

PAOPA: ITS BEHAVIOURAL, COGNITIVE, AND MOLECULAR EFFECTS

CHARACTERIZING THE COGNITIVE, BEHAVIOURAL, AND MECHANISTIC
ACTIONS OF NOVEL ALLOSTERIC MODULATOR PAOPA FOR THE
TREATMENT OF SCHIZOPHRENIA

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1 ABSTRACT

The pathophysiology, etiology, and treatment of schizophrenia remain elusive, but research is closing the gap. Schizophrenia globally affects less than 1% of the population and presents with positive, negative, and cognitive symptoms. As treatment for schizophrenia is not completely and meaningfully effective at treating all of the symptoms, without eliciting side effects, the current thesis aimed to evaluate a new drug candidate. PAOPA is a novel allosteric modulator that increases dopamine binding to the dopamine D₂ receptor. It has previously shown positive findings in preventing and reversing behaviours proposed to model phenotypes of schizophrenia. However, it has not yet been tested to improve cognitive deficits in animal models, nor has its effects on other animal models been investigated. Lastly, its mechanism of action has not yet been comprehensively answered. In three separate studies, PAOPA was tested on ameliorating attentional deficits using the 5-choice serial reaction time task in an amphetamine model, deficits in novel object recognition memory, sensorimotor gating, social interaction, and locomotor activity using a PCP model, and its effects on proteins regulating G protein-coupled receptors (GRK2 and arrestin-3), downstream signalling (ERK1 and ERK2), and synaptic vesicular control (synapsin II) were investigated. Although the sample sizes were too small to draw valid interpretations, the results suggested that PAOPA partially attenuated deficits in attention, novel object recognition memory, social interaction, sensorimotor gating, but not locomotor. Furthermore PAOPA increased the protein expression of GRK2, arrestin-3, ERK1 and 2, and synapsin IIa in the medial prefrontal cortex, striatum, and the nucleus accumbens. The results suggest that PAOPA influences the dopaminergic system in the striatum to change behaviour via receptor internalization

and possibly downstream signalling. The present studies illuminate new insights, and point to future explorations for the potential development of PAOPA as a therapeutic for schizophrenia.

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5 LIST OF ABBREVIATIONS AND SYMBOLS

5-CSRTT, 5-choice serial reaction time task

AMPH, amphetamine-treated rats

ANOVA, analysis of variance

AP, amphetamine- and PAOPA-treated rats

CNTRICS, Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia

D₁R, dopamine D₁ receptor

D₂R, dopamine D₂ receptor

D_{2s}R, dopamine D₂ short receptor

D_{2L}R, dopamine D₂ long receptor

ERK, extracellular signal regulated kinase

GAPDH, glyceraldehyde 3-phosphate dehydrogenase

GPCR, G-protein coupled receptor

GRK, G-protein coupled receptor kinase

HRP, horseradish peroxidase

IgG, immunoglobulin G

i.p., intraperitoneal

ITI, inter-trial interval

MATRICES, Measurement and Treatment Research to Improve Cognition in Schizophrenia

MIF-1, melanocyte-stimulating hormone release-inhibiting factor

mPFC, medial prefrontal cortex

PAOPA, (3*R*)-[2(*S*)-pyrrolidinylcarbonyl]amino]-2-oxo-1-pyrrolidineacetamide)

PLG, L-prolyl-L-leucyl-glycinamide

PPI, pre-pulse inhibition

SAL, saline-treated rats

s.c., sub-cutaneous injection

PCP, phencyclidine

PCPa, treatment group that was used to compare the preventive effects of PAOPA on PCP-induced behavioural abnormalities

PCPb, treatment group that was used to compare the reversal effects of PAOPA on PCP-induced behavioural abnormalities

TBS-T, Tris buffered saline-Tween-20

6 INTRODUCTION

6.1 Schizophrenia

Schizophrenia likely existed in early human civilizations, but Emil Kraepelin has been attributed the distinction of being the first person in modern history to identify the disease in 1887, although interest in the mind and various other psychiatric disorders preceded him (Kyziridis, 2005). The 19th century saw a boom in information about the mind and body and Kraepelin combined the existing and separate loose definitions of schizophrenia into one disorder and labelled it *dementia praecox* (early dementia). At the beginning, schizophrenia was thought of as a decline in cognitive abilities such as social decline, apathy, paranoia, delusions, catatonia, and hebephrenia (Kyziridis, 2005). Later, Eugen Bleuler argued the term was inappropriate due to a lack of evidence for a global decline in cognition. In 1908, Bleuler coined the term that has been used to date: *schizophrenia* (split mind). Bleuler defined the disease as: reduced emotional response to stimuli, disordered pattern of thought, inability to make decisions, and a loss of awareness of external events (Kyziridis, 2005). Bleuler was also the first to divide the disease into *positive* and *negative* clusters.

Since then, growing research and interest in schizophrenia has led to changing and finer definitions and classifications of symptoms. Today,

schizophrenia is still classified as a severe and debilitating mental disease that affects 0.3 – 1% of the global population, depending on the definition of the disease (Kandel et al., 2013a; van Os and Kapur, 2009). The symptoms are usually clustered into three categories. The *positive* symptoms include the well-known hallucinations (seeing or hearing things that do not exist) and delusions (beliefs that are not true) especially of a persecutory type; the *negative* symptoms: blunted affect, anhedonia, alogia, social withdrawal, apathy, and lethargy; the cognitive symptoms: working memory, attention/vigilance, verbal learning and memory, visual learning and memory, reasoning and problem solving, speed of processing, and social cognition (Howes et al., 2015; Nuechterlein et al., 2004; Tamminga and Holcomb, 2005; Young et al., 2012). Diagnosis of the disease is made by matching observable behaviours to symptoms outlined by the Diagnostic and Statistics Manual of Mental Disorders (DSM) published and regularly updated by the American Psychiatric Association and/or the International Classification of Diseases (ICD; van Os and Kapur, 2009).

Once diagnosed the standard treatment for patients are pharmacological drugs mainly targeting the dopamine system. In the early modern history of schizophrenia, mid-20th century medical treatment for the disease proved a challenge (Kyziridis, 2005), likely due to the fact that the precise etiology and pathophysiology was unknown. The serendipitous discovery of chlorpromazine around the mid-20th century gave way to the first generation of antipsychotic drugs,

also called typical antipsychotics. These drugs effectively managed the psychotic state of patients but also produced the now well-known side effects such as tremors, restlessness, loss of muscle tone, and postural disorders—known collectively as extra-pyramidal symptoms (Kyziridis, 2005; van Os and Kapur, 2009). The second generation of antipsychotics—also known as atypical antipsychotics—had fewer motor side effects and similarly managed the psychotic symptoms. Unlike the typical antipsychotics, however, they were better at treating the negative symptoms, although this advantage may not have been meaningful for patients (Kyziridis, 2005; van Os and Kapur, 2009). Furthermore, atypical antipsychotics came with their own side effects that took on a metabolic nature like weight gain, and increased triglycerides and cholesterol. As a result, 1 in 10 patients with schizophrenia on atypical antipsychotics develop a metabolic syndrome and a third of the patient population remains symptomatic (van Os and Kapur, 2009). The main challenge for patients is adhering to treatment and improving functional outcome. Many patients discontinue medication likely due to the stigma associated with the disease and dampened motivation as a result of the dopamine-blocking drugs. The reduced functional outcome is a hindrance for patients for resuming employment and is a result of a lack of interventions, neurocognitive changes, and decreased motivation (van Os and Kapur, 2009). Counselling, psychotherapy, community-case management, and cognitive behavioural therapies are non-pharmacological therapies that have also entered the market; they teach patients how to function in social situations, maintaining hygiene, managing personal finances, and how to

differentiate between reality and illusions (Kyziridis, 2005; van Os and Kapur, 2009). Unfortunately, despite small experimental evidence in support of such therapies, patients do not have easy access to them leaving antipsychotics as the mainstay of treatment (van Os and Kapur, 2009).

The etiology of the disease remains elusive and the subject of continuing research, but environmental and genetic factors have both emerged as contributors. For example, people living in urban environments, immigrants living in a community of a different ethnicity, and people exposed to cannabis have a slightly greater risk of developing schizophrenia (van Os and Kapur, 2009). Genes, which could be under the influence of the environment via epigenetic mechanisms, have an important part to play. Although no single gene is solely responsible for schizophrenia, the disease is highly heritable (approximately 80%) and a small proportion of the incidence is associated with structural changes such as copy number variation and duplications (Howes and Kapur, 2009; van Os and Kapur, 2009). Multiple genes seem to be responsible, affecting various systems. Genes implicated are involved in the dopaminergic pathways, brain development, neurotransmitters such as glutamate and γ -amino-butyric acid (GABA), and synaptic vesicular release (Cesca et al., 2010; Howes and Kapur, 2009; Saviouk et al., 2007).

Schizophrenia patients have reduced gray matter, enlarged ventricles, and alterations of white matter tracts. This structural difference from healthy controls is

in addition to increased dopamine synthesis, release, and resting-state synaptic concentrations for acute psychotic symptoms. Furthermore, patients show increased or decreased brain activity depending on the brain region and cognitive test used. Patients also show a reduced brain response to novel stimuli, and are less able to suppress brain activation to repeated stimuli. One of the theories that attempts to explain how changes in the dopamine system can lead to psychosis proposes that the dysregulation of the dopamine system misattributes salience to irrelevant environmental stimuli. The patient subsequently assigns incorrect interpretations to the newfound salience (van Os and Kapur, 2009).

Impairment in cognition is a major feature of the disease as its onset precedes, are unrelated to and remain after remission of psychosis (Silveira et al., 2012; van Os and Kapur, 2009), likely leads to the pervasive social disability (Kandel et al., 2013a; Keefe and Harvey, 2012), strongly predicts functional outcome (Ragland et al., 2007), and pharmacological treatment range from ineffective to modest (Keefe and Harvey, 2012; Ragland et al., 2007), which parallels the chronic social disability. Patients have broad cognitive impairments 1 standard deviation below normal in a variety of domains (van Os and Kapur, 2009).

6.2 Pharmacological models of schizophrenia

Several rodent models of schizophrenia exist. Perhaps due to the influential role of the dopamine and glutamate hypotheses of schizophrenia, dopamine

agonists and N-methyl-D-aspartate (NMDA) receptor antagonists have been commonly used to model schizophrenic symptoms in animals.

The dopamine hypothesis was originally put forward by the observation that antipsychotics antagonized the dopamine D₂ receptor (D₂R) in the striatum (Howes and Kapur, 2009; Seeman, 2006). Although dopamine is not the only neurotransmitter found deregulated in schizophrenia, its concentration and activity has been reported to be decreased in the frontal cortex and increased in the striatum of patients, the latter of which has been directly associated with positive symptoms (Howes and Kapur, 2009). Amphetamine abuse has been well studied in humans. Amphetamine use has been reported to produce psychotic symptoms in non-abusers, and enhance those in patients (Featherstone et al., 2007). Amphetamine has also been found to increase mesolimbic dopamine activity, and amphetamine-induced dopamine release in patients and animals. Amphetamine sensitization, which refers to an enhanced behavioural response to amphetamine following pre-exposure to the drug, has been robustly found to induce psychotic-like symptoms in animals. Two widely used tests that are proposed to reflect psychotic symptoms are pre-pulse inhibition of the acoustic startle response (PPI) and locomotor activity. PPI is a measure of sensorimotor gating, or the ability to ignore irrelevant environmental stimuli. Schizophrenia patients are less able than the normal population to attenuate their startled response to a startling acoustic stimulus when it is preceded by a neutral acoustic stimulus (pre-pulse; Featherstone et al., 2007). Increased locomotor activity is not a symptom in schizophrenia; rather, the

increased locomotor activity seen in animal models has been proposed to reflect the hyper-dopaminergic state of the striatum in patients (Beyaert et al., 2013; Featherstone et al., 2007). An escalating sensitization of amphetamine, whose molecular structure of amphetamine is similar to that of dopamine (Figure 1), has been found to decrease and increase PPI and locomotor activity, respectively, in treated animals compared to saline-treated animals (Beyaert et al., 2013; Fletcher et al., 2007; Tenn et al., 2003; Tenn et al., 2005). The ability of amphetamine to model negative symptoms, such as social withdrawal, is more controversial and, when reported, usually seen in the withdrawal phase (Featherstone et al., 2007). Interestingly, in an intermittent escalating regimen of amphetamine sensitization, Beyaert et al. (2013) found that the number of interactions and time spent interacting decreased in amphetamine-sensitized animals. With regards to the cognitive deficits, Fletcher et al. (2005; 2007) have reported impairments in executive and attentional functions, which were reversed by a dopamine D₁ receptor agonist, of amphetamine-sensitized rats.



