) paralleled the results observed with combinations of MD-354 and (+)amphetamine (**2**). Apparently, the effect of MD-354 on the motor actions of (+)methamphetamine and (+)amphetamine are similar.

DOM (**10**), a non-stimulant phenylalkylamine structurally similar to amphetamine was used as a control. Supposedly lacking significant central stimulant properties, DOM was not expected to influence "stimulant" measures of activity. However, it has been previously shown that hallucinogens can produce both a stimulant effect in locomotor activity assays as well as stimulus generalization; stimulus effects can be block by serotonin antagonists.[54]

Results of the present investigation showed that DOM (**10**) at a low dose (0.3 mg/kg) significantly increased vertical entries. This is an activity measure that was not affected by administration of either the psychomotor stimulant (+)amphetamine (**2**) or (+)methamphetamine (**3**). Also, DOM doses did not have an effect on movement episodes, which was decreased by varying doses of psychomotor stimulants. This increase in vertical entries, as well as no effect on movement episodes differentiates DOM from the psychomotor stimulants. Other parameters affected by the administration of DOM (**10**) included movement time, movement distance, and margin distance suggesting the drug displayed some stimulant activity, which is consistent with literature that states that low doses of DOM may produce stimulant effects.[3] Yamamoto and Ueki

analyzed the effect of DOM on locomotor activity in mice, and observed increases in locomotor activity at 0.5 -1.0 mg/kg doses injected i.p.[3] However, at 0.1 mg/kg, head twitches, a hallucinogenic parameter was increased.[3] Data observed in the present investigation is consistent with Yamamoto and Ueki's[3] results, although the present study did not analyze head twitches, but rearing as a hallucinogenic parameter.

By increasing vertical entries, the animal is in motion more, which in turn will increase the amount of time the animal is in motion (i.e., movement time). Also, when animals display rearing behavior, it is usually around the margins of the chamber, as the mice look as if they are trying to climb out of the chamber; this can increase both margin distance, margin time, and movement distance. Therefore, as vertical entries increase, it is not uncommon for movement time, movement distance, margin distance, or margin time to also increase. Further analysis of the effects of varying doses of the hallucinogen DOM (**10**) showed results similar to those observed following administration of (+)amphetamine (**2**) and (+)methamphetamine (**3**) (i.e., no effect), as DOM produced saline-like effects for margin time, center distance, center time, and center entries.

Results of combination studies of an effective dose of DOM (**10**) (0.3 mg/kg) with MD-354 (**42**) differed from those observed from combination of MD-354 with (+)amphetamine (**2**) and (+)methamphetamine (**3**). With both phenylalkylamine stimulants, combination of MD-354 with (+)amphetamine or (+)methamphetamine produced effects similar to the stimulant when administered alone. However, in combination with DOM (**10**), MD-354 (**42**) antagonized stimulant parameters as well as other parameters analyzed. In particular, DOM (**10**) is a hallucinogen; vertical entries

should be increased,[123] which was observed with DOM administered alone as well as in combination with MD-354. However, in combination with MD-354 (**42**), the effect of DOM (**10**) on vertical entries, as well as movement time, movement distance, margin distance, center distance and center entries were all completely blocked, resulting in a saline-like effect. The only parameters not affected by this combination were movement episodes, margin time, and center time, which were saline-like when DOM was administered alone. Literature has shown that 5-HT_2 antagonists block the stimulus effect of DOM,[54] therefore, similar results could be observed with a 5-HT_3 partial agonist.

Cocaine (**1**) was evaluated as a non-phenylalkylamine stimulant that has a different mechanism of action than (+)amphetamine or (+)methamphetamine, to determine if MD-354 (**42**) behaves similarly or differently in combination than with phenylalkylamine stimulants. Administration of varying doses of cocaine (**1**) indicated that the stimulant significantly increased the effect on the same four parameters increased by (+)amphetamine (**2**) and (+)methamphetamine (**3**). These four parameters, movement episodes, movement time, movement distance, and margin distance, were significantly increased at doses 10 mg/kg and 30 mg/kg; stereotypy was not observed at the high (30 mg/kg) dose. The present results are consistent with literature data as Mcgeehan *et al.* showed that the average distance traveled was significantly increased following doses of 15 and 30 mg/kg of cocaine (**1**).[139] Cocaine produced a saline-like effect on total vertical entries, total margin time, total center distance, total center time, and total center entries which coincides with the data found in both the (+)amphetamine (**2**) and (+)methamphetamine (**3**) studies. This indicates that all three psychomotor stimulants are

producing similar locomotor effects, regardless of their differences in mechanism of action, since the overall effect is an increase in synaptic dopamine levels.

Studies were conducted to determine if MD-354 (**42**) behaved similar in combination with cocaine (**1**) to combination with (+)amphetamine (**2**). An effective dose of cocaine (10 mg/kg) was used in combination with the 5-HT_3 receptor partial agonist MD-354 (**42**). Potentiation of the effect of cocaine administered alone was observed when in combination with MD-354 for several parameters: movement episodes, movement time, vertical entries, center distance, and center entries (at various time intervals). A decrease in movement episodes and an increase in movement time and vertical entries was observed at all doses of MD-354 (**42**) in combination with cocaine (**1**). Center distance and center entries were only affected at a higher dose of MD-354. These results were observed at different time intervals. Analysis of the overall effect of the combination indicated that MD-354 potentiated the effect of cocaine on center entries at a 6.0 mg/kg dose. These results differed from those observed with a combination of MD-354 (**42**) and (+)amphetamine (**2**), as the combination with MD-354 produced effects similar to those observed with (+)amphetamine alone.

Combination of the same dose of cocaine (**1**) (10 mg/kg) with the 5-HT_3 receptor antagonist ondansetron (**19**) displayed results similar to the combination of MD-354 (**42**) and cocaine. The effect of cocaine was potentiated in combination with ondansetron (**19**) for movement episodes (i.e., movement episodes decreased). The effect of cocaine was also potentiated in combination with ondansetron for center entries, whereas ondansetron decreased the effect of cocaine on margin distance. The effect of cocaine (**1**)

administered alone increases margin distance; however in combination with ondansetron, the effect was blocked, resulting in a saline-like effect. The effect of ondansetron on cocaine varied between different time intervals. However, analysis of the total effect of the combination of drugs on the nine parameters showed that only center entries were affected at 0.5 and 1.0 mg/kg doses of ondansetron (potentiated the effect of cocaine).

Several different 5-HT_3 receptor antagonists (i.e., zacopride, tropisetron, bemesetron, and ondansetron) have been studied in combination with cocaine, usually resulting in attenuation of the effect of cocaine. Le *et al.* analyzed locomotor activity counts in male DBA/2N mice,[143] which differed from the ICR strain of mice used in the present studies. Low doses of ondansetron (**19**), 0.001, 0.01, and 0.1 mg/kg were administered s.c. in combination with cocaine (**1**) to yield slightly different results from those observed in the present investigation, as the combination of ondansetron did not potentiate or antagonize the stimulant effects of cocaine.[143] The differences in the results could be due to the type of rodent used as well as the route of administration. King *et al.* indicated that varying doses of ondansetron (1.0 -16 mg/kg i.p.) attenuated the effect of cocaine (15 mg/kg i.p.) in male Sprague Dawley rats.[130] Locomotion was measured using the Ellinwood and Balster rating scale.[130] Svingos and Hitzemann conducted a study analyzing the effect of zacopride, tropisetron, and bemesetron on an effective (10 mg/kg) dose of cocaine.[129] All three 5-HT_3 receptor antagonists (injected i.p.) attenuated the effect of cocaine in rats when analyzing locomotion (measured as the number of quadrant crossovers).[129] Further support for attenuation of the effect of cocaine by 5-HT_3 receptor antagonists was determined by Reith.[132] The effect of tropisetron (injected s.c), as well

as zacopride (injected i.p.), in combination with a 25 mg/kg dose of cocaine were determined; combination resulted in attenuation of locomotor counts in male C57BL/6ByJ mice.[132] In the present study, ondansetron behaved similarly to the different 5-HT_3 receptor antagonists discussed by attenuating the effect of cocaine on margin distance. However, other parameters were potentiated by the combination of ondansetron with cocaine. The differences in attenuation of effect observed with several 5-HT_3 receptor antagonists and the present study could be due to route of administration, doses, difference in species, as well as the method used for determining locomotor activity.

For the present study, a combination of cocaine (**1**) with ondansetron (**19**) produced effects similar to those observed with combinations of cocaine and MD-354 (**42**). Analysis of data suggests that MD-354 is behaving similar to the 5-HT_3 receptor antagonist ondansetron when administered in combination with an effective dose of cocaine (**1**). This is because combination studies of MD-354 and cocaine, as well as ondansetron and cocaine, produced similar effects on cocaine. This is similar to the results observed with MD-354 (**42**) in combination with (+)amphetamine (**2**), suggesting that in combination with psychomotor stimulants, regardless of mechanism of action, MD-354 either behaves similar to a 5-HT_3 receptor antagonist or is devoid of action.

A combination study was conducted using the 5-HT_3 receptor agonist SR 57227A (**28**) with a 10 mg/kg dose of cocaine (**1**). The dosage was not altered for cocaine as in the (+)amphetamine (**2**) study, as the cocaine dose-response curve suggested that there was a large range of effective doses of cocaine before the threshold of stereotypy was

reached. The results of the combination study showed that SR 57227A (**28**) potentiated the stimulant effect of cocaine by decreasing movement episodes, and increasing movement time and center entries at various time intervals; these same three parameters were affected in a similar manner by the addition of MD-354 (**42**) to cocaine (**1**), suggesting MD-354 is behaving like a 5-HT_3 receptor agonist. However, analysis of the results of the total effect showed that the only parameter affected by the combination was center entries; SR 57227A potentiated the effect of cocaine on center entries when analyzed using an un-paired t-test.

The results of the present study were further investigated as MD-354 (**42**) was seemingly behaving as both a 5-HT_3 receptor agonist and 5-HT_3 receptor antagonist when analyzing the total effect of each 5-HT_3 receptor ligand in combination with cocaine: analysis of all nine parameters showed that the 5-HT_3 receptor ligands displayed no effect on cocaine except with the parameter center entries. Since similar effects were observed when analyzing total parameters, the effect of the combination of 5-HT_3 receptor ligands on cocaine were analyzed at different time intervals. Combination studies of MD-354 and cocaine, ondansetron and cocaine, and SR 57227A and cocaine all potentiated the effect of cocaine on movement episodes and center entries. Since MD-354 is a 5-HT_3 receptor partial agonist, it has the potential to behave as either/both an agonist and antagonist. It was concluded that in combination with cocaine (**1**), MD-354 (**42**) behaved similarly to a 5-HT_3 receptor agonist, based on the evidence provided by other locomotor activity parameters analyzed. Three parameters, movement episodes, movement time, and center entries were potentiated by both ondansetron (**19**) and

SR57227A (**28**), suggesting MD-354 (**42**) worked as both a 5-HT_3 receptor agonist and a 5-HT_3 receptor antagonist. Ondansetron (**19**) also antagonized the effect of cocaine (**1**) on margin distance. This antagonism was distinct, and not observed in combination studies of cocaine with MD-354 (**42**) or SR 57227A (**28**). By actually antagonizing the effect instead of modulating the effect on margin distance, as compared to MD-354 and SR 57227A studies, the antagonist is blocking effect of the drug. Since neither the 5-HT_3 receptor partial agonist or 5-HT_3 receptor full agonist display this or similar effects (i.e., attenuation of effect of cocaine on margin distance), it is supported that MD-354 (**42**) is working through a 5-HT_3 receptor agonist mechanism in combination with cocaine (**1**). Table 5 summarizes the results of the locomotor activity studies conducted in the present investigation.

For the three psychomotor stimulants ((+)amphetamine (**2**), (+)methamphetamine (**3**), and cocaine (**1**)) margin distance was increased when the drug of abuse was administered alone. This could be a result of the drugs inducing anxiogenic-like behavior. However, this could just be a result of stimulation. It is normal for rodents to display anxiogenic-like activity, which is supported by the high values obtained for margin distance and margin time as opposed to center distance and center time. When a psychomotor stimulant was administered, the mice displayed increased locomotion. If the mice are walking more following the administration of a stimulant, and generally tend to walk around the margins, then it is likely the mice will walk more around the margins when administered a stimulant. The stimulants may be producing anxiogenic-like behavior, but also could be displaying thigmotaxis in a stimulated manner.

In conclusion MD-354 (**42**) may either behave as a 5-HT_3 receptor antagonist in combination with phenylalkylamine stimulants, or be devoid of action as it neither potentiates nor antagonizes effective doses of (+)amphetamine (**2**) nor (+)methamphetamine (**3**). However, in combination with a non-phenylalkylamine stimulant, that releases dopamine through a different mechanism of action, MD-354 (**42**) might behave as a 5-HT_3 receptor agonist, by potentiating several stimulant and non-stimulant parameters as seen with the 5-HT_3 agonist SR 57227A. The differences in mode of action of MD-354 may be a result of the mechanism of action of each stimulant to release dopamine.

MD-354 is a 5-HT_3 partial agonist with a low Log P value suggesting it does not cross the BBB. However, drug discrimination studies, which can indicate whether or not a drug acts centrally (i.e., crosses the BBB), have shown that MD-354 serves as a discriminative stimulus[11] and that MD-354 enhances the discriminative stimulus action of (+)amphetamine in rats.[143] Currently it is unknown whether MD-354 works peripherally. Analysis of ex vivo studies indicated that the 5-HT_3 receptor agonist, SR 57227A, crosses the BBB and acts both centrally and peripherally.[111] Since MD-354 neither potentiated nor antagonized the stimulant effect of (+)amphetamine in the locomotor activity assay it is not displaying a central effect, compared to the central acting 5-HT_3 agonist which potentiated the stimulant effect of (+)amphetamine. Although locomotor activity assays are in vivo studies that analyze central effects, the possibility of a peripheral effect of MD-354 can not be excluded.

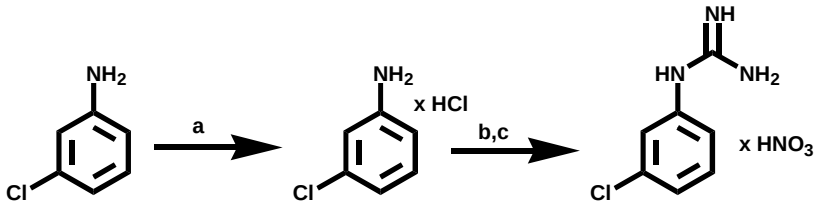
Comparison of MD-354 and (+)amphetamine with MD-354 and cocaine suggests differences in mode of action, as MD-354 potentiated the effect of cocaine on various parameters, at various time intervals. Similar effects were observed with SR 57227A suggesting that in combination with a stimulant that has a different mechanism of action, MD-354 may behave both centrally and peripherally.

In conclusion, in combination with phenylalkylamine stimulants, the 5-HT_3 receptor partial agonist MD-354 may be devoid of action because it is not lipophilic enough to cross the BBB, crosses the BBB, but at a dose too low to cause an effect if MD-354 is a centrally acting agent, or is behaving similar to a 5-HT_3 receptor antagonist. However, in combination with a non-phenylalkylamine stimulant, MD-354 behaves similar to SR 57227A which acts both centrally and peripherally.

B. Synthesis

1. Preparation of phenyl carbamate analogs of MD-354.

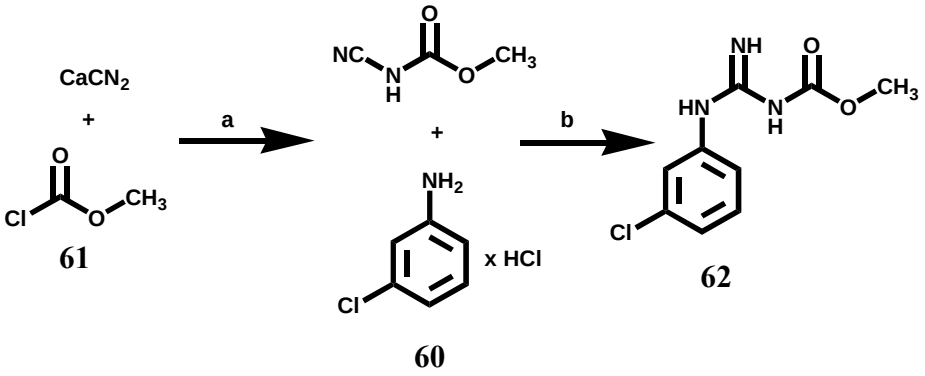
MD-354 (**42**) was prepared as previously described (Scheme 1).[144]



59 **60** **42**

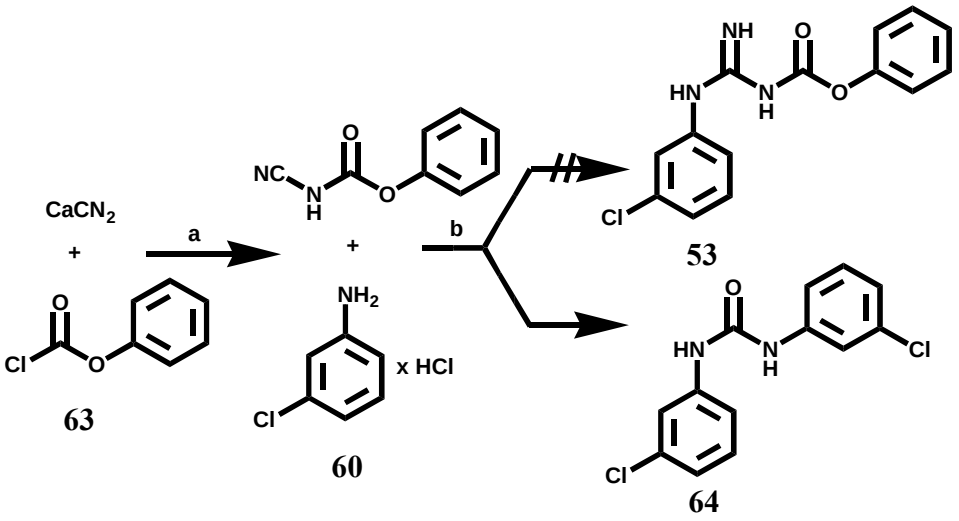
Scheme 1. a. HCl/Et_2O; b. NH_2CN, EtOH, reflux; c. NH_4NO_3, H_2O.

Several different methods were used to synthesize carbamate analogs of MD-354. For the methyl carbamate analog **62**, a one-pot synthesis by Khasanov[145] was employed (Scheme 2).



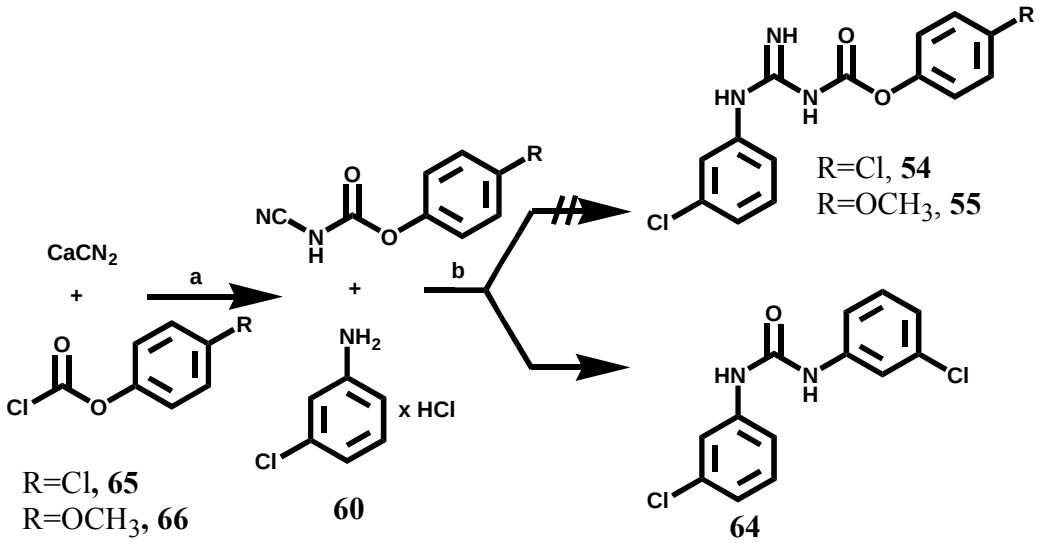
Scheme 2. a. H_2O, 45 °C; b. 40% NaOH, reflux, 4.5 h.

The first attempt to prepare the phenyl carbamate **53** was to follow the same method used in the synthesis of the methyl carbamate **62** (Scheme 3).[145] Calcium cyanamide was allowed to react with phenyl chloroformate (**63**). 3-Chloroaniline hydrochloride (**60**) was added to the filtrate and the pH was adjusted to 4. The precipitate was collected by suction filtration and recrystallized from anhydrous MeOH. ^{1}H NMR spectral analysis indicated that the product was not the desired carbamate **53**, but a dimer of 3-chloroaniline, *N,N'*-bis(3-chlorophenyl)urea (**64**). Urea **64** is a known[146] compound. Comparison of the melting point of **64** with literature data supported this characterization.[146] The structure of **64** was also supported by microanalysis for C, H, and N.



Scheme 3. a. H_2O, 45 °C, 30 min; b. 40% NaOH, reflux overnight.

This same procedure was followed using 4-chlorophenyl chloroformate (**65**) and 4-methoxyphenyl chloroformate (**66**) (Scheme 4). In both instances, the resultant white precipitate was also identified as the 3-chloroaniline dimer **64**.



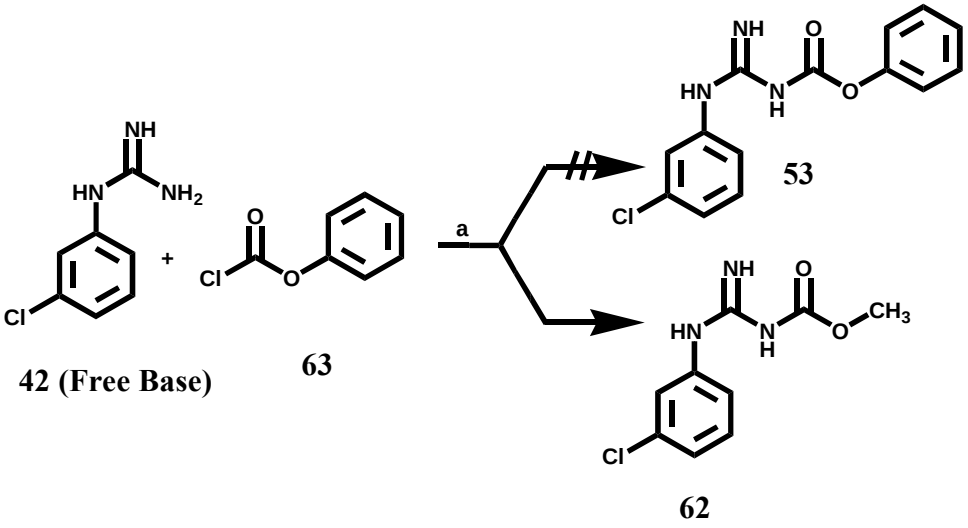
Scheme 4. a. H_2O, 45 °C, 30 min; b. 40% NaOH, reflux overnight.

In general, there are numerous methods available to synthesize ureas. For the most part, ureas are extremely stable, and most of the routes of synthesis are very clean and easy to perform.[147] Interestingly, one of the common methods to synthesize ureas includes carbamates as starting material. Liu *et al.* reported a two-step synthesis which used a chloroformate and an amine to obtain a carbamate, followed by the addition of an amine to form a urea.[147] This reaction could be conducted both in organic and aqueous solvents. Liu *et al.* also stated that the reaction of isocyanates with amines can also produce ureas under mild conditions.[147]

Therefore, with the reaction conditions used in Scheme 3 and Scheme 4, the desired carbamate may have been synthesized, but was quickly converted to the urea, as urea may be a more stable compound than the phenyl carbamate **53**. Further analysis of the reaction mechanism showed that several side products other than the desired phenyl carbamate can be obtained (i.e., isocyanate derivative). If an isocyanate had been formed, it too could have been converted into the urea in the presence of excess amine. Since the reaction yielded several products (tlc), which could include both the isocyanate (and infrared analysis of the crude reaction mixture showed the possible formation of an isocyanate intermediate as evidenced by a band at 2359 cm^{-1}), the desired carbamate, and unreacted amine, conditions employed during purification might have resulted simply in the isolation of one of these products, the urea **64**.

Another attempt was made to synthesize the phenyl carbamate analog of MD-354 using a procedure by Naiman (Scheme 5).[148] In this procedure, MD-354 (free base; **42**) was added to a solution of triethylamine and dry THF. A THF solution of phenyl

chloroformate (**63**) was added to the reaction mixture and the reaction was monitored by tlc. The triethylamine hydrochloride precipitate (mp = 261 °C)[149] was removed by filtration and the solvent was evaporated to obtain an oil which was subjected to flash chromatography. Three products were obtained: two were unstable and decomposed rapidly upon standing as evidenced by tlc. A third product was recrystallized from anhydrous MeOH. ^{1}H NMR spectral analysis of the latter product indicated the presence of three aliphatic protons (signal at δ = 3.60 ppm) that corresponded to the methyl group of methyl carbamate **62**. Melting point and tlc analysis further supported this conclusion: R_f = 0.35 (3:2 hexane:EtOAc).