Figure 1. Molecular structure of amphetamine (left) and dopamine (right). Both structures possess an ethyl group bound to a phenyl and primary amino group.

The NMDA receptor antagonist model has been proposed to model more of the symptoms of the disease—especially the negative and cognitive—than dopamine agonist models (Javitt and Zukin, 1991; Javitt et al., 2012). A reduced activation of NMDA receptors in the frontal cortex has been observed in schizophrenia, and NMDA antagonists like phencyclidine (PCP), and ketamine exacerbate and produce symptoms in patients and healthy volunteers, respectively (Javitt and Zukin, 1991; Javitt et al., 2012; Jentsch and Roth, 1999; Poels et al., 2014a; Poels et al., 2014b; Yee and Singer, 2013).

6.3 *Cognition*

Interest has developed in cognitive impairment in schizophrenia as research has revealed that such impairments are a core feature of the disease, most closely correlate with functional outcome, precede the onset of psychosis, and are not completely effectively treated by antipsychotic medication (Young et al., 2012; Young et al., 2009). The lack of effective treatment galvanized the National Institute of Mental Health (NIMH) based in USA to form an initiative called MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia). Its general purpose is to facilitate the drug development process, especially for new therapy for negative and cognitive symptoms (Marder and Fenton, 2004). One of its goals is the development of a consensus battery of pre-clinical cognitive tests on which pharmacological candidates can be tested. The

Neurocognition Subcommittee of MATRICS identified seven important cognitive domains affected in schizophrenia, which were stated above (Young et al., 2012):

1. Working memory
2. Attention/vigilance
3. Verbal learning and memory
4. Visual learning and memory
5. Reasoning and problem solving
6. Speed of processing
7. Social cognition

The present study investigated attentional impairments in a model of schizophrenia. Attention has been defined as the “*the ability to allocate and sustain the focus of cognitive resources on specific stimuli or information while ignoring or filtering other information*” (Young et al., 2012). Attention is not a domain unto itself—in fact it likely mediates all the other cognitive domains.

One test developed to assess attention in humans was Leonard's five choice serial reaction time task, which entailed subjects tapping one of five randomly illuminated light bulbs as quickly as possible for 30 minutes (Wilkinson, 1963). A more common test is the continuous performance test (CPT). There are several variations of this test, but the principal theme is that subjects must attend to a series of stimuli, such as digits or letters, and respond only when the pre-determined target stimulus appears. The dependent variables in this task are the number of correct,

incorrect, and omitted responses (Nuechterlein and Dawson, 1984). Imaging studies have shown that normal performance on CPT recruits the frontal cortex (Young et al., 2009). In addition, schizophrenic patients perform poorer than normal controls in the test, and antipsychotics do not meaningfully ameliorate performance deficits (Young et al., 2012).

T. W. Robbins and colleagues at the University of Cambridge developed a rodent analogue of Leonard's five choice serial reaction time task and the human CPT called the 5-choice serial reaction time task (5-CSRTT). Briefly, the test is conducted in an operant conditioning chamber, where one side has a horizontal array of five apertures that can illuminate, and the opposite wall containing a magazine for the delivery of food reinforcements. Which aperture is illuminated is decided randomly by a computer software and rodents must monitor and poke their noses into the illuminated apertures in order to receive the food reward. For the purpose of assessing attention, accuracy is the measure of interest, but the number of omissions and time taken to collect the food reward can also give indications of the motivational state of the rodent, which can be a confounding factor in the test. Lesions of the medial prefrontal cortex in rodents have impaired performance in the task, which parallels the increased frontal activation seen in normal human subjects (Young et al., 2012). Furthermore, repeated PCP administration has shown impaired accuracy, which was attenuated by chronic clozapine and not acute clozapine or risperidone treatment (Amitai and Markou, 2010; Amitai et al., 2007). As already mentioned, an amphetamine sensitized regimen has also shown to

produce an impairment in accuracy, which was reversed by D₁R agonist SKF38393 (Fletcher et al., 2007).

6.4 *Allosteric modulators*

Allosteric modulators bind to the allosteric site on receptors, which is topographically distinct from the orthosteric site bound by agonists and antagonists (Christopoulos, 2002; Keov et al., 2011). The binding of an allosteric modulators induces a conformational change in the receptor. This new state of the receptor either has an increased or decreased affinity for its cognate agonist and/or efficacy for eliciting the biological response. Thus, allosteric modulators usually come in two forms: positive allosteric modulators, which increase the affinity of the receptor to its agonist, thereby increasing the frequency of binding; negative allosteric modulators, which decrease the affinity and binding between a receptor and its agonist (Christopoulos, 2002; Keov et al., 2011). With regards to treatment of schizophrenia, allosteric modulators offer three main advantages that theoretically make more suitable than current market antipsychotic drugs. First, allosteric modulators are more specific to receptor subtypes than antipsychotics. This is because the orthosteric site has been evolutionarily conserved across receptor subtypes to allow the same ligand control over the subtypes. As a result, drugs targeting the orthosteric site may inadvertently also target other receptor subtypes and produce unintended side effects. The allosteric site, on the other hand, has not

been under evolutionary pressure to be conserved across receptor subtypes. Therefore, the unique composition of each site can be exploited by allosteric modulators to ensure receptor specificity (Christopoulos, 2002; Conn et al., 2009; Jensen and Spalding, 2004; Keov et al., 2011; May et al., 2007; Urwyler, 2011). Second, the allosteric site becomes saturated, preventing any excess allosteric modulators from binding. This prevents over-stimulation or over-inhibition of the biological response, in contrast to orthosteric binding (Christopoulos, 2002; Jensen and Spalding, 2004; Keov et al., 2011; Lewis et al., 2008). Third, allosteric modulators exert a more subtle, fine-tuned modulation over the biological response, which has been compared to a dimmer switch, in contrast to antipsychotics, which abolish the response like an *off* switch (Christopoulos, 2002; Conn et al., 2009; Jensen and Spalding, 2004; Keov et al., 2011; May et al., 2007; Urwyler, 2011). Finally, allosteric modulators have no intrinsic biological response by themselves—they only exert their effects under the presence of the agonist (Christopoulos, 2002; Conn et al., 2009; Jensen and Spalding, 2004; Lewis et al., 2008)—further reducing the chances of side effects. These four features of allosteric modulation offer attractive benefits in the treatment of schizophrenia. Indeed, allosteric modulators are already in the market for the treatment for hyperparathyroidism and HIV: cinacalcet and maraviroc, respectively (Conn et al., 2009; Urwyler, 2011)

Yu et al. (1988) developed the novel allosteric modulator PAOPA (3(*R*)-[2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide) based on the

endogenous brain tripeptide PLG (L-prolyl-l-leucyl-glycinamide or melanocyte-stimulating hormone release-inhibiting factor, MIF-1;

Figure 2).

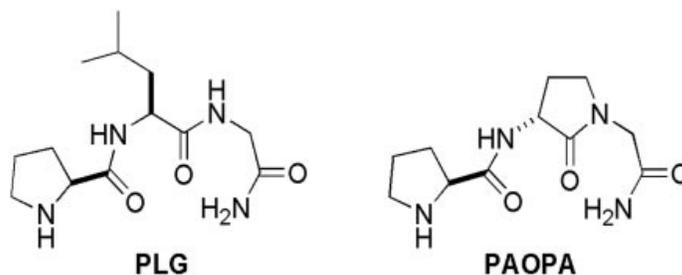


Figure 2. Molecular structure of PLG (left) and PAOPA (right). PAOPA is a conformationally-constrained analogue of PLG.

Studies since have observed the ability of PLG and PAOPA to modulate the dopamine D₂ short receptor (D_{2S}R), D₂ long receptor (D_{2L}R), and the dopamine D₄ receptor, the latter to a lesser degree (Verma et al., 2005), and PAOPA to attenuate positive and negative-like symptoms in pre-clinical animal models of schizophrenia (Beyaert et al., 2013; Dyck et al., 2011a). Whether these pre-clinical observations translate into positive clinical effects remains to be seen, but PAOPA has offered more hope in specifically targeting schizophrenia-like symptoms and not non-specific effects. For example, vacuous chewing movement is a model of human tardive dyskinesia, which is a side effect of haloperidol treatment. Two previous studies from the laboratory found that PLG and PAOPA were able to attenuate haloperidol-induced chewing movements (Castellano et al., 2007; Sharma et al., 2003), and PAOPA was also found to attenuate haloperidol-induced catalepsy

(Costain et al., 1999). Therefore, it seems that PAOPA can elicit its pharmacological therapeutic-like effects in rodents without inducing extra-pyramidal side effects.

6.5 *Summary*

Schizophrenia still poses a significant disease burden (number of healthy years lost to a disease), and strain on the economy (Kandel et al., 2013a). The disease accounts for 30% of hospitalizations and the majority of patients suffering from schizophrenia have life-long social disability, which include educational, occupational, and economical/financial disabilities that are not completely restored with current antipsychotics (Goldberg and Weinberger, 1988; Kandel et al., 2013a; Keefe and Harvey, 2012; Ragland et al., 2007). The ability of PAOPA to prevent and/or reverse positive and negative-like symptoms has been investigated in amphetamine and MK-801 models of the disease, but its effects on cognition and other models, as well as its mechanism of action are not completely understood.

To address these issues, the present thesis investigated the ability of PAOPA to prevent and/or reverse attentional deficits in an amphetamine model, its ability to prevent and/or reverse schizophrenia-like symptoms in a PCP model, and lastly its effects on specific molecular targets in three separate studies. These studies have been divided by objectives, methods, results, and discussion. At the end, limitations

of the three studies are presented, and a conclusion is made regarding the future potential of PAOPA for the treatment of schizophrenia.

7 Study one – An investigation of the ability of PAOPA to ameliorate amphetamine-induced impairments in attention

7.1 Brief Summary

The purpose of this study was to investigate the ability of PAOPA to prevent and/or reverse amphetamine-induced impairments in accuracy. The 5-choice serial reaction time task was employed to assess impairments in attention. Rats were first trained to criterion performance in 5-CSRTT. They were then sensitized to amphetamine by administering amphetamine regularly and increasing the dose each week for 5 weeks. After 10 weeks, animals were tested on 5-CSRTT by increasing the difficulty of the task. Their locomotor activity was also measured. In general, the sample size was too small to give any significant results. However, 5-CSRTT revealed impairment of attentional performance in the amphetamine-sensitized group compared to saline. The performance of rats treated with PAOPA and amphetamine concomitantly showed performance that seemed to fall between saline- and amphetamine-treated rats, suggesting that PAOPA partially prevented deficits. However, the performance of AP rats was not significantly different from amphetamine-treated rats or saline-treated rats. This was likely due to the low

power of this group. The results from this study unfortunately cannot make any conclusions about the ability of PAOPA to prevent and reverse attentional deficits in an amphetamine-sensitized model of cognitive-like symptoms in schizophrenia. However, the data suggests a possible effect that warrants further study with a larger sample size.