Scheme 5. a. Et_3N, THF; crystallized from MeOH.

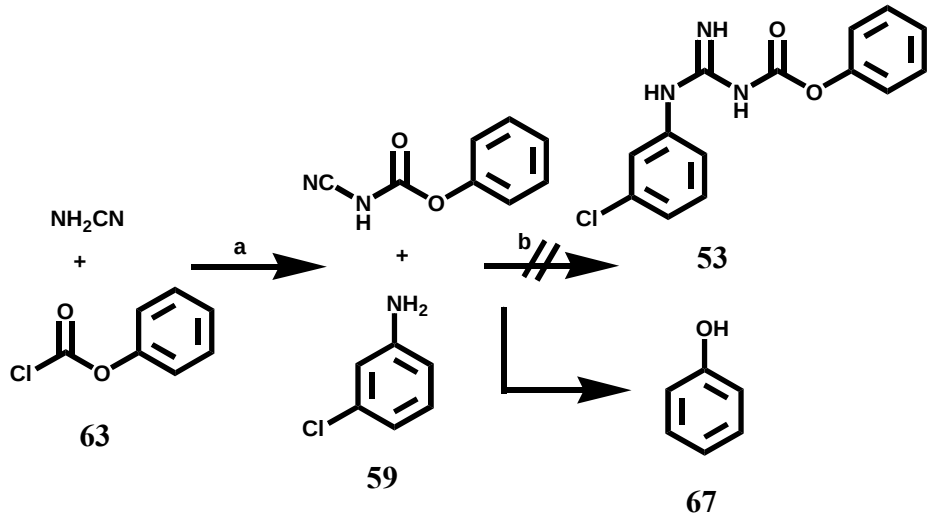
Looking back at the previous reaction, tlc analysis indicated that a significant amount of starting material was still present after the 15-min reaction time. Therefore, the reaction was repeated (Scheme 5), but allowed to continue until all the starting material was consumed (17 h). Several additional products were formed (tlc). The

resultant oil was crystallized from anhydrous MeOH several times to yield a white solid. ^{1}H NMR spectral analysis and melting point indicated that the methyl carbamate **62** was obtained, again with three aliphatic proton signals present in the ^{1}H NMR spectrum. Based on this information, it was thought that the desired product could more easily be isolated using a shorter reaction time; this was employed in future trials. However, higher yield of the methyl carbamate **62** was obtained during purification using recrystallization methods (17%) as opposed to flash chromatography (3%).

Synthesis of the methyl carbamate **62** from phenyl chloroformate **63** indicated that methanolysis might be occurring. Two separate purification techniques were employed to determine if heat was necessary for this to occur. The reaction was repeated and the resultant oil was divided into two batches. One batch crystallized upon addition of hot MeOH, which yielded the methyl carbamate. The other batch was subjected to MeOH at room temperature. A solid material precipitated from the addition of MeOH, but could not be purified. This supported the conclusion that heat is necessary for methanolysis to occur because methyl carbamate **62** was not isolated from the latter reaction.

The next attempt to synthesize the phenyl carbamate analog of MD-354 used the procedure of Gotz and Zeeh[150] (Scheme 6). This method consisted of adding cyanamide to water while maintaining a pH of 7-8. Phenyl chloroformate (**63**) was added to the reaction mixture, and was allowed to stir, followed by the addition of 3-chloroaniline (**59**); pH was adjusted to 3. Basification of the filtrate resulted in immediate precipitation of a white solid (NaCl; mp > 300 ˚C). A second product was present on tlc which was

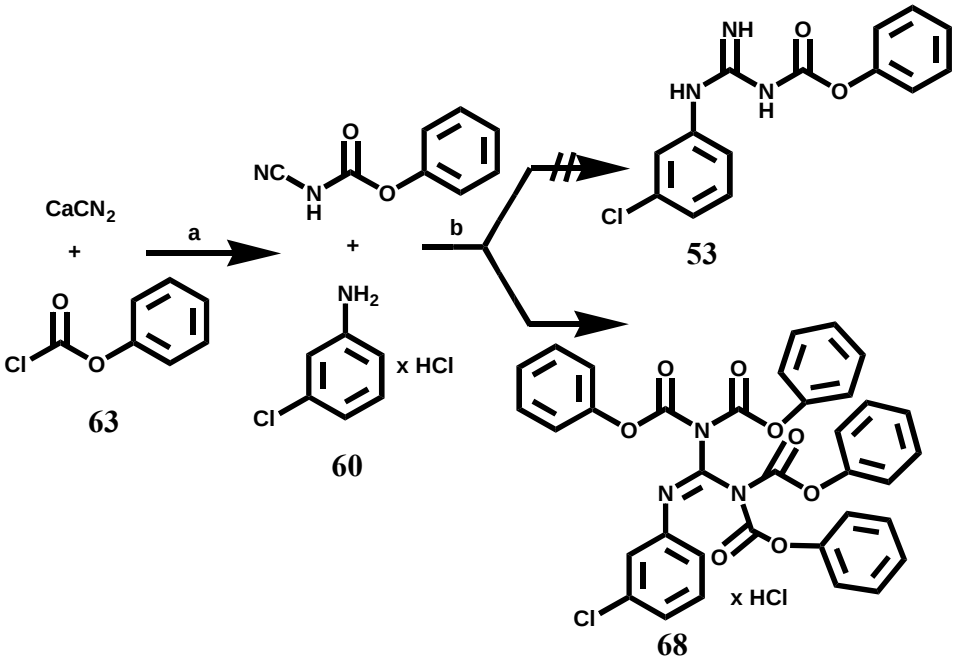
determined to be phenol (**67**); R_f = 0.83 (3:2 hexane:EtOAc). Synthesis of phenol and NaCl prevented the reaction from proceeding to completion.



Scheme 6. a. H_2O, 50% NaOH, conc. HCl (pH 7-8), 45 °C, 1 h; b. conc. HCl (pH 3), reflux, 45 min.

Further analysis of the literature suggested that commercially available phenyl chloroformate is not very stable, and could be hydrolyzed to phenol or decompose in the presence of water, heat, or air, all three of which were present in the previous attempts.[151] Therefore, the reaction conditions for the previous reactions were modified. Using the method of Khasanov[145] (Scheme 7), acetone rather than water was used as solvent in the first step of the reaction, and the reaction was conducted at room temperature instead of at 45 °C. A calcium cyanamide/acetone mixture was added to phenyl chloroformate (**63**) and the reaction mixture was allowed to stir. 3-Chloroaniline hydrochloride (**60**) was added to the filtrate and stirring was continued; the pH was not changed. The solution was evaporated under reduced pressure to yield a white solid. Recrystallization attempts

were made with several solvents (i.e., ethyl acetate, acetonitrile, and acetone) with acetone as the only solvent able to dissolve the product without heat. Once again, ^{1}H NMR spectral analysis suggested the product was not the desired phenyl carbamate **53**. Instead, ^{1}H NMR and elemental microanalysis supported the conclusion that the product obtained was the tetra-carbamate analog of MD-354 as its hydrochloride salt (i.e., **68**). This conclusion was further supported by the IR spectrum that showed a carbamate peak(s) at 1586 cm^{-1}.



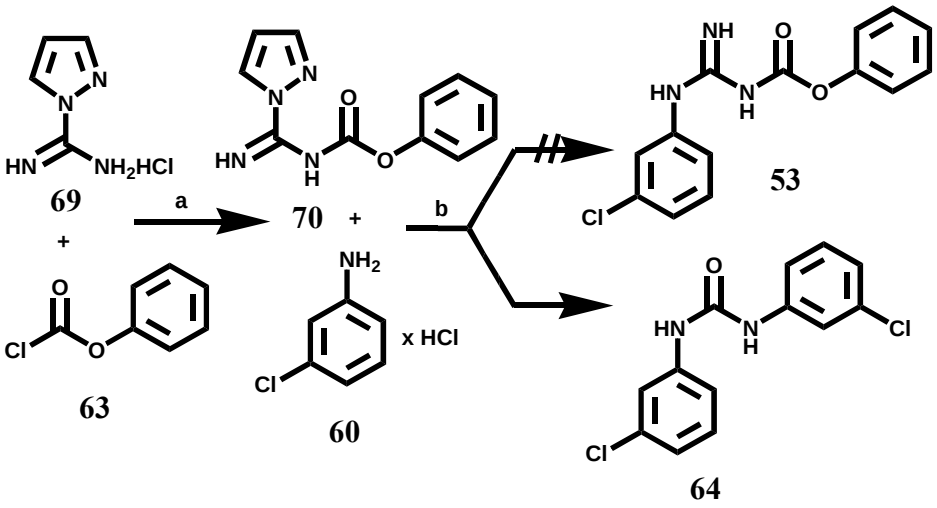
Scheme 7. a. acetone, rt, N_2, 45 min; b. rt, N_2, 4 h.

The formation of tetra-carbamate **68** could be due to the fact that the reaction was not run in a 1:1 ratio of starting materials. Instead, a 2:1 carbamate:amine ratio was used, which could result in a dicarbamate product. When running the reaction, it was extremely difficult to add the $CaCN_2$/acetone mixture to phenyl chloroformate (**63**)

because calcium cyanamide was not soluble in the solvent. Therefore, it is likely that not all of the calcium cyanamide was added, causing even more of an excess of phenyl chloroformate. The basicity of the amines in the presence of excess acid chloride could result in multiple couplings, creating tetra-carbamate **68**.

Another attempt to synthesize the phenyl carbamate analog of MD-354 was made using protecting groups (Scheme 8).[152,153] In this method, 1H-pyrazole-1-carboxamide hydrochloride (**69**) was allowed to stir with diisopropylethylamine and CH_2Cl_2. A solution of phenyl chloroformate (**63**) and CH_2Cl_2 was added to the mixture. Once the reaction was complete (tlc), the product was extracted using aqueous sodium bicarbonate and brine. The extract was dried (sodium sulfate), filtered, and solvent was evaporated under reduced pressure to yield a white, fluffy solid, the phenyl ester **70** of the pyrazole. The product was characterized by ^{1}H NMR spectral analysis and elemental analysis.

The ester **70** and 3-chloroaniline hydrochloride (**60**) were allowed to stir in refluxing diisopropylethylamine (DIEA). Once all starting materials were consumed (tlc), the solvent was evaporated under reduced pressure and the resultant crude product was isolated using column chromatography: 3:2 hexane:EtOAc. The crude product was recrystallized from acetone to yield the urea **64**.

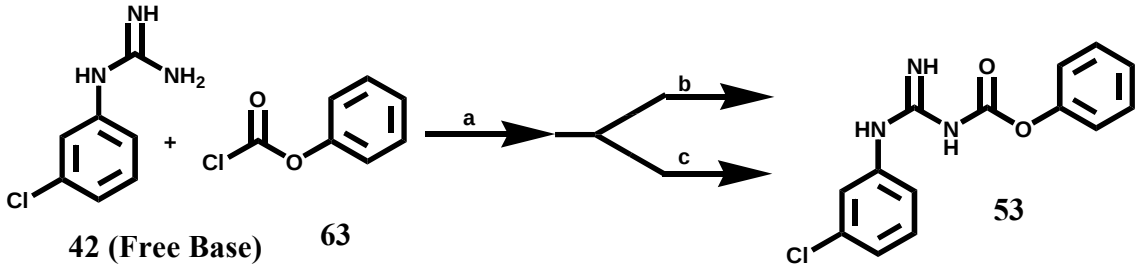


Scheme 8. a. DIEA, DCM, rt, 2 h; b. DIEA, reflux overnight.

Once again, ureas are easily synthesized by the addition of amine to carbamates. Since characterization of intermediate **70** indicated that the desired carbamate had been formed, then the phenyl carbamate **53** should be synthesized. However, the more stable urea was isolated. The literature states that the reaction of protected pyrazole with simple primary amines is rapid and needs to be monitored closely.[153] However, reduction in nucleophilicity drastically increased reaction time sometimes preventing the reaction from proceeding (e.g. the amine *p*-nitroaniline suppressed reaction time completely).[153] Even though *m*-chloroaniline is more nucleophilic than *p*-nitroaniline,[154] the electron-withdrawing group may be strong enough to suppress the reaction, resulting in the urea **64** instead of the desired phenyl carbamate **53.**

Based on previous synthetic attempts to prepare phenyl carbamate **53** that indicated the occurrence of methanolysis, the concept of alcoholysis was analyzed. The theory was put to use with the simple reaction of acid chloride and amine in dry THF to

synthesize a carbamate. Different bulky alcohols (i.e, isoporopanol and n-butanol) would then be used to isolate bulky carbamates, such as the isopropyl carbamate or n-butyl carbamate. A THF solution of phenyl chloroformate (**63**) was added to a solution of MD-354 (free base; **42**), triethylamine, and dry THF (Scheme 9). The triethylamine hydrochloride was removed by filtration and the filtrate was evaporated under reduced pressure to yield a yellow oil. The oil was divided into two separate batches; isopropanol was added to one and n-butanol was added to the other at room temperature. The precipitates from each batch were collected and washed with Et_2O. It was expected that one reaction would yield the isopropyl carbamate whereas the other would provide the butyl counterpart. Comparison of the two products indicated the same material was formed in both cases (melting point, tlc, and ^{1}H NMR spectral analysis). ^{1}H NMR spectral analysis and elemental analysis supported the conclusion that the structure was that of the desired phenyl carbamate **53**.