8 HYPOTHESES

The following were the study's hypotheses:

- 1) Rats sensitized to amphetamine would show reduced percent accuracy and increased number of premature responses compared to saline-treated rats when challenged with a variable stimulus duration and variable inter-trial interval, respectively
- 2) Rats sensitized to amphetamine and treated with PAOPA concomitantly would show no difference in percent accuracy and number of premature responses compared to saline-treated rats when challenged with a variable stimulus duration and variable inter-trial interval, respectively
- 3) Rats sensitized to amphetamine would show reduced percent accuracy and increased number of premature responses compared to rats sensitized to amphetamine and treated with PAOPA concomitantly when challenged with a variable stimulus duration and variable inter-trial interval, respectively

- 4) Rats sensitized to amphetamine would show reduced percent accuracy and increased number of premature responses compared to PAOPA-treated rats when challenged with a variable stimulus duration and variable inter-trial interval, respectively
- 5) Rats treated with PAOPA would show no difference in percent accuracy and number of premature responses compared to saline-treated rats when challenged with a variable stimulus duration and variable inter-trial interval, respectively
- 6) Amphetamine-sensitized rats would show increased percent accuracy and decreased number of premature responses when administered a one-time PAOPA dose 30 minutes prior to testing compared to amphetamine-sensitized rats administered saline 30 minutes prior to testing and when challenged with a variable stimulus duration and variable inter-trial interval
- 7) Amphetamine-sensitized rats would show no difference in percent accuracy and number of premature responses when administered a one-time PAOPA dose 30 minutes prior to testing compared to saline-treated rats administered saline 30 minutes prior to testing and when challenged with a variable stimulus duration and variable inter-trial interval

9 MATERIALS AND METHODS

9.1 *Animals*

Ten Sprague-Dawley rats were purchased from Charles Rivers Laboratories (Quebec) and housed in the central animal facility at McMaster University. Since the various tests would take long periods of time, the animals were kept under reversed light cycle with lights off at 7:30 am and on at 7:30 pm so that all of the testing could be completed during the day. The animal procedures were approved by the McMaster University Animal Research Ethics Board and were in accordance with the stipulations of the Canadian Council on Animal Care.

9.2 *Habituation and Feeding*

Upon arrival rats were first allowed to habituate to their new environment for one week. Then experimenters handled the rats for three days and introduced the 5-CSRTT food reinforcement, which the rats would later use during training and testing, by placing approximately 50 or so pellets in ramekins in each rat's cage. On each day of handling, experimenters replenished the pellets. The next day, rats were food restricted to 15 or 16 g of food per day and started training on the 5-CSRTT. After this point rats were weighed weekly to ensure their weights did not change above 15 g.

9.3 5-CSRTT Training

The room housing the 5-CSRTT chambers were kept dimly illuminated using red light and training started at approximately 11 am each day. The task takes place in an operant sound-attenuating chamber. On one end of the chamber are five apertures that can be illuminated and can detect entries of a rat's nose. On the opposite end is a receptacle into which the food reinforcement—45-mg dustless precision pellets (F0021, Cederlane, Mississauga, ON)—is dispensed. Before training on 5-CSRTT was commenced, the rats needed to first learn some simple behaviours such as poking their noses into an aperture that was illuminated and collecting the food reinforcement. A new program was developed to automate this acquisition, which was previously done manually by hand-baiting each aperture and illuminating it. The program that runs the final 5-CSRTT procedure was modified to a set of simple events: when the program is started, three pellets are released into the receptacle, which is illuminated. It remains illuminated until the rat pokes its nose inside it, which likely infers that it collected the food pellets, starting the first trial. Next, one of the five apertures illuminates with the location random. The aperture stays illuminated until the rat pokes its nose inside it; nose-pokes into apertures not illuminated have no effect. Once the illuminated aperture is selected, three pellets are released into the receptacle. Collecting these pellets initiates the next trial. An acquisition session is completed once the rat completes 10 trials or 10 minutes have elapsed. Rats were trained on this acquisition once a day, five days

per week, until all rats performed the sessions perfectly. Following acquisition, rats were trained on the complete 5-CSRTT task.

Rats were trained on the complete 5-CSRTT procedure following the protocol outlined by (Bari et al., 2008) and shown in Table 1.

Table 1. 5-CSRTT training schedule. From (Bari et al., 2008)

Training stage	Stimulus duration (s)	ITI (s)	LH (s)	Criterion to move to next stage
1	30	2	30	≥ 30 correct trials
2	20	2	20	≥ 30 correct trials
3	10	5	10	≥ 30 correct trials
4	5	5	5	≥ 50 correct trials > 85% accuracy
5	2.5	5	5	≥ 50 correct trials > 85% accuracy < 15% omissions
6	1.25	5	5	≥ 50 correct trials > 85% accuracy < 15% omissions
7	1	5	5	≥ 50 correct trials > 85% accuracy < 15% omissions

Since the rats were under a reversed light cycle, the house light of the 5-CSRTT chambers was left off throughout the session. A session started with the illumination of the receptacle and the release of one pellet into it. Once the rat collected the pellet, the first trial began (Figure 3).

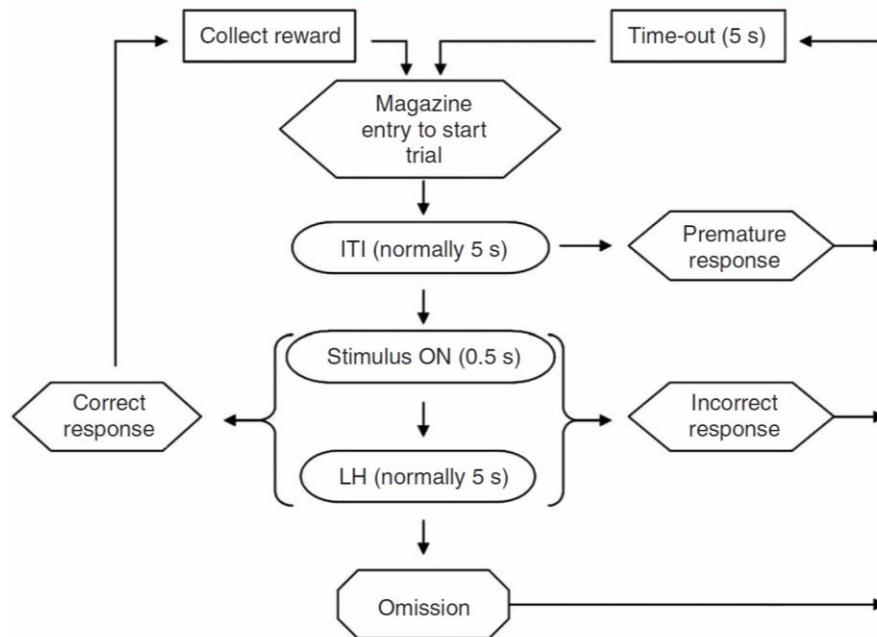


Figure 3. Trial schedule. From (Bari et al., 2008).

A trial starts with an inter-trial interval (ITI), in which the rat must wait and not respond. If the rat does not respond during ITI, one of the five apertures will randomly be illuminated. If the rat responds during ITI, the response is recorded as a premature response, and the house light is turned on for 5 seconds—known as punishment or time out. Responses made during time outs are recorded as time out responses and restart the 5-s time out. Once the time out elapses without a response, the ITI is restarted. When one of the five apertures is illuminated, the rat has two opportunities to make a correct selection: either while the aperture is still illuminated for a set time (stimulus duration) or after the stimulus duration when the program waits for a set time (limited hold) to record a response. A correct response made in either of these two periods is rewarded with a pellet released into

an illuminated receptacle and head entry into the receptacle starts the next trial. If the rat makes an incorrect response during the stimulus duration or the limited hold, an incorrect response is recorded and the rat is punished with a 5-s time out. If by the end of the limited hold the rat has not made a response, an omission is recorded and the rat is punished with a 5-s time out. The next trial is initiated after either of these time outs. A session is complete after 100 trials or 30 minutes. Rats were trained once a day, six days per week, Monday to Saturday. Rats first started on relatively “easy” parameters of the task—a 30-s stimulus duration, a 30-s limited hold, and a 2-s ITI—and easy criteria to advance to the next level of training—for example, a rat would need at least 30 correct trials to pass from the first training level to the second. As rats advanced the training levels the stimulus duration and limited hold were progressively shortened and the ITI increased, until at the final training level (T7) the stimulus duration was 1 s, the limited hold 5 s, and the ITI 5 s. Rats were continuously trained on T7 until they performed at ≥ 50 correct responses, $> 85\%$ accuracy, and $< 15\%$ omissions for seven consecutive training sessions. Once this was achieved the sensitization period was started and followed that of Fletcher et al. (2007): during the 5-week sensitization and 5-week withdrawal, rats performed 5-CSRTT (T7) three times a week on Tuesdays, Thursdays, and Saturdays.

9.4 *Drugs and Sensitization*

D-amphetamine sulfate was purchased from Toronto Research Chemicals (Toronto, ON). Amphetamine and PAOPA solutions were prepared in the morning on the day of the injections and dissolved in saline (0.9% NaCl). PAOPA was prepared at a concentration of 1 mg/mL and the dose injected was 1 mg/kg (Beyaert et al., 2013). Amphetamine sensitization closely followed the design of Fletcher et al. (2007), and was an extension of Beyaert et al. (2013), and Tenn et al. (2003): in the first week of the sensitization, amphetamine was prepared at a concentration of 1 mg/mL and the dose injected was 1 mg/kg. In subsequent weeks, the concentration of the solution prepared and the dose injected was increased by 1 mg/mL and 1 mg/kg, respectively, each week for 4 weeks. Saline was purchased from McMaster University Medical Science Store (Hamilton, ON). The treatment groups were: saline ($n = 2$), amphetamine ($n = 3$), amphetamine and PAOPA ($n = 3$), and PAOPA ($n = 2$). Drugs were injected via the intraperitoneal route three times a week on Mondays, Wednesdays, and Fridays for five weeks. The five-week sensitization period was followed by a five-week withdrawal period where the rats were not injected with any drug. During this time, rats only performed 5-CSRTT (T7) on Tuesdays, Thursdays, and Saturdays. After the last week of withdrawal, the ability of PAOPA to prevent or reverse amphetamine-induced impairments in accuracy or impulsivity were tested. Testing always occurred on Tuesdays and Fridays with Mondays, Wednesdays, and Thursdays serving as standard training

(1-s stimulus duration and 5-s ITI) days so that the rats would not adapt to the augmented difficulty of 5-CSRTT testing. Animals did not perform 5-CSRTT on weekends.

9.5 Experiment 1: Effect of stimulus duration manipulation on percent accuracy

To test the ability of PAOPA to prevent amphetamine-induced impairments in accuracy, the rats' attentional performance on 5-CSRTT was challenged by manipulating the stimulus duration using a Latin square design. On a testing day, each rat performed a session of 5-CSRTT using one of five stimulus durations (2, 1, 0.5, 0.25, or 0.13 s). The order of the varied stimulus duration was counterbalanced within each treatment group as far as possible. As testing was done on Tuesdays and Fridays, it took two and a half weeks to complete Experiment 1.

9.6 Experiment 2: Effect of variable inter-trial interval on premature responses

To test the ability of PAOPA to prevent amphetamine-induced impairments in premature responses, the rats' ability to inhibit premature responses was challenged by varying the inter-trial interval within a single session. The previously standard ITI of 5 s was varied randomly between 3.5, 5.5, 7.5, and 9.5 s. Testing occurred on the Friday of the third week after withdrawal.

9.7 *Experiment 3: Effect of variable stimulus duration on percent accuracy*

Since the present study had a few experiments to test after manipulating stimulus duration and since manipulating stimulus duration a session at a time takes a relatively long time to complete, the ability of a single session of variable stimulus durations to elucidate amphetamine-induced impairments in accuracy was investigated 8 weeks after drug cessation. It is possible to configure the 5-CSRTT program to randomly vary the stimulus duration for a single session. Although such an experiment has the disadvantage of yielding less data than manipulating stimulus duration one session at a time, it has the advantage of assessing the same question in a greatly reduced period of time (five sessions in two and a half weeks versus one session in one day).

Variable stimulus durations were repeated 10, 13, and 14 weeks after drug cessation to investigate the duration of any amphetamine-induced impairments on percent accuracy.

9.8 *Experiment 4: Effect of an acute PAOPA administration on percent accuracy*

To assess whether PAOPA could reverse amphetamine-induced impairments in accuracy, amphetamine-treated rats were administered 1 mg/kg PAOPA or 1 mL/kg saline 30 minutes prior to the start of 5-CSRTT. The stimulus

duration was varied within the session as described above. The consequent results were compared against the previous performance (Experiment 3) of amphetamine-treated rats in a session of variable stimulus duration. Testing took place one week after the first variable stimulus duration session.