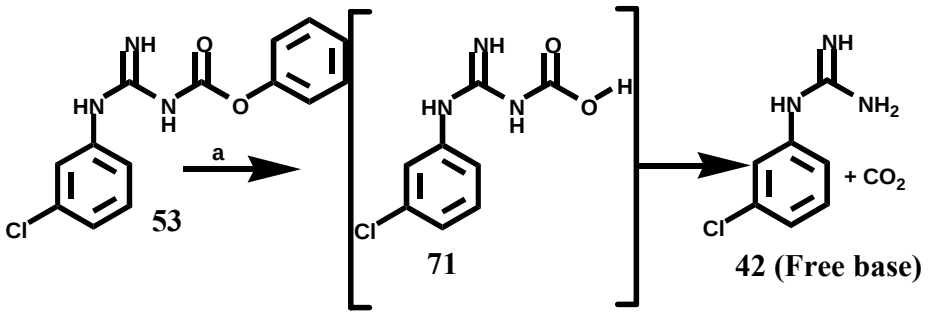


Scheme 9 . a. ET_3N, THF, N_2, 15 min; b. isopropanol; c. n-butanol

The purpose of using the bulky alcohols in this reaction was to support the idea that alcoholysis was occurring. However, synthesis of the phenyl carbamate **53** instead of either the isopropyl carbamate or the butyl carbamate indicated that this was not the case. Several factors may contribute to this. It was shown that for methanolysis to occur, heat was necessary. However, at no point was heat used in the purification of the phenyl carbamate **53** (Scheme 9). Therefore, alcoholysis may not have been able to occur since heat was not present to hydrolyze the phenyl carbamate **53**. Also, alcoholysis may have been prevented due to the actual bulkiness of isopropanol and *n*-butanol. Since MeOH is a much sterically smaller (less bulky, and/or more acidic) compound, it could attack the phenyl carbamate **53** and convert it to the methyl carbamate **62**. However, since the other alcohols employed were bulkier, steric interactions due to the structure of the phenyl carbamate **53**, may have prevented the alcohols from attacking the compound, which in turn prevented alcoholysis. Therefore, the phenyl carbamate **53** was isolated instead of the isopropyl or butyl carbamates. Also, alcoholysis may not have occurred due to the polarity of the bulky alcohols; the bulky alcohols are less polar and less acidic than MeOH, therefore they are less reactive. The less polar nature of isopropanol and *n*-butanol as opposed to MeOH, may require more extreme conditions, such as the use of heat, to allow alcoholysis to occur.

The stability of the final product was of concern since so many attempts showed it to be unstable. Therefore, the final product was heated with H_2O. Within 15 min, the phenyl carbamate **53** began to hydrolyze and by 30 min there was no phenyl carbamate present as evidenced by tlc. Analysis by tlc indicated that the phenyl carbamate **53** may

have been hydrolyzed to its carbamic acid **71** which decarboxylated to form starting material, *m*-chlorophenylguanidine (**42,** free base), R_f = 0.77 (MeOH) (Scheme 10). The phenyl carbamate also hydrolyzed to MD-354 in room temperature H_2O; hydrolysis occurred at a slower rate than in refluxing H_2O. It is important to note that heat and water were not necessary to decompose the phenyl carbamate; in a separate reaction, the product decomposed while in EtOAc in a sealed vial at room temperature. These data support the idea that carbamate **53** is relatively unstable. Therefore, the carbamate was not evaluated in animal studies, as it may hydrolyze or decompose too quickly to attain any reliable results.



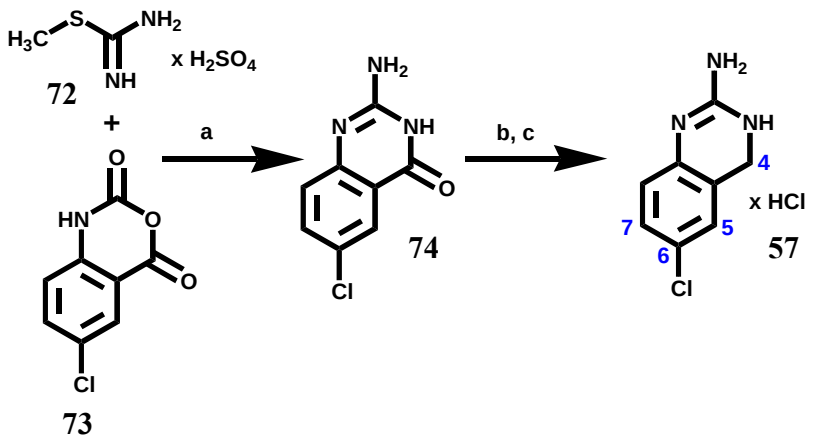
Scheme 10. a. H_2O, reflux

The same procedure was applied to the synthesis of both the 4-chlorophenyl carbamate **54** and the 4-methoxyphenyl carbamate **55**. However, attempts at purification were unsuccessful. Also, it was noted that the crude products were extremely unstable, and decomposed in sealed vials (tlc). This lack of apparent product stability was the basis for not continuing with the pharmacological aspects of this study.

2. Preparation of a conformationally-constrained analog of MD-354.

2-Amino-6-chloroquinazoline hydrochloride (**57**) was synthesized using a general method described by Grosso and Nichols (Scheme 11).[155] Commercially available 5-chloroisatoic anhydride (**73**) and S-methylisothiourea (**72**) were heated at reflux in 1,4-dioxane. The resulting quinazolinone **74** was reduced with diborane in refluxing THF to obtain the free base **57**, which was converted to its hydrochloride salt using an HCl/ether solution.

The progress and purity of each step of the reaction was monitored by tlc using 3:2 hexane:EtOAc as eluent. ^{1}H NMR spectroscopy of the final target indicated the presence of two aliphatic proton signals at δ = 4.49 ppm that correspond to the methylene group of **57** after the reduction of lactam **74**. Similarly, the IR spectrum showed absence of the lactam group at 1679 cm^{-1} seen in **74**. The physicochemical properties of **57** were compared with those of a sample previously synthesized in our laboratory but incorrectly assigned structure **56**. Both compounds possessed identical melting points, R_f = 0.26 (H_2O), IR, ^{1}H NMR, and microanalytical properties.



Scheme 11. a. 1,4-Dioxane, Na_2CO_3, reflux, overnight; b. BH_3-THF, reflux, N_2, 2.5 h; c. HCl/Et_2O, recrystallized from EtOH.

The conformationally-constrained analog of MD-354 (i.e., **57**) was synthesized to confirm the structure of a compound previously synthesized in our laboratories, and whose structure may have been misassigned. Previously, it was believed that 2-amino-7-chloroquinazoline hydrochloride (**56**) had been obtained; however, analysis of the synthesis indicated that the position of the chloro group was in question. Therefore, two reactions were performed using different starting materials, 5-chloroisatoic anhydride and 4-chloroisatoic anhydride, to yield the respective 6- and 7-chloroquinazolines. The 7-chloro analog, **56**, has been independently synthesized in our laboratory by an unequivocal route and has been shown to be different in structure from that of **57** (Ownby and Dukat, unpublished data as shown in Scheme 11).

^{1}H NMR spectral analysis indicated that the difference between the 6-chloro quinazoline and the 7-chloroquinazoline was a shift of two aromatic hydrogen signals upfield. These data were compared to the ^{1}H NMR data of the previously reported

compound, which supported the conclusion that the structure had been misassigned and that the 2-amino-6-chloroquinazoline hydrochloride (**57**) had originally been synthesized.

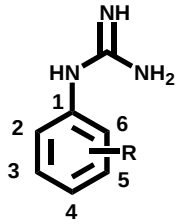
C. Molecular Modeling

QSAR studies were conducted on 5-HT_3 receptor agonists and partial agonists to evaluate Dukat's current working pharmacophore accounting for the binding of arylguanidines and arylbiguanides at 5-HT_3 receptors.[14] Since many of the arylguanidine and arylbiguanide ligands are *meta*-substituted, and because there are two *meta*-positions (3 and 5), rotameric binding can occur. Therefore, constrained analogs were included to better account for the possibility of rotameric binding.

An arginine fragment obtained from SYBYL was used to construct a model of MD-354 (**42**), which served as the template for all of the arylguanidine analogs shown in Table 6. The crystal structure of *m*-chlorophenylbiguanide was obtained from the Cambridge Database (Identification name FERDOW), and was used as a template to construct the arylbiguanide analogs shown in Table 7. Since there are two *meta*-positions, selection of substitution was based on K_i values; *meta*-substituted analogs with K_i values $\geq$700 nM were labeled as 5-substituted analogs, whereas those with K_i values <700 nM were labeled as 3-substituted analogs. This classification was based on the constrained analogs used in the model; the 5-chloroquinazoline bound with an affinity >700 nM, therefore *meta*-substituents with higher affinity were placed in the 3-position. Also, for analogs that were di- and tri-substituted, substitution positions were determined based on binding affinity. For example the 3,6-dichloro arylbiguanide analog was

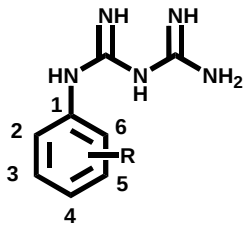
labeled as such, instead of 2,5-dichloro arylbiguanide, because the binding affinity was less than 700 nM, which was consistent with monosubstituted 3-chloro analogs.

Table 6. Arylguanidine 5-HT_3 receptor agonists/partial agonists and their respective binding affinities used in the present QSAR studies.[113,115]



#	R=	pKi	#	R=	pKi
41	H	5.63	**83**	4-C_2H_5	6.11
42	3-Cl	7.49	**43**	3,4-fused phenyl	7.74
47	5-CF_3	5.61	**84**	4-C_6H_5	8.15
75	5-OH	5.69	**48**	3,4-Cl	8.51
76	3-CN	6.91	**51**	3-CF_3, 4-Cl	7.44
77	5-OCH_3	5.80	**85**	4,5-OCH_3	5.57
78	4-CH_3	6.3	**46**	5-CH_3	5.19
45	4-Cl	6.49	**86**	4-$C(CH_3)_3$	5.68
79	4-CF_3	6.64	**87**	4-C_6H_5	5.60
80	4-OCH_3	6.00	**49**	3,5-Cl	8.30
81	3-Cl, 5-OCH_3	7.74	**50**	3,4,5-Cl	9.15
82	4-$CH_2C_6H_5$	6.60			

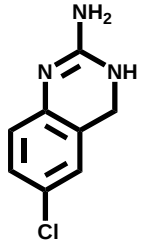
Table 7. Arylbiguanide 5-HT_3 receptor agonists/partial agonists and their respective binding affinities used in the present QSAR studies.[115]



#	R=	pKi	#	R=	pKi
29	H	5.92	**89**	3-Cl, 4-CH_3	6.65
30	3-Cl	7.75	**39**	3,5-Cl	8.74
37	3,5,6-Cl	9.40	**90**	3,5-CF_3	5.92
38	3,4-Cl	7.92	**91**	3,6-Cl	8.54
40	3,4,5-Cl	8.57	**92**	2,3-Cl	8.33
32	6-Cl	7.21	**93**	4,6-F	6.59
33	4-Cl	6.68	**94**	4-F	6.21
88	4-CH_3	6.05	**95**	4-CF_3	6.16
31	3,4-fused phenyl	7.85			

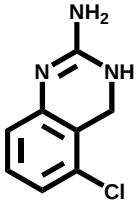
The structures of 43 arylguanidines and arylbiguanides (with K_i values spanning a 10,000-fold range), including three constrained analogs (**57, 58,** and **96**; Figure 58) were constructed and minimized using Gasteiger Hückel algorithms. These 43 analogs were used as a training set and aligned using a Least Squares Method: the Fit Atom function. Alignment was based on three common points of the structure of MD-354 (**42**): the aryl

3-position, the aryl 5-position, and the carbon atom of the guanidine moiety (as shown in Figure 59).