9.9 *Experiment 5: Confirmation of sensitization using locomotor activity*

To confirm amphetamine sensitization and replicate prior work of Beyaert et al. (2013), rats' locomotor activity was assessed at various time points during sensitization and six weeks after drug cessation, during 5-CSRTT testing.

Locomotor Activity

Increased locomotor activity (hyper-locomotor activity) is proposed to be a manifestation of positive-like symptoms in rodents (Beyaert et al., 2013). AccuScan computerized locomotor activity chambers (AccuScan Instruments, Columbus, OH) used infrared sensors to track the distance animals travelled. Rats were placed in the locomotor activity chamber and baseline was recorded for 1 hour. Next, all rats received a 1 mg/kg amphetamine challenge and were put back in the locomotor activity chambers to record locomotor activity for 2 hours. At the end of 2 hours, rats were transferred back to their home cages, and the locomotor chambers cleaned with 75% ethanol.

9.10 Analyses

Data from 5-CSRTT experiments (1 – 4) were analyzed using repeated measures two-way analysis of variance (ANOVA) with Bonferroni post-hoc tests. Depending on the experiment, either the stimulus duration (Experiments 1, 3, and 4) or ITI (Experiment 2) was one factor and treatment the second factor. Data from locomotor activity was analyzed for total distance travelled using one-way ANOVA and Tukey's post-hoc tests.

10 RESULTS

10.1 5-CSRTT

Rats took approximately two months to complete training and perform stably at T7. Once trained, rats were divided into the four treatment groups and did not differ in percent accuracy ($F=0.1225$, $p=0.9462$), percent omissions ($F=0.5046$, $p=0.6815$), premature responses ($F=0.2587$, $p=0.8546$), perseverative responses ($F=1.727$, $p=0.1789$), correct response latency ($F=0.3658$, $p=0.7781$), or reward latency ($F=1.115$, $p=0.3560$; Figure 34 to Figure 39).

10.2 Administration of and withdrawal from amphetamine

During drug administration (A1 – A5) and withdrawal (W1 – W5) there were no significant differences between the four treatment groups in percent accuracy ($p=0.9952$), percent omissions ($p=0.1040$), perseverative responses ($p=0.3469$), premature responses ($p=0.6638$), or correct response latency ($p=0.3143$; Figure 34 to Figure 38 in Appendix A). Although there was no main effect of treatment ($p=0.2112$) on reward latency (Figure 39 in Appendix A), post-tests revealed a significant difference between amphetamine- and PAOPA-treated rats (AP) on the one hand and saline ($p<0.01$) and amphetamine ($p<0.01$) treated rats on the other during the fourth week of administration, where AP rats took longer than both groups to collect the food reward. Aside from these two differences, no other significant differences were observed during the drug administration and withdrawal periods.

10.3 Experiment 1: Effect of stimulus duration manipulation on percent accuracy

Manipulating stimulus duration between 0.125 and 2 s had no main effect of treatment ($p=0.5882$) on percent accuracy (Figure 4). There was a main effect of subjects matching ($p=0.0036$) and stimulus duration ($p<0.001$) where decreasing stimulus duration decreased the percent accuracy for all treatment groups. Stimulus duration manipulation had no effect on percent omissions ($p=0.4079$), perseverative responses ($p=0.6060$), correct response latency ($p=0.4744$), or reward latency

($p=0.6185$)—although for reward latency some non-significant consistent difference may have appeared between treatment groups (Figure 40 to Figure 44. There was a main effect of subjects matching ($p<0.001$) for reward latency (Figure 44 in Appendix A) and a main effect of stimulus duration for correct response latency ($p=0.0040$; Figure 43 in Appendix A) and reward latency ($p=0.0322$) where decreasing the stimulus duration generally tended to decrease both correct response latency and reward latency. Stimulus duration manipulation did however have a main effect of treatment ($p=0.0138$) on the number of premature responses (Figure 41 in Appendix A) and Bonferroni post-tests revealed a significant decrease in premature responses for AP rats compared to saline rats at the 0.25 s stimulus duration ($t=3.414$, $p<0.01$). A student's t-test was performed between saline- and amphetamine-treated rats' accuracy for the 0.125 s stimulus duration; although a significance was not observed, the p value approached the 0.05 threshold ($t=2.771$, $df=3$, $p=0.0695$).

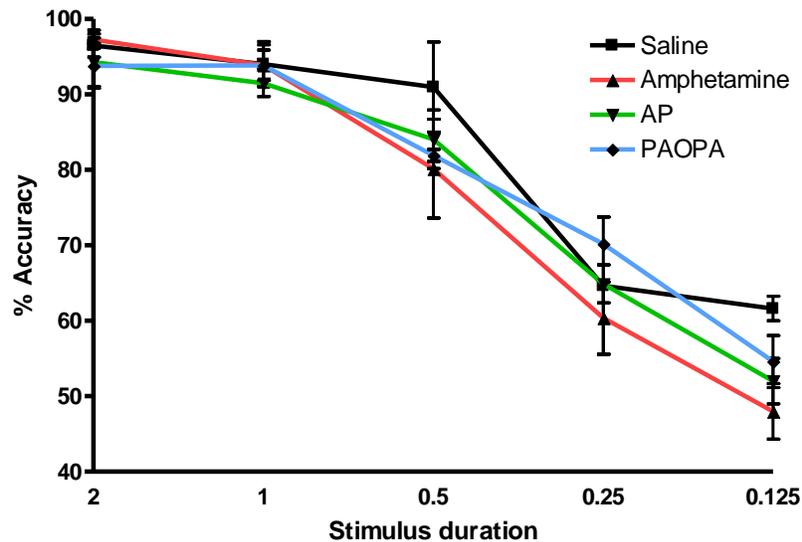


Figure 4. Effect of stimulus duration manipulation on percent accuracy. There was no main effect of treatment. Stimulus duration significantly decreased percent accuracy for all treatment groups ($p < 0.0001$). There was a main effect of subjects matching ($p = 0.0036$). A student's t-test comparing saline and amphetamine treatments at the 0.125 s stimulus duration revealed a p value ($p = 0.0695$) close to the 0.05 threshold. Data represents mean \pm standard error of mean.

10.4 Experiment 2: Effect of variable inter-trial interval on premature responses

There was no main effect of treatment ($p = 0.2330$) on the number of premature responses made when ITI was varied within a single session. Bonferroni post-tests revealed a significant difference between saline treated and AP rats ($t = 3.126$, $p < 0.05$) and amphetamine treated and AP rats ($t = 3.641$, $p < 0.01$) for total ITIs (Figure 5).

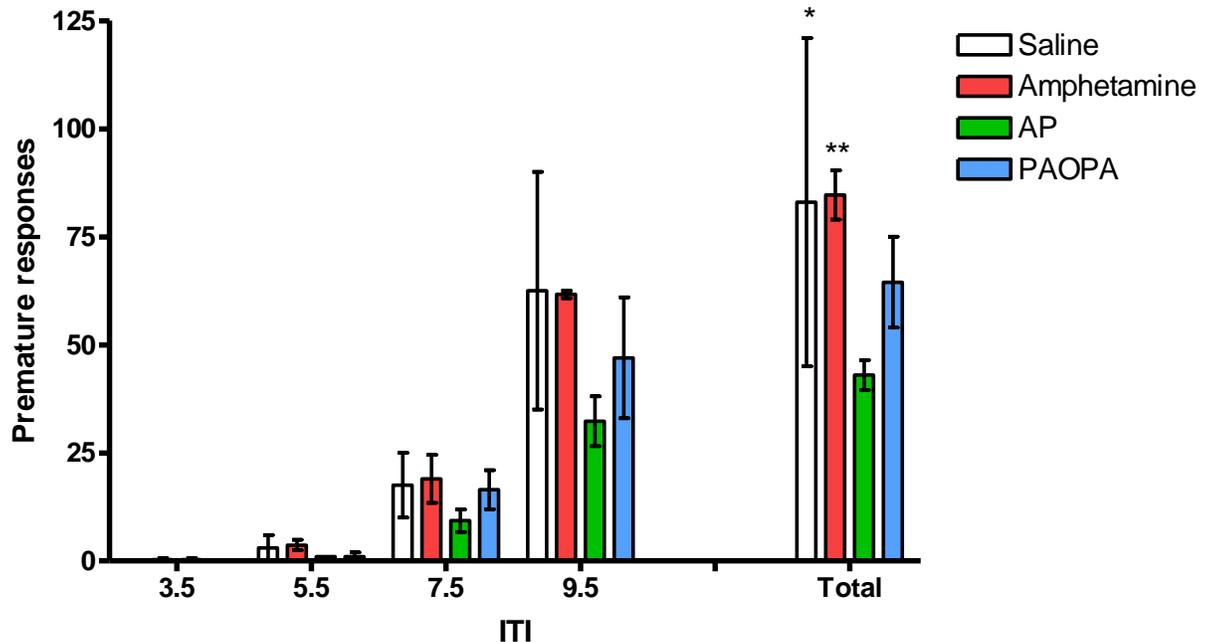


Figure 5. The effects of variable ITI on premature responses. ITI was varied between 3.5, 5.5, 7.5, and 9.5 s within a single session. There was a main effect of ITI ($p < 0.0001$) and subjects ($p = 0.0147$) on premature responses. When premature responses for all ITIs were summed, AP rats had lower premature responses than saline ($t = 3.126$, $p < 0.05$), and amphetamine ($t = 3.641$) treated rats. Data represents mean \pm standard error of mean.

Variable ITI did not elicit any main effects of treatment in percent accuracy ($p = 0.1469$), percent omissions ($p = 0.0770$), correct response latency ($p = 0.1029$), or reward latency ($p = 0.4764$; Figure 45 to Figure 48 in Appendix A). However, Bonferroni post-tests revealed significant differences for percent accuracy, percent omissions, and correct response latency for various treatment groups at specific ITIs. For percent accuracy (Figure 45, Appendix A), there was a main effect of interaction ($p = 0.0134$), ITI ($p < 0.0001$), and subject matching ($p = 0.0012$). AP rats

performed better than saline (9.5 s, $t=3.363$, $p<0.01$), amphetamine (9.5 s, $t=4.247$, $p<0.01$), and PAOPA (7.5 s, $t=3.198$, $p<0.05$) treated rats at specific ITIs. For percent omissions (Figure 46, Appendix A), there were no main effects. Bonferroni post-tests revealed significant differences between PAOPA and amphetamine treated rats (9.5 s, $t=4.113$, $p<0.01$), and PAOPA and AP rats (9.5 s, $t=3.253$, $p<0.05$), where PAOPA rats had higher percent omissions than amphetamine and AP rats. For correct response latency (Figure 47, Appendix A), there was a main effect of ITI ($p<0.0001$). In addition, amphetamine-treated rats had lower correct response latencies than AP ($t=2.875$, $p<0.05$) and PAOPA ($t=3.095$, $p<0.05$) treated rats at an ITI of 9.5 s. Finally, for reward latency (Figure 48, Appendix A), there was only a significant main effect of subject matching ($p<0.0001$).

10.5 Experiment 3: Effect of variable stimulus duration on percent accuracy

When the stimulus duration was varied within a single session—unlike Experiment 1, when it was varied one session at a time—there was still no main effect of treatment of percent accuracy ($p=0.5938$; Figure 6). However, there was a significant main effect of stimulus duration ($p<0.0001$)—indicating that percent accuracy decreased with decreasing stimulus duration—and subjects ($p=0.0076$). Bonferroni post-tests did not reveal any significant differences between treatment groups. A student's t-test between saline and amphetamine treated rats at the shortest stimulus duration (0.125 s) revealed a p value ($t=2.2391$, $df=3$, $p=0.0966$)

that was mildly greater than the 0.05 threshold and the 0.0695 value determined in Experiment 1.

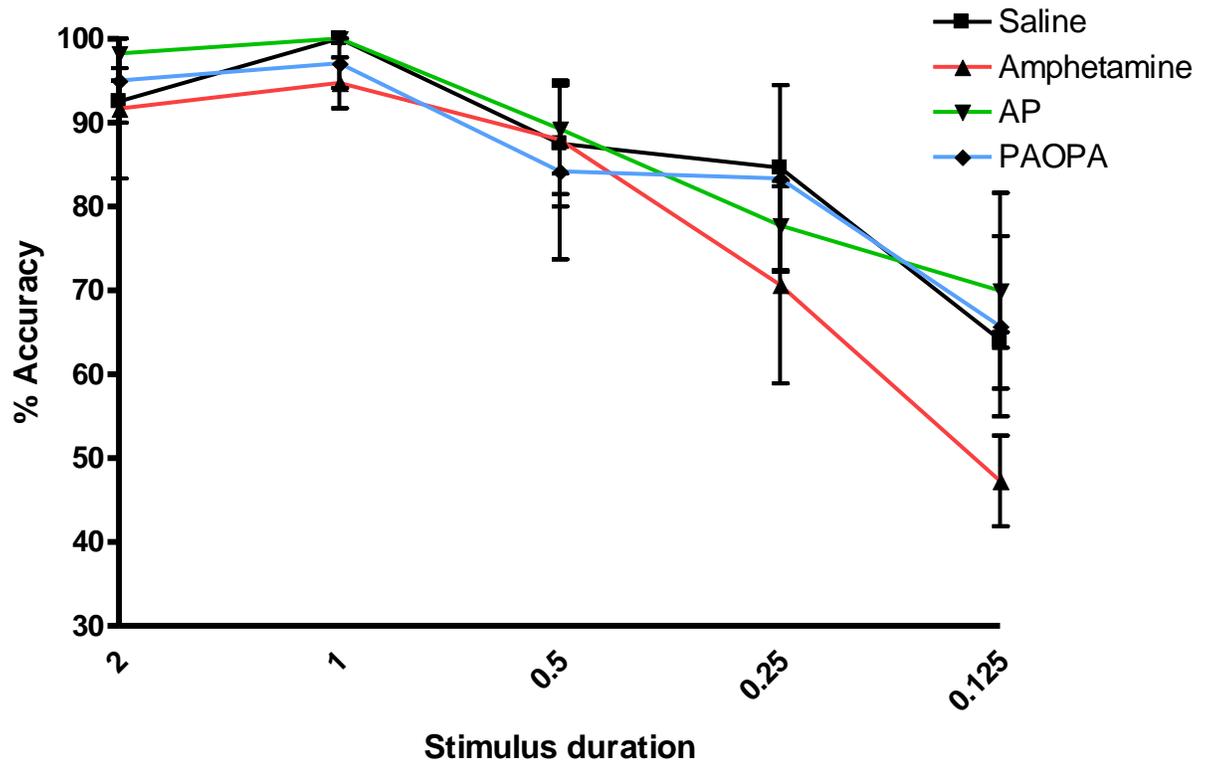


Figure 6. The effect of varying stimulus duration on percent accuracy. Stimulus duration was varied within a single session (2, 1, 0.5, 0.25, 0.125 s). There was no main effect of treatment ($p=0.5938$) nor any differences revealed by Bonferroni post-tests. There were significant main effects of subjects ($p=0.0076$) and stimulus duration ($p<0.0001$), the latter indicating that decreasing the stimulus duration decreased the percent accuracy for all treatment groups. A student's t-test revealed a p value ($t=2.391$, $df=3$, $p=0.0966$) that was mildly greater than the 0.05 threshold. Data represents mean \pm standard error of mean.

Varying stimulus duration also had no significant main effect of treatment on percent omissions ($p=0.0799$; Figure 49, Appendix A), although this seemed to

approach significance. There was a main effect of stimulus duration ($p=0.0092$), which indicates that decreasing the stimulus duration tended to increase percent omissions, and subjects ($p=0.0496$). Bonferroni post-tests revealed that the AP treatment group had higher percent omissions than the saline (0.125 s, $t=4.015$, $p<0.01$; 0.25 s, $t=2.868$, $p<0.05$), amphetamine ($t=3.078$, $p<0.05$), and PAOPA ($t=3.327$, $p<0.05$) treatment groups.

The duration of amphetamine-induced impairments in percent accuracy was also investigated. The data for each session are shown in Figure 50 to Figure 54, and summarized in Figure 55 and Figure 56 in Appendix A.

10.6 Experiment 4: Effect of an acute PAOPA administration on percent accuracy

Nine weeks after drug cessation, the ability of a one-time 1 mg/kg PAOPA intraperitoneal injection to reverse amphetamine-induced impairments in percent accuracy was assessed and is presented in Figure 7. There was no significant main effect of treatment ($p=0.4700$), although stimulus duration ($p<0.0001$) and subjects (0.0019) had significant main effects. Bonferroni post-tests also did not reveal any significant differences between treatment groups.

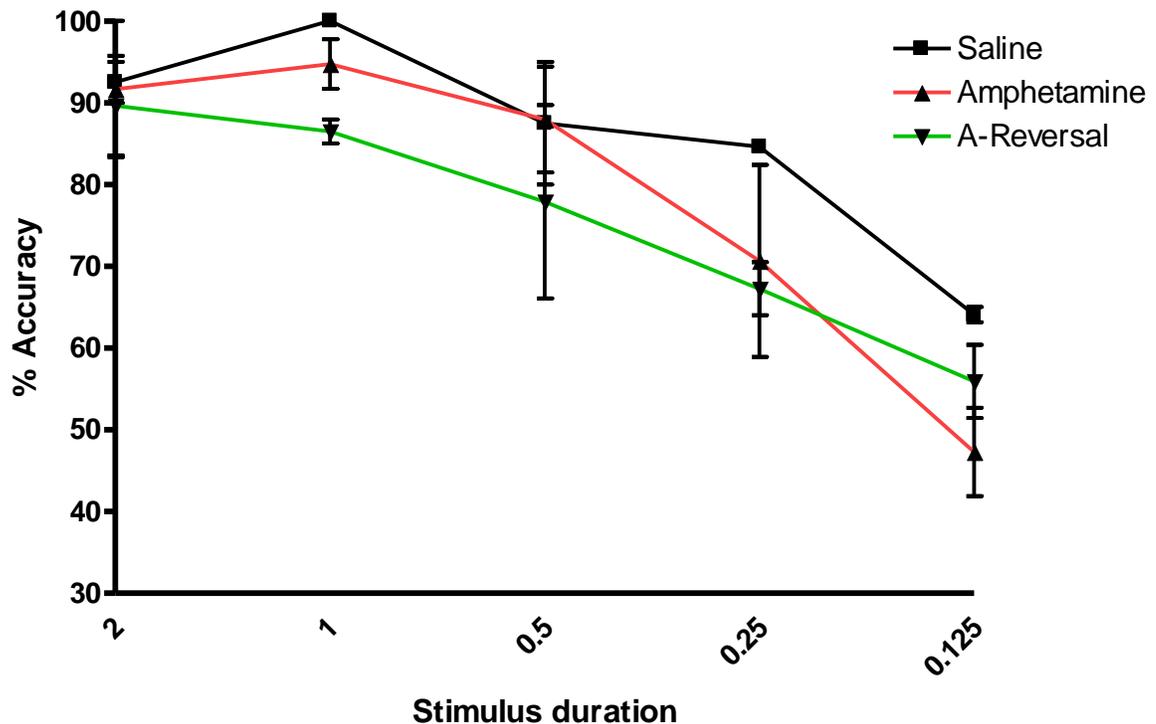


Figure 7. The effect of one-time PAOPA administration on amphetamine-induced impairments in accuracy. 1 mg/kg PAOPA was administered i.p. to amphetamine-treated rats (A-Reversal, green line). Testing was performed 9 weeks after drug cessation. The performance was compared to the previous performance, which took place 8 weeks after drug cessation, of amphetamine (red line) and saline (black line) treated rats. The stimulus duration was varied within a single session (2, 1, 0.5, 0.25, and 0.125 s). There was no main effect of treatment; there was a main effect of stimulus duration ($p < 0.0001$) and subjects ($p = 0.0019$). Bonferroni post-tests did not reveal significant differences between treatment groups. Data represents mean \pm standard error of mean.

With regards to effects on percent omissions, there were no main effects of treatment, or stimulus duration (Figure 57). Subjects had a significant main effect ($p = 0.0230$) on percent omissions.

10.7 Experiment 5: Confirmation of sensitization using locomotor activity

Six weeks after drug cessation, locomotor activity was assessed to confirm amphetamine sensitization. Figure 8 illustrates the data. There was a significant main effect of treatment ($p=0.0002$), amphetamine challenge ($p<0.0001$), and interaction ($p=0.0008$). Bonferroni post-tests revealed no significant differences between treatment groups during baseline. However, after the amphetamine challenge, post-tests revealed that amphetamine treated rats had significantly greater locomotor activity than saline ($t=5.916$, $p<0.001$), AP ($t=3.239$, $p<0.05$), and PAOPA ($t=7.743$, $p<0.001$) treated rats. AP rats also had significantly greater locomotor activity than saline ($t=3.019$, $p<0.05$) and PAOPA ($t=5.452$, $p<0.001$) treated rats. Finally, PAOPA treated rats had the lowest locomotor activity of all (saline, $t=2.890$, $p<0.05$). Figure 58 in Appendix A illustrates locomotor activity during recording.

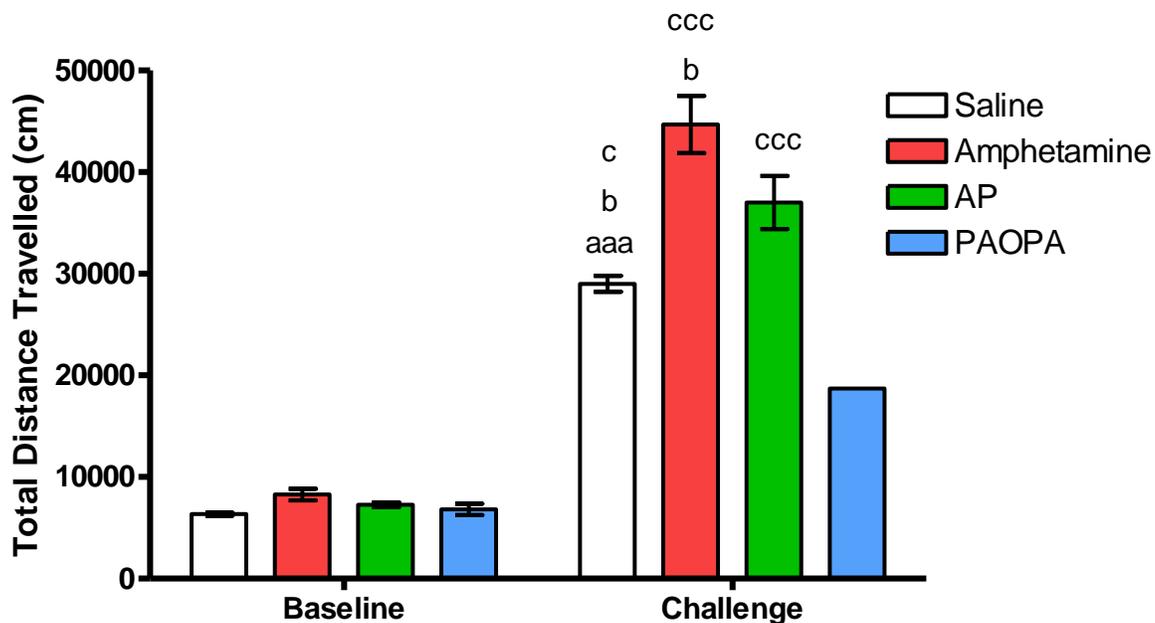


Figure 8. Confirmation of amphetamine sensitization. Six weeks after drug cessation, rats were tested for amphetamine sensitization. Locomotor activity was recorded for 1 hour for baseline values. A 1 mg/kg amphetamine injection was given i.p. and locomotor activity recorded for two more hours. There was a significant main effect of amphetamine challenge ($p < 0.0001$) and interaction ($p = 0.0008$). Bonferroni post-tests indicated significant differences after the challenge. A superscript “a” denotes significant difference to amphetamine rats; “b” to AP rats; and “c” to PAOPA rats. ^{aaa}, $p < 0.001$; ^b and ^c, $p < 0.05$; ^{ccc}, $p < 0.001$. Data represents mean \pm standard error of mean.