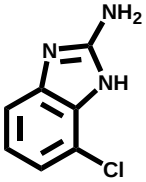
57

pKi = 7.10



58

pKi = 6.13



96

pKi = 6.14

Figure 58. Constrained analogs and their respective binding affinities used in further evaluation of the 5-HT_3 receptor working pharmacophore model.[13,135]

CoMFA studies were conducted on the training set of 43 arylguanidine and arylbiguanide derivatives. Analysis indicated there was a 63% steric contribution and a 37% electrostatic contribution (r^2 = 0.681, q^2 = 0.693) with 5 as the optimal number of components. Since the predictability factor (q^2) was above 0.60, the model was considered to be acceptable and could be used to predict binding affinities of arylguanidine or arylbiguanide analogs. In general, models with r^2 values above 0.60 have good predictability, however, the closer to 1 the r^2 value, the better the predictability.

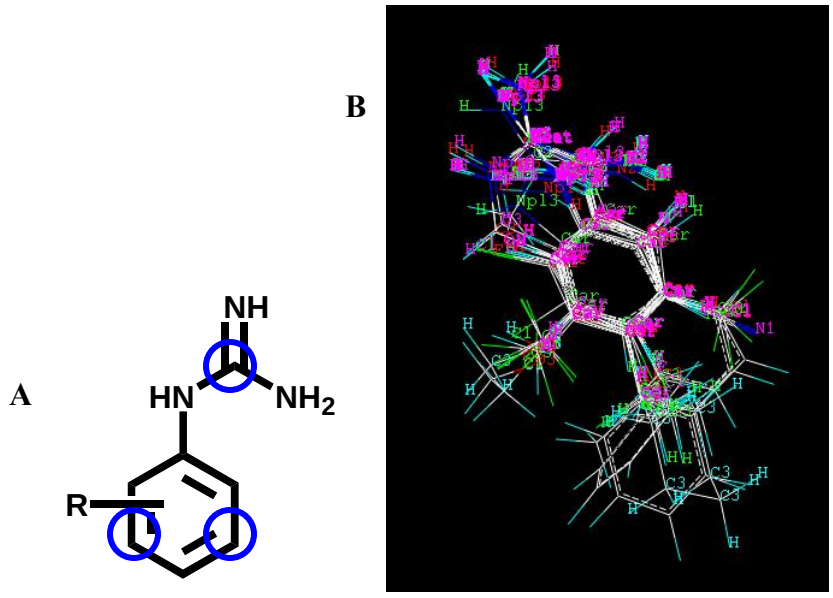
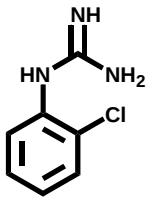


Figure 59. A) Three points used for alignment of all 43 training-set compounds. B) All 43 compounds aligned in CoMFA.

These results differ from those observed in the previous pharmacophore model as the previous model only used a total of 33 compounds instead of 43 as employed in the current investigation.[113] In the previous model, similar compounds were used for both arylguanidines and arylbiguanides; mono-, di-, and tri-substituted compounds, as well as the 2-napthyl analogs. However, the previous model did not include bulky substituents at the 4-position or any of the constrained analogs.[113] It did, however, include substitution within the guanidine moiety, which is not included in the current investigation.[113] The same three points, aryl 3-position, aryl 5-position, and the cationic carbon attached in the guanidine moiety were used for alignment.[113] However, with the previous model, CoMFA studies resulted in $r^2 = 0.851$ and $q^2 = 0.584$.[113]

The difference in predictability of the model generated in the present studies versus that of the previous model not only could be due to the addition of constrained analogs to the training set, but also the function of these constrained analogs. Currently, is not known whether these constrained analogs behave in an agonist or antagonist manner. 5-HT_3 receptor agonists and antagonists may bind to the receptor in a different manner. If this is the case, and both models are supposed to be representative of arylguanidine and arylbiguanide 5-HT_3 receptor agonists and partial agonists, the addition of ligands with unknown functionality may decrease the predictability of the model.

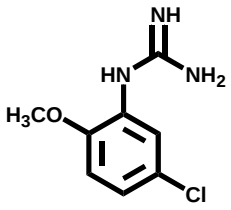
Validation of the model was completed using a test set of five (arbitrarily selected) compounds, whose known pKi values were compared to the pharmacophore model's predicted values (Figure 60). The predictability of the validation set was high as some affinities were predicted within 2- to 3-fold.



44

Actual pKi: 6.72 (K_i = 190 nM)

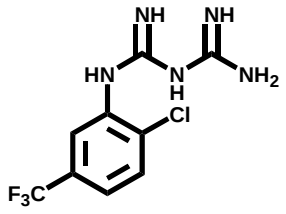
Predicted pKi: 6.23 (K_i = 588 nM)



97

Actual pKi: 6.10 (K_i = 794 nM)

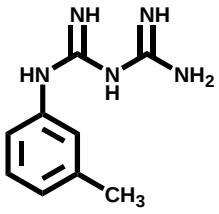
Predicted pKi: 6.13 (K_i = 741 nM)



98

Actual pKi: 6.52 (K_i = 302 nM)

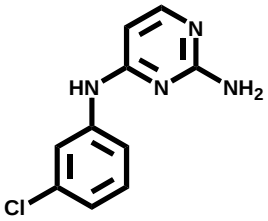
Predicted pKi: 6.00 (K_i = 1000 nM)



99

Actual pKi: 6.11 (K_i = 776 nM)

Predicted pKi: 5.95 (K_i = 1122 nM)



100

Actual pKi: 5.84 (K_i = 1445 nM)

Predicted pKi: 5.10 (K_i = 7943 nM)

Figure 60. Predicted pKi and K_i values of five arbitrarily selected test-set compounds from the CoMFA model compared to their known[113,115] pKi and K_i values.

Figure 61 displays the region in the pharmacophore model where electrostatic interactions are favorable versus unfavorable. There was only a small area near the aryl 5- and aryl 6-positions where negatively charged substituents were favored. The aryl 3- and aryl 4-positions possessed a large region favoring positively charged substituents. These interactions were also favored around the guanidine moiety.

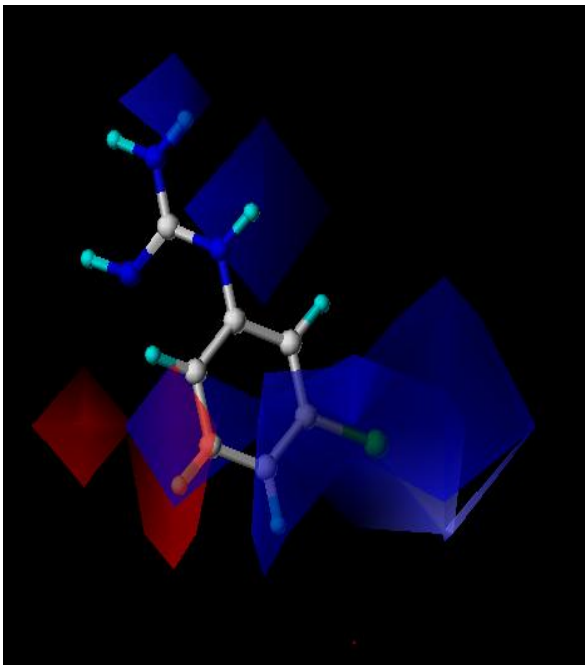


Figure 61. CoMFA results analyzing only electrostatic interactions (n = 43). Blue is a region were positively charged substituents are favored, whereas red represents regions where negatively charged substituents are favored. MD-354 (**42**) is displayed in the model.

CoMFA results also displayed regions where steric interactions are favorable versus unfavorable (Figure 62). A small area of bulk tolerance was observed near the aryl 3-position. However, this area did not extend too far away from the aromatic region. It was observed that steric interactions were unfavorable to a large extent at the aryl 4-position. Therefore, bulky substituents are not favored and, in fact, compounds display lower binding affinities when they bear such substituents near this region.

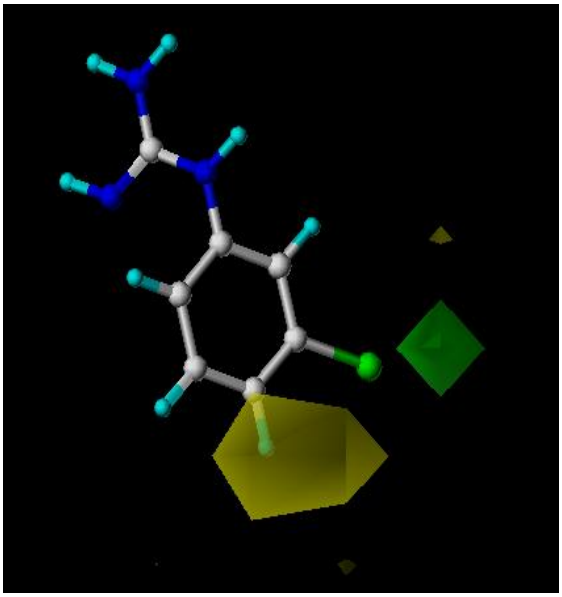


Figure 62. CoMFA results analyzing regions of steric tolerance (n = 43). Green areas are regions where bulky groups are favored and yellow represents areas where bulky substituents are unfavored. MD-354 (**42**) is the compound represented in the model.

Based on the CoMFA studies, there are more regions of favorable electrostatic interactions than favored steric interaction. This could be due to the number of arylguanidines and arylbiguanides that had variable electrostatic substituents versus the amount of sterically hindered 5-HT_3 receptor agonists/partial agonists employed in the training set. Figure 63 displays the full results of the CoMFA study, which differ from Dukat's pharmacophore model as the previous model provided more information about substitution within and around the guanidine moiety. However, that model included analogs with substitution within the guanidine moiety as opposed to the present model that did not include these types of analogs.

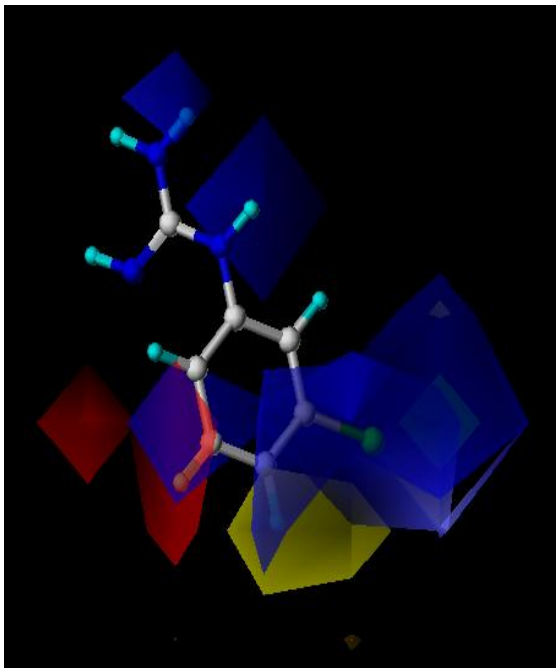


Figure 63. CoMFA results including favorable and unfavorable regions of steric and electrostatic interactions (n = 43). Blue areas are regions where positively charged substituents are favored, whereas red areas are regions where negatively charged substituents are favored. Green areas are regions where bulky groups are favored and yellow areas are regions where bulky substituents are unfavored. MD-354 (**42**) is compound displayed in the model.

This model differs from results of the previous CoMFA model as bulky substitutents are favored close to the aryl 3- and aryl 5-positions, whereas bulky substituents are unfavored around the majority of the aryl-positions (further out from the aryl 3- and aryl 5-positions) and around the guanidine moiety.[113] The present model suggests that bulky substituents are favored near the aryl 3-position, and disfavored around the aryl 4-position, however, this is the only information the present investigation

provides relating favorable and unfavorable regions or steric bulk. Therefore, the present investigation is not as informative as the previous model on the location of where regions of bulk are preferred versus unfavorable regions.

Also, the present investigation displays large regions where electrostatic interactions are favored (i.e., positively charged substituents improve affinity). This is observed near the aryl 3-, aryl 4-, and aryl 5-positions, as well as within the guanidine moiety. The previous model also displays favorable electrostatic interactions within the guanidine moiety, as well as near the aryl 5-position, however these interactions are unfavored near the aryl 3-position.[113] This could be due to assignment of *meta*-substituents to the 3- versus 5-position based on binding affinity relative to the constrained analogs. Once again, constrained analogs were not present in the previous study.

CoMSIA studies can provide information on steric, electrostatic, hydrophobic, H-bond donor, and H-bond acceptor regions. All five aspects were analyzed in the present study (n = 43), however the r^2 values were low ($r^2 < 0.50$) on all parameters analyzed except hydrophobic interactions. With this parameter, slightly better predictability was observed ($r^2 = 0.51$, $q^2 = 0.50$), as opposed to H-bond donor/acceptor, electrostatic, and steric parameters. Although this predictability is not too reliable, as r^2 is not greater than 0.60, it still can be used to give some insight into regions where hydrophobic interactions are favorable versus unfavorable (Figure 64).