Amphetamine sensitization was confirmed again twice, 14 weeks after drug cessation. In the first test (Figure 59, Appendix A), there was no significant main effect of treatment ($p = 0.8303$) and additionally Bonferroni post-tests did not reveal any differences between treatment groups. However, in the second test, which took place two days later, there was a significant main effect of treatment ($p = 0.0498$).

Bonferroni post-tests revealed that the amphetamine group was not statistically different from the saline group ($t=2.374$, $p>0.05$). The only differences found were between PAOPA rats and amphetamine ($t=4.732$, $p<0.001$) and AP ($t=2.807$, $p<0.05$) treated rats (Figure 9).

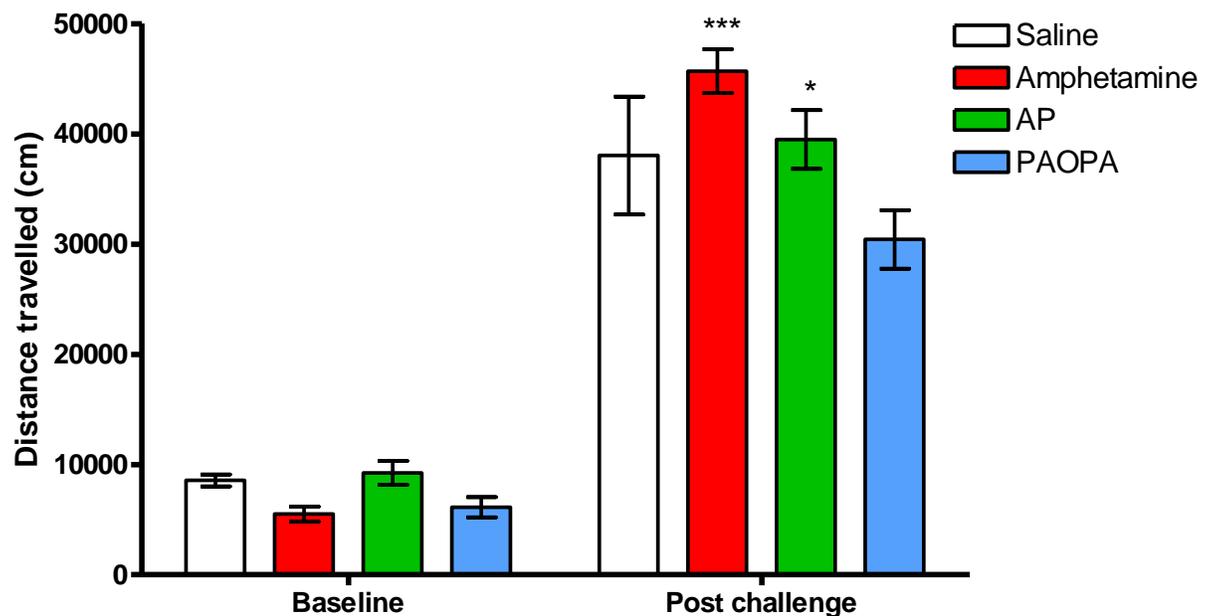


Figure 9. Confirmation of amphetamine sensitization, 14 weeks after drug cessation. Baseline activity was recorded for one hour prior to a 1 mg/kg amphetamine i.p. injection, which was followed by two hours of recording. There was a significant main effect of treatment ($p=0.0498$), and amphetamine challenge ($p<0.0001$). Bonferroni post-tests only indicated significant differences for PAOPA treated rats, which had lower locomotor activity than amphetamine ($t=4.732$, $*p<0.001$) and AP ($t=2.807$, $*p<0.05$) treated rats. Data represents mean \pm standard error of mean.**

On the first day of drug administration, rats injected with amphetamine or amphetamine and PAOPA had significantly greater locomotor activity than both saline and PAOPA treated rats (Figure 10).

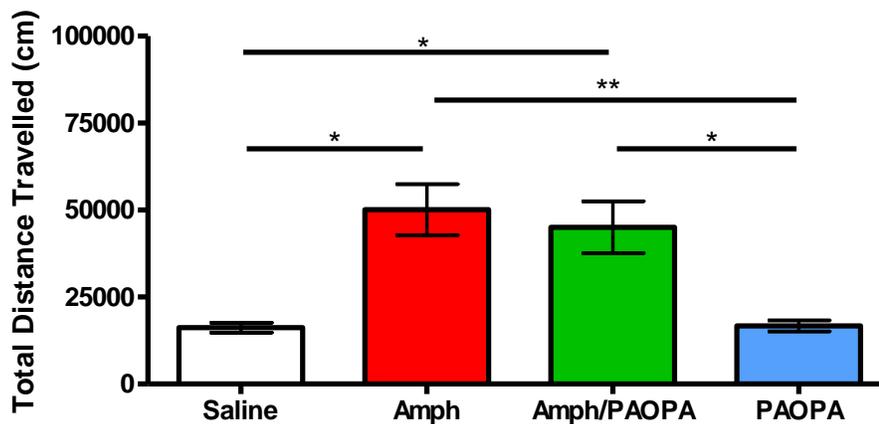


Figure 10. Effect of acute drug administration on locomotor activity. On the first day of drug administration, rats were recorded for locomotor activity for 3 hours after injections. One-way ANOVA indicated a significant difference between treatments ($F=9.007$, $p=0.0014$). Tukey's multiple comparison tests revealed that amphetamine treated rats had greater locomotor activity than saline ($p<0.05$), and PAOPA ($p<0.01$) treated rats. Furthermore, AP rats had greater locomotor activity than saline ($p<0.05$) and PAOPA ($p<0.05$) treated rats. Data represents mean \pm standard error of mean.

In addition, observation of the interval data (Figure 11), indicated that the greater locomotor activity of amphetamine and AP groups occurred at the beginning of recording.

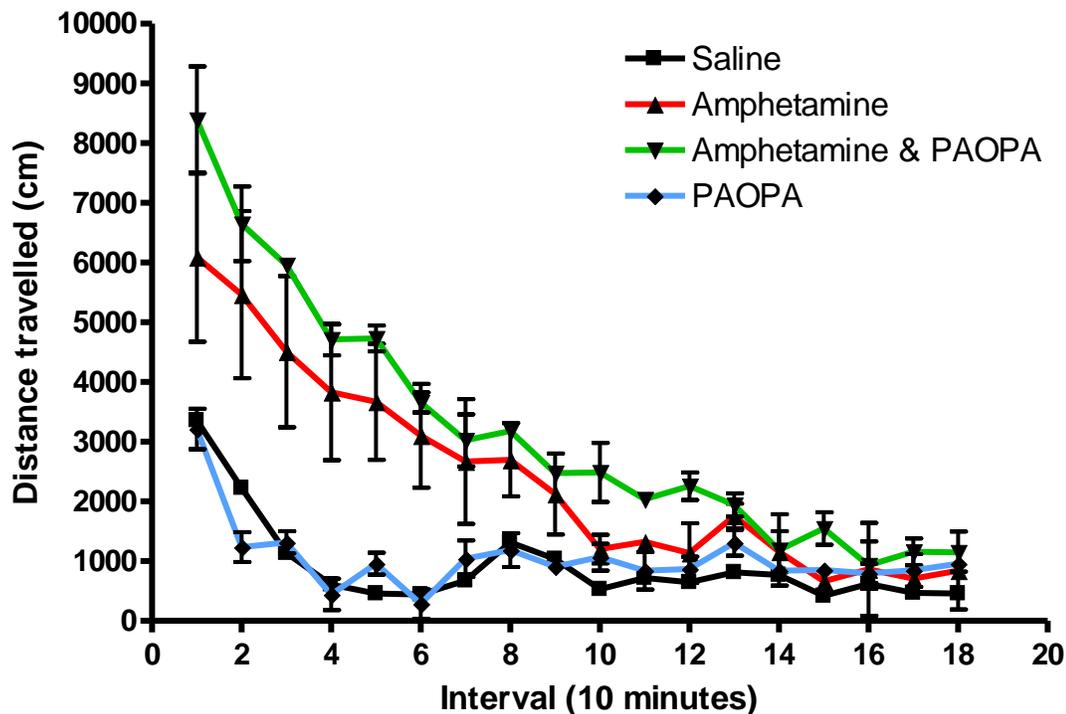


Figure 11. The effect of acute drug administration on locomotor activity. Rats were recorded for 3 hours for locomotor activity after the first drug administration. A cursory observation of the data suggests that the enhanced locomotor activity of amphetamine (red) and AP (green) groups occurred at the beginning of recording. Data represents mean \pm standard error of mean.

Rats were again recorded for locomotor activity after the last day of drug administration. Tukey's multiple comparison tests revealed that the AP group had significantly greater locomotor activity compared to all other groups (saline, $p < 0.01$; amphetamine, $p < 0.05$; PAOPA, $p < 0.05$; Figure 62 in Appendix A). Observation of the interval data (Figure 12) suggested that this enhancement occurred towards the end of recording.

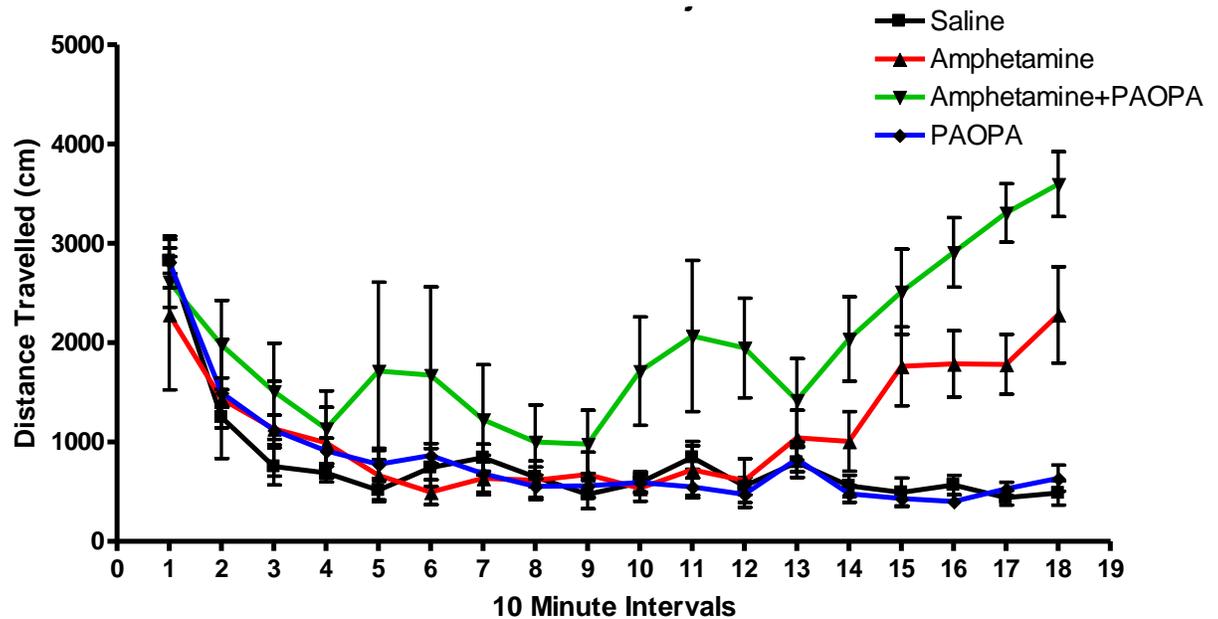


Figure 12. Effect of final drug administration on locomotor activity. Rats were recorded for 3 hours for locomotor activity after the final drug administration. A cursory observation of the data suggests that the enhanced locomotor activity of AP (green) occurred towards the end of recording. The data also suggests that amphetamine-treated rats had a delayed non-significant enhancement of locomotor activity. Data represents mean \pm standard error of mean.

In the first week of withdrawal, no differences were observed between treatment groups ($F=0.2258$, $p=0.8776$; Figure 13).