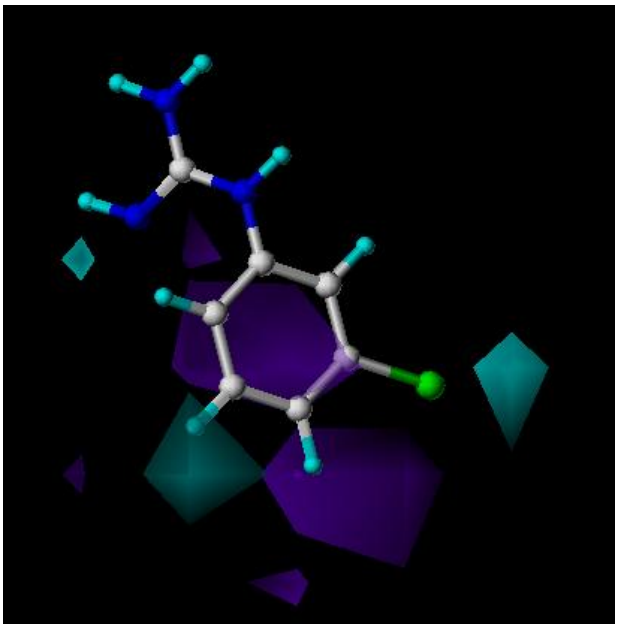
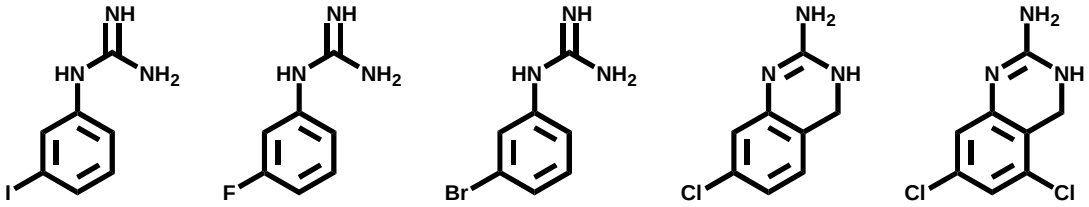


Figure 64. CoMSIA results showing regions of hydrophobic interaction (n = 43). Cyan areas are regions were hydrophobic interactions are favored whereas those areas shown in purple are regions where hydrophobic interactions are unfavorable. MD-354 (**42**) is the compound displayed in the model.

Results from CoMSIA studies indicated that hydrophobic interactions are favored near the aryl 3-position, the aryl 5-position, and near the terminal amine in the guanidine moiety. There was a much larger area of unfavorable hydrophobic interactions, which was observed near the aryl 4-position as well as in the center of the aromatic ring; typically, compounds with nonpolar substituents at these positions would be predicted not to bind well.

From these findings, the binding affinities of five new compounds recently synthesized in our laboratories, whose binding affinities have not yet been determined,

were predicted as shown in Figure 65. The binding affinities were predicted using CoMFA as this model displayed higher predictability than the CoMSIA model.



101	**102**	**103**	**56**	**104**
7.43	6.68	7.51	7.53	7.76

Figure 65. Predicted pKi values of five new arylguanidine analogs using CoMFA analysis.

These predictions will be compared to the actual binding affinities of these compounds once radioligand binding data become available.

This information further extends the current working pharmacophore model for 5-HT_3 arylguanidine and arylbiguanide agonists and partial agonists. Evidence from the current investigation supports the idea that binding is sensitive to electronic and lipophilic substituents at the aryl 3- and aryl 5-positions as well as lipophilic substituents at the aryl 4-position. Information provided from this model gives insight into what features are necessary to bind with high affinity to the 5-HT_3 receptor. Based on this, design of novel compounds can be facilitated.

QSAR studies are extremely helpful in designing novel ligands that could bind with high affinity, by understanding which regions of the receptor prefer which type of

substituents (i.e., electron-withdrawing versus electron-donating groups). The present investigation shows that electrostatic factors are preferred in the majority of the model except at aryl 5- and aryl 6- positions. However, di-substituted ligands, preferably electron-withdrawing groups at the 3- and 5- positions bind with higher affinity than mono-substituted ligands. Therefore, design of novel di-substituted ligands should be considered.

Not only are QSAR studies useful in design of ligands, but very helpful in predicting the binding affinities of ligands. Even though some of the compounds in Figure 65 have already been synthesized (i.e., all but **104**), binding data have not yet been obtained. Nevertheless, binding affinities have been predicted and compared to each other. This information can also help in the design of new ligands, as analysis of what may increase or decrease binding affinity, such as more electronegative substituents, can help to determine new analogs to be synthesized without having to wait for binding studies to be conducted.

V. Conclusions

In locomotor activity assays, the goal was to determine the effect, if any, MD-354 might have on the locomotor effects of different psychomotor stimulants. This could provide information on whether MD-354 is acting through a 5-HT_3 receptor agonist or antagonist mechanism. Since MD-354 is a 5-HT_3 partial agonist, it has the potential to behave as either an agonist or antagonist. The three 5-HT_3 receptor ligands MD-354 (**42**), the 5-HT_3 receptor antagonist ondansetron (**19**), and the 5-HT_3 receptor agonist SR 57227A (**28**) all produced saline-like effects in locomotor activity assays when administered alone. Effective doses of the four drugs of abuse tested ((+)amphetamine (**2**), (+)methamphetamine (**3**), DOM (**10**), and cocaine (**1**)) were determined and used in combination with the 5-HT_3 receptor ligands. MD-354 (**42**) neither potentiated nor antagonized the stimulant effect of either (+)amphetamine (**2**) or (+)methamphetamine (**3**). These results were similar to those observed with (+)amphetamine in combination with ondansetron (**19**). They suggest that MD-354 might be acting as an antagonist, or is devoid of action. These results differed from the combination of (+)amphetamine with SR 57227A, as the 5-HT_3 agonist potentiated the stimulant effect of (+)amphetamine on the parameters of movement episodes, movement time, movement distance, and margin distance. Combination of MD-354 (**42**) with cocaine (**1**) resulted in the potentiation of stimulant parameters consistent with results observed with a combination of SR 57227A

(**28**) and cocaine. This suggests that MD-354 (**42**) is working through a 5-HT_3 receptor agonist mechanism. These results could be due to the fact that psychomotor stimulants display increased locomotion due to increased synaptic dopamine levels, and 5-HT_3 receptor agonists can indirectly cause release of dopamine. However (+)amphetamine (**2**) and (+)methamphetamine (**3**) increase dopamine levels through a different mechanism of action than cocaine (**1**). This could be the reason MD-354 has different effects between the phenylalkylamine stimulants versus the non-phenylalkylamine stimulant. Combination of MD-354 (**42**) with the phenylalkylamine control non-stimulant DOM (**10**) resulted in antagonism of the effect that hallucinogens commonly have on various parameters. This antagonism versus potentiation, or no effect at all, could be due to the fact that DOM is not a stimulant and does not work through releasing dopamine.

Various trials were employed to synthesize the more lipophilic phenyl carbamate analogs of MD-354, which were to be used in animal studies as prodrugs of MD-354. Lack of stability of the carbamates made isolation of the phenyl carbamate analog of MD-354, **53,** extremely complex. However, by avoiding heat, air, and water the phenyl carbamate analog of MD-354, **53,** could be isolated using bulky alcohols to precipitate the product. However, upon exposure to heat and water, the phenyl carbamate analog **53** was rapidly hydrolyzed to starting material MD-354 (**42**). This lack of stability precluded animal studies from being conducted. Other phenyl carbamate analogs were even more unstable, and could not be isolated as they decomposed upon standing.

The current working pharmacophore model for arylguanidine and arylbiguanide 5-HT_3 receptor agonists and partial agonists was further developed by the addition of

constrained analogs to the model to help account for rotameric binding that can occur based on the presence of two *meta*-substitution positions. This model indicated that the 3- and 5-positions are sensitive to lipophilic and bulky substituents. Information from the model can be used to design high affinity 5-HT_3 receptor ligands. CoMFA studies were used to predict the binding affinity of several analogs that have been or are being synthesized in our laboratory, but for which binding data are not yet available. Once binding data are available, their binding affinities might further validate this model.

In conclusion, the 5-HT_3 receptor partial agonist MD-354 may behave as either an agonist or is devoid of action when administered with psychomotor stimulants that have different mechanisms of action. It is indicated that this is a central action, although peripheral action can not be excluded. The effect of MD-354 on DOM locomotor effects is intriguing and requires further investigation. These effects are very interesting, but cannot be explained at this time. In regards to synthesis, the more lipophilic carbamate analogs of MD-354 were unstable preventing use in animal studies as prodrugs of MD-354 that could possibly cross the BBB. As such, this is the first study to demonstrate that phenyl carbamates of guanidines are not stable compounds. QSAR studies were conducted including conformationally-constrained analogs to help improve the current working 5-HT_3 receptor agonist/partial agonist pharmacophore model for arylguanidines and arylbiguanides.

VI. Experimental

A. Synthesis

Melting points were taken on a Thomas-Hoover melting point apparatus in glass capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 spectrometer with tetramethylsilane (TMS) as an internal standard. Peak positions are given in parts per million (δ). Infared spectra were obtained on a Nicolet Avatar 360 FT-IR spectrophotometer. Microanalyses were performed by Atlantic Microlab Inc. (Norcross, GA) for the indicated elements and results are within 0.4% of calculated values. Chromatographic separations were performed on silica gel columns (Silica Gel 62, 60-200 mesh, Sigma-Aldrich). Flash chromatography was performed on a CombiFlash Companion/TS (Teledyne Isco Inc., Lincoln, NE). Reactions were monitored by thin-layer chromatography (tlc) on silica gel GHLF plates (250 μ, 2.5 x 10 cm; Analtech Inc., Newark, DE).

***m*-Chlorophenylguanidine nitrate (42).** *m*-Chloroaniline HCl (**60**) (2.36 g, 14.4 mmol), cyanamide (1.21 g, 28.8 mmol), and absolute EtOH (12 mL) were combined and heated at reflux overnight. The solvent was removed under reduced pressure and the crude product was dissolved in H_2O (6 mL). Excess NH_4NO_3 (2.66 g, 33.2 mmol) was added to form a precipitate that was collected and recrystallized from H_2O. The resulting HNO_3 salt was collected by filtration, rinsed with anhydrous Et_2O (3 x 10 mL), and

recrystallized a second time from H_2O to yield 1.57 g (31%) of product as white crystals **42**: mp 170-171 °C (lit.[144] mp 171-172 °C). IR (KBr, cm^{-1}) 3420, 3333, 3195, 1659, 1217 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.22 (m, 1H, ArH), 7.33-7.39 (m, 3H, ArH, NH), 7.47 (t, J=7.8 Hz, 1H, ArH), 7.52 (br.s, 3H, NH), 9.72 (br.s, 1H, HNO_3).

Phenyl [[(3-chlorophenyl)amino](imino)methyl]carbamate (53). Method A. A solution of phenyl chloroformate (**63**) (0.08 mL, 0.63 mmol) in dry THF (3 mL) was added in a dropwise manner to a mixture of *m*-chlorophenylguanidine (**42,** free base) (0.11 g, 0.63 mmol) and Et_3N (0.18 mL, 1.26 mmol) in dry THF (3.5 mL) on ice under a N_2 atmosphere. The reaction mixture was allowed to stir for 25 min at room temperature, then filtered twice to ensure all $Et_3N{\cdot}HCl$ was collected. The solvent was evaporated under reduced pressure. A few drops of isopropanol were added to the resultant crude product and the solution was placed in the refrigerator for 1 h. The precipitate was collected by filtration and washed with anhydrous Et_2O. The solid was dried under vacuum for 48 h to yield 0.04 g (19%) of a white crystalline product, **53**: mp 159-161 ºC; IR (KBr, cm^{-1}) 3463, 3288, 3051, 1668; ^1H NMR (DMSO-d_6) δ: 7.09-7.27 (m, 6H, ArH), 7.37-7.42 (t, 2H, ArH), 7.61 (s, 1H, ArH), 9.28 (3H, NH, D_2O exchangeable). Anal. calcd. for $C_{14}H_{12}N_3O_2Cl{\bullet}0.25Et_2O$: C, 58.45; H, 4.74; N, 13.63 Found: C, 58.38; H, 4.42; N, 13.77.

Method B. A solution of phenyl chloroformate (**63**) (0.38 mL, 3.05 mmol) in dry THF (11 mL) was added in a dropwise manner to a mixture of *m*-chlorophenylguanidine (**42,**

free base) (0.52 g, 3.05 mmol) and Et_3N (0.85 mL, 6.10 mmol) in dry THF (16 mL) in an ice bath under a N_2 atmosphere. The reaction mixture was allowed to stir for 25 min at room temperature, then filtered twice to ensure all Et_3N·HCl was collected. The solvent was evaporated under reduced pressure. A few drops of n-butanol were added to the resultant crude product and the solution was placed in the refrigerator for 1 h. The precipitate was collected by filtration and rinsed with anhydrous Et_2O. The solid was dried under vacuum for 48 h to yield 0.03 g (3%) of a white crystalline product, **53**: mp 159-160 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.07-7.29 (m, 6H, ArH), 7.38-7.42 (t, 2H, ArH), 7.62 (s, 1H, ArH), 9.28 (3H, NH, D_2O exchangeable).

Method C. A solution of cyanamide (2.5 g, 60 mmol) in H_2O (25 mL) was basified with 50% NaOH (pH 7-8)**.** Phenyl chloroformate (**63**) (9.4 g, 60 mmol) was added in a dropwise manner and the reaction mixture was allowed to stir at 43 ºC for 1 h. An unidentified precipitate was collected by filtration; mp >300 ºC. A second product was determined to be phenol (**67**) by tlc analysis compared to commercially available phenol; R_f = 0.83 (3:2 hexane:EtOAc).

2-Amino-7-chloro-3,4-dihyroquinazoline hydrochloride (56).[156] BH_3-THF complex (12.2 mL, 1 M, 6.1 mmol) was added in a dropwise manner to **105** (0.6 g, 3.07 mmol) under a N_2 atmosphere. The reaction mixture was heated at reflux for 2.5 h. After cooling the reaction mixture to room temperature, a 6 N solution of HCl (2.1 mL) was added in a dropwise manner releasing a gas. Then, a 6 N solution of NaOH (8.4 mL) was added to

basify the mixture. Upon standing, a white precipitate formed and was removed by filtration. The solvent was removed under reduced pressure and the residue was dissolved in hot H_2O (20 mL), and extracted with hot $CHCl_3$ (20 mL x 3). The solid at the interface was collected and dried under reduced pressure for 4 h to give 0.15 g (28%) of a white solid (**56**, free base): mp 178-180 °C, ^{1}H NMR (DMSO-d_6) δ: 3.45 (br.s., 1H, NH, D_2O exchangeable), 4.30 (s, 2H, CH_2), 6.11 (br.s., 1H, NH, D_2O exchangeable), 6.60 (s, 1H, ArH), 6.73 (d, 1H, ArH), 6.89 (d, 1H, ArH).

A solution of **56** (free base) (0.13 g, 0.72 mmol) in EtOH was cooled in an ice/water bath. Gaseous N_2 was bubbled through the solution for 5 min to remove any moisture followed by bubbling of gaseous HCl for 15 min. The solvent was removed under reduced pressure to give a white solid. The solid was recrystallized from absolute EtOH to afford 0.08 g (53%) of a white crystalline solid **56:** mp 249-251 °C; IR (KBr, cm^{-1}): 3300, 3190, 2979, 2927, 2855, 1700, 1618, 1493, 1091; ^{1}H NMR (DMSO-d_6) δ: 4.45 (s, 2H, CH_2), 7.07 (d, 1H, ArH), 7.17 (dd, 1H, ArH), 7.25 (d, 1H, ArH), 7.77 (s, 2H, NH_2, D_2O exchangeable), 8.63 (s, 1H, NH, D_2O exchangeable), 11.02 (s, 1H, NH^+, D_2O exchangeable). Anal. Calcd. for $C_8H_8N_3Cl \cdot HCl \cdot 0.25\ H_2O$: C, 43.17; H, 4.30; N, 18.89 Found: C, 43.41; H, 4.05; N, 18.50.

2-Amino-6-chloro-3,4-dihydroquinazoline hydrochloride (57). The quinazolinone **74** (0.52 g, 2.65 mmol) was added to 1M BH_3-THF (11 mL) and heated at reflux under N_2 gas for 2.5 h. The borate complex was then hydrolyzed by the dropwise addition of 6 N HCl (2 mL), then basified with 6 N NaOH (8 mL). The mixture was

concentrated, dissolved in boiling H_2O, and extracted with hot $CHCl_3$ (3 x 10 mL). Solvent was removed under reduced pressure and the resultant solid was rinsed with Et_2O to yield 0.26 g (54%) of white solid **57** (free base): mp 225-230 ºC; IR (KBr, cm^{-1}) 3422, 3102; (DMSO-d_6) δ: 4.28 (s, 2H, CH_2Ar), 5.63 (br.s, 2H, NH, D_2O exchangeable), 6.23 (br.s, 1H, NH, D_2O exchangeable), 6.55-6.58 (d, 1H, ArH), 6.91 (s, 1H, ArH), 6.96-6.99 (d, 1H, ArH).

A solution of HCl/Et_2O (20 mL) was added in a dropwise manner to a stirred solution of **57** (free base) in hot EtOH (~10 mL). The reaction mixture was allowed to stir at room temperature for 10 min. The solvent was evaporated under reduced pressure. The resultant crude product was recrystallized from hot absolute EtOH to yield 0.06 g (19%) of off-white crystals **57**: mp 237-238 ºC; ^{1}HNMR (DMSO-d_6) δ: 4.49 (s, 2H, CH_2Ar), 6.99-7.02 (d, 1H, ArH), 7.30-7.38 (d, 2H, ArH), 7.80 (br.s, 2H, NH, D_2O exchangeable), 8.69 (s, 1H, NH, D_2O exchangeable), 11.16 (s, 1H, NH^+, D_2O exchangeable). Anal. Calcd. for $C_8H_8N_3Cl \cdot 1.25HCl \cdot 0.25H_2O$: C, 41.47; H, 4.24; N, 18.13 Found: C, 41.89; H, 3.96; N, 18.05.

Methyl [[(3-chlorophenyl)amino](imino)methyl]carbamate (62). Method A. A solution of calcium cyanamide (0.64 g, 8.04 mmol) and H_2O (4 mL) was added in a dropwise manner to methyl chloroformate (**61**) (0.56 mL, 7.32 mmol) and the reaction mixture was allowed to stir at 45 ºC for 20 min. The solid was removed by filtration and then 3-chloroaniline hydrochloride (**60**) (0.60 g, 3.66 mmol) was added. The reaction mixture was made slightly more basic (pH 4) by the addition of 40% NaOH and heated at

reflux for 4.5 h. The solution was filtered and solvent evaporated under reduced pressure. The crude product was recrystallized from MeOH to yield 0.07 g (9%) of a white crystalline solid **62**: mp 132-134 ºC (lit.[145] 138-140 ºC); tlc similar to that of previously synthesized sample[12] R_f = 0.83 (9:1 CH_2Cl_2:MeOH); IR (KBr, cm^{-1}) 3443, 3350, 1741, 1669; ^{1}H NMR (DMSO-d_6) δ: 3.6 (s, 3H, OCH_3), 7.11-7.15 (d, 1H, ArH), 7.24-7.36 (m, 2H, ArH),7.48 (3H, NH, D_2O exchangeable) 7.69 (s, 1H, ArH).

Method B. A solution of phenyl chloroformate (**63**) (0.34 mL, 2.70 mmol) in dry THF (10 mL) was added in a dropwise manner to a mixture of *m*-chlorophenylguanidine (**42,** free base) (0.46 g, 2.70 mmol) and Et_3N (0.75 mL, 5.41 mmol) in dry THF (15 mL) in an ice bath under a N_2 atmosphere. The reaction mixture was allowed to stir for 15 min at room temperature, then filtered. The solvent was evaporated under reduced pressure to yield a yellow oil. Flash chromatography (3:2 hexane:EtOAc) was conducted to separate the products. The isolated crude product was recrystallized from acetone to yield 0.02 g (3%) of a white solid: mp 129-132 ºC (lit.[135] 128-129 ºC); ^{1}H NMR (DMSO-d_6) δ: 3.55 (s, 3H, OCH_3), 7.05-7.09 (d, 1H, ArH), 7.26-7.31 (m, 2H, ArH), 7.59 (s, 2H, NH, D_2O exchangeable) 7.78 (s, 1H, ArH), 9.19 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_9H_{10}N_3O_2Cl \cdot 0.25$ acetone: C, 48.35; H, 4.79; N, 17.35 Found: C, 48.63; H, 4.61; N, 17.94.

Method C. A solution of phenyl chloroformate (**63**) (0.45 mL, 3.55 mmol) in dry THF (13 mL) was added in a dropwise manner to a mixture of *m*-chlorophenylguanidine (**42**,

free base) (0.60 g, 3.55 mmol) and Et_3N (9.90 mL, 7.10 mmol) in dry THF (20 mL) in an ice bath under a N_2 atmosphere. The reaction mixture was allowed to stir overnight (17 h) at room temperature then filtered. The solvent was evaporated under reduced pressure to give a yellow oil. A solid precipitated upon the addition of hot MeOH and was recrystallized from MeOH to yield 0.13 g (17%) of a white crystalline solid **62**: mp 136-138 ºC (lit.[145] 138-140 ºC); ^{1}H NMR (DMSO-d_6) δ: 3.48 (s, 3H, OCH_3), 7.02-7.13 (d, 1H, ArH), 7.25-7.36 (m, 2H, ArH), 7.53 (s, 2H, NH, D_2O exchangeable), 7.78 (s, 1H, ArH), 9.15 (s, 1H, NH, D_2O exchangeable).

***N,N'*-bis(3-chlorophenyl)urea (64). Method A.** A solution of calcium cyanamide (0.54 g, 7.74 mmol) and H_2O (2 mL) was added in a dropwise manner to phenyl chloroformate (**63**) (0.77 mL, 6.02 mmol). The reaction mixture was allowed to stir at 45 ºC for 20 min. The solid was removed by filtration and 3-chloroaniline hydrochloride (**60**) (0.50 g, 3.05 mmol) was added to the solution. The mixture was made slightly more basic (pH 4) by the addition of 40% NaOH and heated at reflux overnight. The solution was filtered and solvent evaporated under reduced pressure. The crude product was recrystallized from MeOH to yield 0.07 g (8%) of white crystalline solid **64**: mp 246-248 ºC (lit.[146] 245 ºC); ^{1}H NMR (DMSO-d_6) δ: 7.04-7.07 (dd, 2H, ArH), 7.30-7.33 (m, 4H, ArH), 7.73 (s, 2H, ArH), 8.99 (s, 2H, NH, D_2O exchangeable).

Method B. A solution of calcium cyanamide (0.64 g, 8.04 mmol) and H_2O (2 mL) was added in a dropwise manner to 4-chlorophenyl chloroformate (**65**) (1.02 mL, 7.32 mmol).

The reaction mixture was allowed to stir at 45 ºC for 30 min. The solid was removed by filtration and 3-chloroaniline hydrochloride (**60**) (0.60 g, 3.66 mmol) was added to the solution. The mixture was made slightly more basic (pH 4) by the addition of 40% NaOH and was heated at reflux overnight. The solution was filtered and solvent evaporated under reduced pressure. The crude product was recrystallized from MeOH to yield 0.03 g (3%) of white crystalline solid **64**: mp 241-243 ºC (lit.[146] 245 ºC); ^{1}H NMR (DMSO-d_6) δ: 7.04-7.07 (dd, 2H, ArH), 7.30-7.33 (m, 4H, ArH), 7.73 (s, 2H, ArH), 8.99 (s, 2H, NH, D_2O exchangeable).

Method C. A solution of calcium cyanamide (0.54 g, 6.70 mmol) and H_2O (2 mL) was added in a dropwise manner to 4-methoxyphenyl chloroforomate (**66**) (0.91 mL, 6.10 mmol). The reaction mixture was allowed to stir at 45 ºC for 30 min. The solid was removed by filtration and 3-chloroaniline hydrochloride (**60**) (0.50 g, 3.05 mmol) was added to the solution. The mixture was made slightly more basic (pH 4) by the addition of 40% NaOH and heated at reflux overnight. The solution was filtered and solvent evaporated under reduced pressure. The crude product was recrystallized from MeOH to yield 0.05 g (6%) of a white crystalline solid **64**: mp 239-240 ºC (lit.[146] 245 ºC); ^{1}H NMR (DMSO-d_6) δ: 7.04-7.07 (dd, 2H, ArH), 7.30-7.33 (m, 4H, ArH), 7.72 (s, 2H, ArH), 8.98 (s, 2H, NH, D_2O exchangeable).

Method D. A solution of phenyl chloroformate (**63**) (0.43 mL, 3.41 mmol) and CH_2Cl_2 (1 mL) was added in a dropwise manner to a solution of 1H-pyrazole-1-carboxamide

hydrochloride (**69**) (0.50 g, 3.41 mmol), DIEA (1 mL), and CH_2Cl_2 (3 mL). The reaction mixture was allowed to stir at room temperature for 2 h, then extracted using $NaHCO_3$ and brine; the extract was dried for 1 h with Na_2SO_4. The solvent was evaporated under reduced pressure to yield 0.53 g (68%) of a white solid, **70**: mp 101-105 ºC. ^{1}H NMR (DMSO-d_6) δ: 6.63 (s, 1H, ArH), 7.19-7.28 (m, 4H, ArH), 7.41-7.46 (t, 1H, ArH), 7.97 (s, 1H, ArH), 8.46-8.47 (d, 1H, ArH), 8.90 (s, 1H, NH, D_2O exchangeable), 9.25 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{11}H_{10}N_4O_2$: C, 57.39; H, 4.38; N, 24.34 Found: C, 55.56; H, 4.22; N, 23.46.