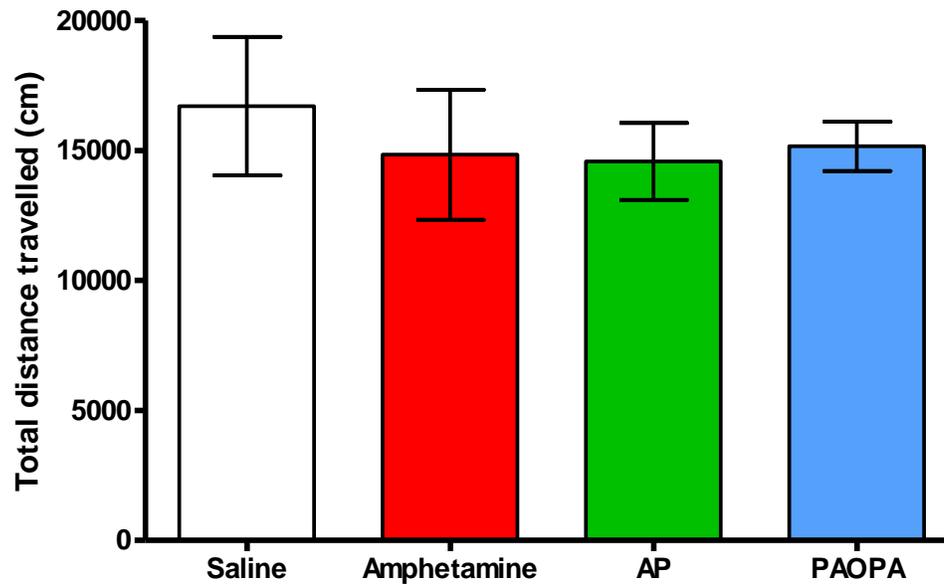


Figure 13. Locomotor activity in the first week of withdrawal. Rats were recorded for 3 hours for locomotor activity. Shown above is total distance travelled. No significant differences were observed between treatment groups ($F=0.2258$, $p=0.8776$).

Similarly, in the first week after withdrawal—five weeks after drug cessation—no differences were observed between treatment groups ($F=1.925$, $p=0.1550$; Figure 14).

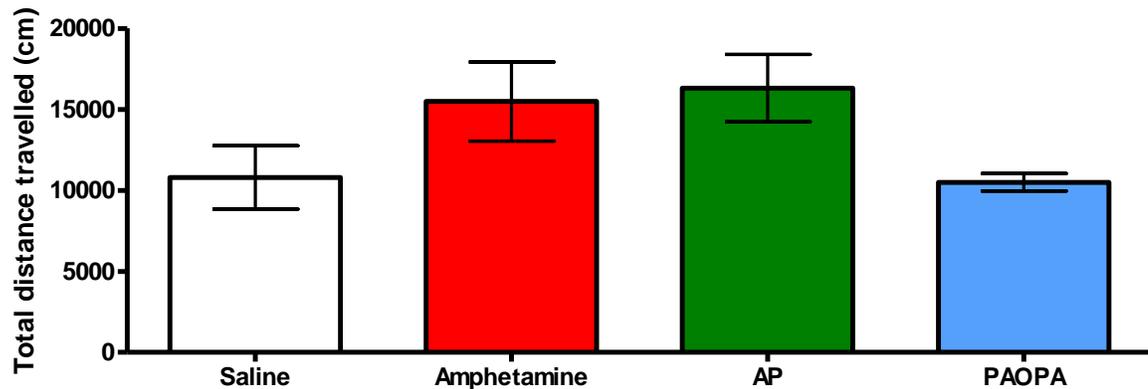


Figure 14. Locomotor activity in the first week after withdrawal. Five weeks after drug cessation, rats were recorded for 3 hours for locomotor activity. Shown above is total distance travelled. No significant differences were observed between treatment groups ($F=1.925$, $p=0.1550$).

11 DISCUSSION

This study lacked the sample size required to draw firm conclusions with regard to the ability of dopamine D₂ receptor allosteric modulator, PAOPA, to prevent or reverse amphetamine-induced impairments in percent accuracy, which was used as a measure of attention. Indeed, the sample size was too low to even observe the amphetamine-induced impairment in percent accuracy that Fletcher et al. (2007) observed. However, this pilot study can perhaps provide three new insights that can guide a future study with sufficient power to comprehensively investigate the therapeutic-like effects of PAOPA administration on impairments in accuracy/attention. First—based on simple observation—it appeared that PAOPA

may have had a partial attenuation and reversal of amphetamine-induced impairments in accuracy. Second, conducting a single session of variable stimulus duration can potentially provide the same results as manipulating the stimulus duration one session at a time over the course of two-and-a-half weeks. Third, it seemed there may be a short limit on the duration of amphetamine-induced impairment in accuracy.

This study did not find many significant differences during the drug administration and withdrawal period in percent accuracy, percent omissions, perseverative responding, premature responding, correct response latency, and reward latency. In fact, only one difference was found and by Bonferroni post-tests: rats receiving concurrent amphetamine and PAOPA injections (AP rats) on average took longer to collect the food reward than both saline ($p < 0.01$) and amphetamine ($p < 0.01$) treated rats during the fourth week of administration (Figure 39, Appendix A). On the surface, these results are dissimilar to those found by Fletcher et al. (2007), who reported a consistent increase in percent omissions and perseverative responding in amphetamine-treated rats, starting in the second week of administration and persisting thereafter. However, examination of Figure 35 (percent omissions) and Figure 36 (perseverative responding) in Appendix A indicates that percent omissions and perseverative responses were slightly (non-significantly) higher in amphetamine-treated rats compared to saline-treated rats. Percent omissions were not as increased in this study as in Fletcher et al.'s (2007)

study; this may be due to the greater training sessions (75 days vs 50 days) and reduced daily feeding (15 – 16 g vs 18 g) the present study had.

Over the course of two-and-a-half weeks, the stimulus duration was varied one session at a time between 2, 1, 0.5, 0.25, and 0.125 s to challenge the rats' attentional system and detect any differences in accuracy between the treatment groups. Decreasing the stimulus duration reliably decreased accuracy for all treatment groups ($p < 0.0001$; Figure 4). Repeated measures two-way ANOVA or Bonferroni post-tests did not reveal any significant differences between treatment groups, which may be a reflection of the small sample size. Figure 4 shows that the amphetamine group had a slightly lower accuracy at the shortest stimulus duration (0.125 s) than the saline group, and that of the AP and PAOPA group fell somewhere in between. Although non-significant, if real, these results would indicate that PAOPA has a partial attenuation on amphetamine-induced impairment in accuracy.

It took a relatively long time to assess accuracy using the method of Fletcher et al. (2007)—that is, changing the stimulus duration one session at a time and testing for five sessions, twice a week, over two-and-a-half weeks. In the present study, the Med-PC program was modified to vary the stimulus duration in a single session. The purpose of this was to drastically reduce the testing time from a little over two weeks to one day. The finding is presented in Figure 6 and demonstrates that a single session of variable stimulus duration was able to produce similar results

to repeated sessions. Decreasing the stimulus duration reliably decreased accuracy ($p < 0.0001$) similar to Experiment 1. Again, there appeared to be a reduction in accuracy by amphetamine-treated rats compared to saline-treated rats and a *t*-test comparing saline and amphetamine groups at the shortest stimulus duration gave a *p* value of 0.0966. In this experiment, however, the AP and PAOPA groups appeared to be closer to the saline group in accuracy than the amphetamine group at the shortest stimulus duration. Concerning omissions, there was no significant main effect of treatment ($p = 0.0799$) but it appeared to approach the threshold. In fact, Bonferroni post-tests revealed that the AP group had significantly greater omissions than all the other three treatment groups. Figure 49 (Appendix A) also suggests that the AP group was most affected by the variable stimulus duration as the group's omissions tended to rise with decreasing stimulus duration. This would suggest that with decreasing stimulus duration, the AP group was less motivated to respond. The reward latency, which was unfortunately not collected for this experiment, would be needed to confirm this hypothesis: if the AP group was less motivated then an increased reward latency would be observed relative to the other treatment groups. The lack of motivation on these trials do not affect interpretations of accuracy data, as the calculation of accuracy ignored omitted trials and only included trials in which responses were made.

The final part of the series of experiments examining accuracy tested whether a one-time 1 mg/kg PAOPA i.p. administration to amphetamine-sensitized

rats could reverse their impairments in accuracy. Their performance from this test was compared against the prior performance of the saline and amphetamine groups in the first variable stimulus duration test (Experiment 3). There were no main effects of treatment and Bonferroni post-tests did not further identify any differences. However, it seems that the amphetamine group given PAOPA (amphetamine-reversal) performed poorer than both the saline and amphetamine groups at 1- and 0.5-s stimulus duration, similar at the 0.25-s stimulus duration, and better at the shortest (0.125-s) stimulus duration. These results are incongruous with the previous tests of prevention and suggest that an acute PAOPA administration potentially exacerbated amphetamine-induced impairments in accuracy. It is strange that this happened for two of the stimulus durations and not for the shortest one where the attentional system is challenged the most.

The present study also tested the duration of effect of amphetamine-induced impairments in accuracy. Figure 55 shows that there may be a limit to how long the impairment lasts. Its presence seemed to wane 10 weeks after drug cessation—or 5 weeks after animals completed withdrawal—until the mean accuracies of amphetamine and saline groups were near identical at 13 weeks after drug cessation (8 weeks after completion of withdrawal). Conversely, there seemed to be an increase in omissions by the amphetamine group with the passage of time, suggesting that omitting and accurately performing in a trial can be dissociated. The increase in omissions may signal that the amphetamine-induced neurochemical

changes are decreasing the ability or the willingness of animals to perform on the task. The waning effect of amphetamine sensitization on accuracy bespeaks the relevance of testing accuracy in a single session in contrast to multiple sessions over two weeks. On the flip side, the advantage of faster testing should be weighed against the disadvantage of fewer data collected: with five separate sessions, 100 trials are collected for each stimulus duration, whereas with a single session only 20 trials are collected. This disadvantage could potentially be resolved by increasing the sample size of the study.

Fletcher et al. (2007) found that when saline and amphetamine-sensitized rats were tested on a session with variable inter-trial intervals (ITIs), amphetamine-sensitized made more premature responses in trials preceded by a 7.5 or 9.5-s interval and—considering the overall performance—made more premature responses in total. When the same experiment was conducted in the present study, no main effect of treatment was found ($p=0.2330$), but increasing ITI had a main effect ($p<0.0001$) of increasing premature responses (Figure 5), similar to Fletcher et al. (2007). Bonferroni post-tests only revealed that the AP group made significantly fewer premature responses than the saline ($p<0.05$) and amphetamine groups ($p<0.01$). While the present study found the same result as Fletcher et al. (2007) of increasing premature responses for all groups by increasing ITI, the lack of difference between saline and amphetamine treated rats is likely due to the large variability in the saline group. The saline group had a sample size of $n=2$, and one

rat had a very high number of premature responses (>100), which likely skewed the average, whereas the other had a low number (approximately 30). The rat with the high number of responses could not justifiably be considered an outlier with only two rats in the group. Consequently, with the amphetamine treated rats showing a comparable average to the saline group, it is difficult to suggest that PAOPA treatment prevented amphetamine-induced impairments in anticipatory responding in the AP group. If the average premature responses of saline group was indeed skewed to a high number, then this would suggest that PAOPA prevented an amphetamine-induced increase. This can only be confirmed with a future study with a large enough sample size to account for variability and outliers. Turning the focus to percent accuracy (Figure 45 in Appendix A), again there was a large variability in the saline group, which performed comparably to the amphetamine group. Both of these groups, along with the PAOPA group, performed worse than the AP group in accuracy at longer ITIs. The AP group also had greater correct response latencies (Figure 47 in Appendix A) than the saline and amphetamine groups. Taken together, this would suggest that as the ITI increased the AP group adapted a strategy of waiting longer before making a correct response and that this resulted in more correct responses. Turning to the PAOPA group, they had greater omissions (Figure 46 in Appendix A) than the amphetamine ($p < 0.01$) and AP ($p < 0.05$) groups, and furthermore had greater correct response latency than the amphetamine group ($p < 0.05$). This might suggest that PAOPA attenuated the motivation of the PAOPA group to perform on the task, but caution should be paid. If general motivation was

affected then their reward latency (Figure 48) would be expected to be greater than the other groups, which it was not. The combination of these results might suggest that the PAOPA group was less motivated in making responses to the trials but still had motivation to collect the food reward.