The pyrazole ester (**70**) (0.35 g, 1.52 mmol) was added to a mixture of *m*-chloroaniline hydrochloride (**60**) (0.75 g, 4.56 mmol) and DIEA (0.9 mL). The reaction mixture was heated at reflux overnight. The solvent was evaporated under reduced pressure to yield a brown oil. The product was isolated using column chromatography (3:2 hexane:EtOAc) and recrystallized from acetone to yield 0.10 g (23 %) of a white solid, **64**: mp 244-246 ºC (lit.[146] 245 ºC); ^{1}H NMR (DMSO-d_6) δ: 7.04-7.06 (d, 2H, ArH), 7.27-7.35 (t, 4H, ArH), 7.71 (s, 2H, ArH), 9.01 (s, 2H, NH, D_2O exchangeable). Anal. Calcd for $C_{13}H_{10}N_2OCl_2$: C, 55.54; H, 3.59; N, 9.96 Found: C, 55.51; H, 3.47; N, 9.82.

***N,N,N',N'*-Tetrakis(phenylcarbamate)-3-chlorphenylguanidine hydrochloride (68).** A solution of calcium cyanamide (1.07 g, 13.4 mmol) and acetone (15 mL) was added in a dropwise manner to phenyl chloroformate (**63**) (1.53 mL, 12.2 mmol) and allowed to stir at room temperature under an N_2 atmosphere for 20 min. The solid was removed by filtration and 3-chloroaniline hydrochloride (**60**) (1.00 g, 6.10 mmol) was added to the

solution; stirring was allowed to continue for another 5 h. The solution was filtered and solvent evaporated under reduced pressure. The crude product was recrystallized from acetone to yield 0.19 g (5%) of a white solid **68**: mp 84-87 ºC; IR (KBr, cm^{-1}) 3257, 1710, 1586; ^{1}H NMR (DMSO-d_6) δ: 7.11-7.14 (d, 2H, ArH), 7.24-7.48 (m, 20H, ArH), 7.64 (s, 2H, ArH), 10.50 (s, 1H, NH^+). Anal. Cald. For $C_{35}H_{24}N_3O_8Cl \cdot HCl$: C, 59.83; H, 3.80; N, 6.34 Found: C, 61.25; H, 4.02; N, 6.0.

2-Amino-6-chloro-4-dihydroquinazolinone (74). S-Methylisothiouronium sulfate (**72**) (2.04 g, 7.31 mmol) and Na_2CO_3 (1.19 g, 11.24 mmol) were added to dry 1,4-dioxane (34 mL). The suspension was heated until all of the isothiourea was dissolved. 5-Chloroisatoic anhydride (**73**) (2.00 g, 10.12 mmol) was added, and the reaction mixture was heated at reflux for 21.5 h. The reaction mixture was allowed to cool to room temperature and poured into 15 mL of H_2O. The mixture was allowed to stir for 20 min at room temperature. The solid paste **74** was collected by suction filtration and dried under vacuum at 60 ºC for 24 h to yield 1.02 g (71%) of yellow solid **74**: mp>300 ºC; tlc similar to that of previously synthesized product[13] R_f = 0.26 (H_2O); IR (KBr, cm^{-1}) 3412, 3174, 3081, 1679; ^{1}H NMR (DMSO-d_6) δ: 6.56 (br.s, 2H, NH, D_2O exchangeable), 7.20-7.23 (d, 1H, ArH), 7.56-7.60 (d, 1H, ArH), 7.80 (s, 1H, ArH), 11.22 (br.s, 1H, NH, D_2O exchangeable). The product was used in the synthesis of **57**.

***m*-Fluorophenylguanidine nitrate (101).**[156] A mixture of *m*-fluoroaniline hydrochloride (1.00 g, 6.78 mmol) and cyanamide (0.38 g, 9.04 mmol) was heated at reflux in absolute

EtOH (5 mL) for 6 h. The solvent was removed under reduced pressure to give a pale yellow oil which was dissolved in H_2O (2 mL), and NH_4NO_3 (1.00 g, 12.5 mmol) was added in excess. The solvent was removed under reduced pressure and the residue was recrystallized (H_2O x 4). The light brown crystals were collected by filtration and washed with cold Et_2O (3 x 5mL) to give 0.81 g (55 %) of a solid product, **101**: mp 145-146 °C (H_2O), 146-147 °C (EtOH) (lit.[157] 165 °C EtOH); IR (KBr, cm-1): 3342, 3330, 3195, 1669, 1597, 1493, 1369, 1143; ^{1}H NMR (DMSO-d_6) δ: 7.21-7.04 (m, 3H, ArH), 7.53-7.45 (m, 5H, ArH, NH_2, ex with D_2O), 9.72 (s, 1H, NH, D_2O exchangeable). Anal. calcd for $C_7H_8FN_3 \cdot HNO_3$: C, 38.89; H, 4.20; N, 25.92 Found: C, 39.08; H, 4.15; N, 25.89.

***m*-Iodophenylguanidine nitrate (102).**[158] *m*-Iodoaniline hydrochloride (4.4 g, 17.22 mmol) and cyanamide (2.1g, 50.0 mmol) were added to absolute EtOH (20 mL). The reaction mixture was heated at reflux for 24 h and cooled to room temperature. Distilled water (5.0 mL) was added to the solution followed by addition of NH_4NO_3 (10.0 mL). A white precipitate was promptly formed and recrystallized from distilled H_2O. The product was further purified by flash chromatography (9:1 CH_2Cl_2:MeOH) to afford white crystals 0.40 g (7%) **102**: mp 166–168 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.25 (t, J = 8.40 Hz, 1H, ArH), 7.27-7.30 (m, 1H, ArH), 7.49 (br s, 5H, NH_2, D_2O exchangeable), 7.62-7.63 (m, 1H, ArH), 7.65-7.69 (m, 1H, ArH). Anal. Calcd for $C_7H_8N_3I \cdot HNO_3$: C, 25.94; H, 2.80; N, 17.29 Found: C, 25.73; H, 2.72; N, 16.98.

***m*-Bromophenylguanidine nitrate (103).**[158] 3-Bromoaniline hydrochloride (4.0 g, 19.2 mmol) and cyanamide (2.1g, 50.0 mmol) were added to absolute EtOH (20 mL). The reaction mixture was heated at reflux for 24 h and cooled to room temperature. Distilled water (5.0 mL) was added to the solution followed by addition of NH_4NO_3 (10.0 mL). A white precipitate was promptly formed and recrystallized from distilled H_2O. The product was further purified by flash chromatography (9:1 CH_2Cl_2:MeOH) to afford white crystals 0.72 g (14%) **103**: mp 160–162 ºC; ^{1}H NMR (DMSO-d_6) δ: 7.26 (ddd, J = 7.80 Hz, 1H, ArH), 7.41 (t, J = 7.80 Hz, 1H, ArH), 7.47-7.52 (m, 7H, ArH and NH_2, D_2O exchangeable). Anal. Calcd for $C_7H_8N_3Br \cdot HNO_3$: C, 30.34; H, 3.27; N, 20.22 Found: C, 30.14; H, 3.16; N, 19.95.

2-Amino-7-chloro-4-dihydroquinazolinone (105).[156] 4-Chloroisatoic anhydride (1.00 g, 5.06 mmol) was dissolved in acetonitrile (24 mL, 80%), then S-methylthioisourea sulfate (**72**) (1.4 g, 5.06 mmol) and Na_2CO_3 (0.58 g, 5.47 mmol) were added to the solution. The resulting solution was heated at reflux for 5 h. The reaction mixture was allowed to cool to room temperature over 0.5 h. The suspension was filtered and washed with acetonitrile (80%, 25 mL x 3). The solvent was evaporated under reduced pressure then dissolved in CH_2Cl_2 (10 mL) and extracted with H_2O (10 mL x 3). The layers were separated and the organic layer was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The residue was purified by column chromatography (9:1:0.1, CH_2Cl_2:MeOH:NH_4OH). The reaction gave 0.64 g (65%) of a pale pink solid **105**: mp >300 °C (lit.[159] >300 °C); IR (KBr, cm^{-1}): 3401, 3133, 1597, 1442, 1101; ^{1}H NMR

(DMSO-d_6) δ: 6.58 (br.s., 2H, NH_2, D_2O exchangeable), 7.11 (dd, 1H, ArH), 7.21 (d, 1H, ArH), 7.87 (d, 1H, ArH), 11.10 (br.s., 1H, NH, D_2O exchangeable). The product was used in the synthesis of **56**.

B. Behavioral Studies

1. Animals

Male ICR mice (19-29 g) were used throughout the study (Harlan Laboratories; Indianapolis, IN). Mice were housed in groups of 5-6, with free access to food and water in a temperature-controlled environment under a standard 12:12 h dark/light cycle in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved facility. The experiments were conducted in accordance to standards set by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University. Mice were allowed to adapt to the testing environment at least 1 h prior to any treatment, and weighed the same day as the experiments.

2. Drugs

(+)Amphetamine sulfate and cocaine hydrochloride (Lot 11K1085J) were purchased from Sigma-Aldrich Chemical Company (Milwaukee, WI). (±)1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane (DOM) hydrochloride was obtained from the National Institute on Drug Abuse (NIDA; Rockville, MD). (+)Methamphetamine hydrochloride was a gift from Dr. R. A. Glennon (Virginia Commonwealth University). SR 57227A (4-amino-(6-chloro-2-pyridyl)-1piperidine) hydrochloride was purchased

from Tocris (Batch 1A/45893; Ballwin, MO). Ondansetron hydrochloride (Zofran®, Lot CO99723; GlaxoSmithKline) was purchased from MCVH-Pharmacy. Solutions were prepared fresh daily; all drugs were dissolved in 0.9% saline and administered to mice in a total volume of 10 ml/kg body weight by intraperitoneal (i.p.) injections.

3. Locomotor Activity Assays

Mice, naïve to the test apparatus, were placed in individual Tru-Scan Activity System (Coulbourn Instruments, Allentown, PA) photocell activity cages (40 cm^3). Tests were conducted between 0800 h and 1730 h. The mice were treated with either saline (0-min pre-injection time), MD-354 (1.0, 3.0, 6.0, or 10 mg/kg; 0-min pre-injection time or 30-min pre-injection time), ondansetron (0.1, 0.5, or 1.0 mg/kg; 30-min pre-injection time), SR 57227A (1.0, 3.0, or 10 mg/kg; 30-min pre-injection time), alone or in combination with (+)amphetamine (2.0 or 3.0 mg/kg; 0-min pre-injection time), (+)methamphetamine (1.5 or 3.0; 0-min-preinjection time), cocaine (10 mg/kg; 0-min pre-injection time) and DOM (0.3 mg/kg; 0-min pre-injection time). Other mice were treated with (+)amphetamine (0.3, 1.0, 3.0, or 6.0 mg/kg; 0-min pre-injection time), (+)methamphetamine (0.3, 1.0, 1.5, 3.0, or 10 mg/kg; 0-min pre-injection time), cocaine (1.0, 3.0, 10, or 30 mg/kg; 0-min pre-injection time) or DOM (0.3, 1.0, or 3.0 mg/kg; 0-min pre-injection time). The mice were only tested once and each dose of test agent (or combination of drugs) was studied in 6-8 mice (n = 6-8 mice/treatment). The behavioral analysis examined nine measures of activity: movement episodes, movement time (s),

movement distance (cm), vertical entries, margin distance (cm), margin time (s), center distance (cm), center time (s), and center entries.

4. Statistical Analysis

Data for each measure of activity were analyzed statistically by a one-way analysis of variance (ANOVA) followed by a Newman-Keuls post-hoc comparison test. t-Tests were also employed in some analysis instead of a one-way ANOVA, when data suggested a statistical significance which was not supported by a one-way ANOVA or post-hoc comparison.

C. Molecular Modeling

The computational studies were performed on a Silicon Graphics workstation using SYBYL (SYBYL Molecular Modeling Package, Version 7.3, 2007; Tripos Inc., St. Louis, MO) software. Compound **42** was built using an arginine fragment in the program. Compound **30** was built from its crystal structure downloaded from the Cambridge Database. All of the compounds we constructed using standard bond lengths and angles within the Build/Sketch command followed by minimization (MINIMIZE) and calculation of charges by the Gasteiger-Hückel algorithm. The compounds were individually superimposed using FIT ATOM on templates **42** and **31** to perform a least squares fit. The linearly independent points (aryl 3-position, aryl 5-position, and the carbon atom in the guanidine moiety) were used in FIT ATOM. CoMFA and CoMSIA studies were conducted.

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