There have been a few studies on the ability of antipsychotics to prevent or reverse attentional impairments in animal models of schizophrenia using 5-CSRTT. Carli et al. (2011a; 2011b) investigated the ability of a single oral administration of aripiprazole, olanzapine, clozapine, sertindole, and haloperidol to prevent impairments in accuracy induced by the NMDA receptor antagonist 3-(*R*)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP), infused bilaterally (50 ng per hemisphere) into the medial prefrontal cortex (mPFC). 1 and 3 mg/kg aripiprazole, 1 mg/kg olanzapine, 0.02, 0.08 and 0.32 mg/kg sertindole, and 1.25 and 2.5 mg/kg clozapine all prevented deficits induced by one-time administration of CPP. On the other hand, the classical typical antipsychotic haloperidol (0.1 mg/kg) did not prevent CPP-induced deficits in accuracy. In another study of clozapine and haloperidol, Baviera et al. (2008) found that 2.5 mg/kg clozapine—and not 0.03 mg/kg haloperidol (both i.p.)—prevented attentional impairments by 100 ng CPP infused into the mPFC. Paine and Carlezon (2009) administered four concentrations of clozapine (0.16 – 1.25 mg/kg sub-cutaneous; s.c.) and haloperidol (0.008 – 0.063 mg/kg, s.c.) 30 minutes prior to NMDA receptor antagonist MK-801 (0.25 mg/kg, i.p.) administration and then tested on 5-CSRTT 30 minutes later. Similar to Carli

et al. (2011a; 2011b), they found that only the 0.16 mg/kg dose of clozapine was able to prevent MK-801-induced impairments in accuracy. However, one study (Amitai et al., 2007) examining the ameliorative effects of chronic 4 mg/kg clozapine did not find as robust of an attenuation by the antipsychotic as the others. In that study, accuracy was assessed on 8 days of saline administration followed by 5 days of 2mg/kg PCP administration. During both those periods, animals received either vehicle or clozapine administered by osmotic minipumps. Only the first day of PCP administration showed a significant amelioration by clozapine, even though on average there seemed to be a (non-significant) trend of amelioration. Finally, Amitai and Markou (2009) were unable to find any ameliorative effects of 5 mg/kg or 10 mg/kg quetiapine—administered via osmotic minipumps—to prevent attentional impairments by 2 mg/kg s.c. PCP. Although the sample size of the present study prohibits any valid interpretations from being made, there is good preliminary evidence to suggest that PAOPA can potentially prevent or reverse impairments in accuracy similar to atypical antipsychotics.

The present study also replicated the locomotor activity results of Beyaert et al. (2013). They reported that an amphetamine challenge given after three weeks of drug sensitization and three weeks of withdrawal increased the locomotor activity of all rats; the locomotor activity of the amphetamine treatment group was significantly greater than that of saline, AP, and PAOPA treatment groups. The present study found similar results. After five weeks of drug sensitization and five

weeks of withdrawal—two weeks longer for each phase than Beyaert et al. (2013)—an amphetamine challenge enhanced the hyper-locomotor activity of amphetamine-treated rats compared to saline, AP, and PAOPA-treated rats. Two discrepancies of note are that the AP group was also significantly higher than the saline and PAOPA groups, and the PAOPA group was significantly lower than the saline group. The first discrepancy could be explained by the fact the drug sensitization was two weeks longer. Consequently, the amphetamine treatment group was sensitized to a greater degree than in the study by Beyaert et al. (2013) and PAOPA only partially attenuated this enhanced sensitized state. The second discrepancy could also be explained by the extended duration of drug administration. PAOPA acutely is a positive allosteric modulator that can induce internalization of the dopamine D₂ receptor when administered chronically. Indeed, in the third study of this thesis, chronic PAOPA treatment resulted in an increase in the protein expression of G protein-coupled receptor kinase 2 and arrestin-3, which are involved in the regulation of dopamine D₂ receptor expression (Krupnick and Benovic, 1998). The dopamine D₂ receptor (D₂R) inhibits the indirect pathway of the basal ganglia, the result of which is disinhibition of the thalamus and frontal motor areas and increased locomotion. By decreasing the expression of D₂R, the output of the basal ganglia would theoretically decrease, resulting in reduced activation of the motor system (Kandel et al., 2013b; Purves et al., 2008).

In the present study, locomotor activity was assessed at an additional six time points: after the first drug administration (Figure 10 and Figure 11), after the last drug administration (Figure 12 and Figure 62, Appendix A), the first week of withdrawal (Figure 13 and Figure 63, Appendix A), after the completion of withdrawal (Figure 14), and twice 9 weeks after completion of withdrawal (Figure 59 to Figure 61, Appendix A and Figure 9). After the first acute injection, amphetamine administration—to the amphetamine group and the AP group—expectedly increased locomotor activity compared to the saline and PAOPA groups; PAOPA had no effect on locomotor activity (Figure 10). Figure 11, which shows the 10-minute interval data recorded during the 3 hours, illustrates that the increase in activity occurred at the beginning of recording when the animals had just been injected. After the final drug administration, Tukey's multiple comparison test revealed that only the AP group had significantly greater locomotor activity compared to the rest of the groups, although the amphetamine group seemed intermediate (Figure 62, Appendix A). What is interesting is that the increase in activity occurred towards the end of recording (Figure 12), in contrast to the first drug administration and suggesting a toleration of amphetamine administration. Figure 12 also demonstrates that the amphetamine group's locomotor activity started to rise towards the end. If recording had been continued past the 3 hours, Figure 62 (Appendix A) might have shown similar locomotor activity for the AP and amphetamine groups.

The first week of withdrawal did not see any differences in locomotor activity. After the completion of withdrawal, there may have been slight differences in locomotor activity—the amphetamine and AP groups might have been hyper-locomotive—but these were not significant (Figure 14). It was not until a 1 mg/kg amphetamine challenge was administered one week later that significant differences emerged (Figure 8 and Figure 58, Appendix A) as has already been described. The changes started to fade 3 weeks later, when locomotor activity was tested twice. In the first test (Figure 59 and Figure 60 in Appendix A), there were no statistical differences between groups although the trend of enhanced activity in the amphetamine group appeared to be there (Figure 59, Appendix A). Two days later, significant differences emerged but only for the PAOPA group. It was significantly lower than the amphetamine and AP groups. The amphetamine treatment group seemed to be higher in locomotor activity than the saline group, but this was not significant. These results indicate that by Week 20—14 weeks after drug cessation—the amphetamine-induced hyper-locomotor activity waned and correlate with results from 5-CSRTT variable stimulus duration testing where (non-significant) amphetamine-induced decrements in accuracy faded 13 weeks after drug cessation.

Taken together, these results demonstrate good support for amphetamine-sensitization being able to induce impairments in accuracy, and preliminary results

of possible prevention and reversal of impairments in accuracy by PAOPA warrant a further study with a large enough sample size to reject or support this observation.

12 Study Two – An investigation of the ability of PAOPA to ameliorate PCP-induced behavioural deficits

12.1 Brief Summary

The purpose of this study was to test the ability of PAOPA to prevent and/or reverse behavioural and cognitive deficits in an animal model (PCP) that has not been used before in the laboratory. PCP was administered sub-chronically for 7 days. Rats were allowed a washout period of another week to avoid any residual effects of the drug on later tests. Following the one-week washout, rats were tested on the novel object recognition task, social interaction, pre-pulse inhibition (PPI), stereotypy, and locomotor activity. PCP induced deficits in all tests of behaviour and cognition and PAOPA showed partial amelioration in all measures except for locomotor activity.

13 HYPOTHESES

It was hypothesized that:

- 1) PCP-treated rats would show increased locomotor activity, decreased percent PPI, decreased social interaction (number and duration of interactions), decreased novel object recognition memory, and increased stereotypy compared to saline-treated rats

- 2) PCP-treated rats would show the differences described above compared to rats treated PCP and PAOPA concomitantly
- 3) Rats treated PCP and PAOPA concomitantly would show no differences in the behaviours described compared to saline-treated rats
- 4) Rats pre-treated with PCP and administered PAOPA shortly before testing would show no differences in behaviours described compared to saline-treated rats
- 5) Rats pre-treated with PCP and administered saline shortly before testing would show the differences described in Hypothesis 1 compared to rats pre-treated with PCP and administered PAOPA shortly before testing

14 MATERIALS AND METHODS

14.1 Animals

Thirty four animals were purchased from Charles River Laboratories (Quebec) and housed in the central animal facility at McMaster University. Rats were kept under constant temperature and humidity conditions, had free access to food and water, and kept under a reversed 12:12-hour light cycle with lights on at 7 am and off at 7 pm. All animal procedures were approved by the McMaster University Animal Research Ethics Board and were in accordance with the stipulations of the Canadian Council on Animal Care. Upon arrival at the central

animal facility, rats were allowed to habituate to the new environment. The thirty four animals were divided first into two cohorts to facilitate testing: some tests are too long to test all thirty four animals in one day. Experimenters handled animals over the course of five days (Days 1 to 5), starting by just letting the animals sniff the experimenter's gloved hands and ending with putting them in a weighing container followed immediately by wrapping them in a small towel as if they were being injected.

14.2 Baseline

For both cohorts, locomotor and PPI baselines were performed to separate rats into treatment groups that did not significantly differ in locomotor activity or PPI scores. Locomotor activity was initially used to separate rats into treatment groups and the PPI score of the resulting treatment groups were compared to ensure no significant difference. If a significant difference was found, the rats would be shuffled between treatment groups until there was no significant difference in locomotor activity and PPI scores between groups. Baseline was performed on Day 5 for locomotor (3 hours) and Day 7 for PPI.

14.3 Treatment Groups

To make sure rats were not accidentally mistaken, on Day 7 rats were physically labelled by punching circles into one of their ears. This was done by

anaesthetizing the rats with isoflurane in a container, maintaining anaesthesia with isoflurane using a nose cone, and using an ear clipper to punch a hole. The number of circles and the ear pierced (left or right) coded the treatment group. There were four treatment groups: saline (n=6), which received saline throughout the study, PCPa (n=10), which received 5 mg/kg PCP twice a day (9 am and 9 pm) for 7 days and then saline during testing, PCPb (n=10), which received 5 mg/kg PCP twice a day (9 am and 9 pm) for 7 days and then PAOPA during testing, and PCP/PAOPA (n=8), which received 5 mg/kg PCP and 1 mg/kg PAOPA twice a day (9 am and 9 pm) for 7 days and then saline during testing. The purpose of having two subgroups within the PCP treatment group was to examine the preventative and reversal effects of PAOPA on PCP-induced behavioural impairments. In other words, to examine the preventative effects of PAOPA the behaviour of rats in the saline, PCPa, and PCP/PAOPA groups would be analyzed and compared; to examine the reversal effects of PAOPA, which would be more clinically relevant, the behaviour of rats in the saline, PCPa (negative control), and PCPb groups would be analyzed and compared. Both PCP subgroups were pre-treated with 5 mg/kg PCP twice a day for 7 days, but only the PCPb group was administered PAOPA approximately 1 hour prior to behavioural testing. Two holes in the left ear coded for saline, 1 hole in the right ear coded for the PCPa group, 2 holes in the right ear coded for the PCPb group, and 1 hole in the left ear coded for the PCP/PAOPA group.

14.4 Drugs

PCP was purchased from Toronto Research Chemicals (Toronto, CA) and stored in -20°C. PCP and PAOPA were prepared daily by dissolving in saline (0.9% NaCl) to make a concentration of 2.5 mg/mL PCP and 0.5 mg/mL PAOPA. On the days of injections, rats were weighed in the morning and their weights noted. One experimenter wrapped rats while the other injected the appropriate solution via the intraperitoneal (I.P.) route. Saline, PCP, and PAOPA were administered in a volume of 2 mL/kg to yield a net dose of 5 mg/kg PCP and 1 mg/kg PAOPA. Rats were injected twice daily (9 am and 9 pm) for 7 days on Days 8 to 14 and then given a washout period of 7 days on Days 15 to 21. During the drug washout period, rats were habituated to the various chambers and mazes of the behavioural tests (described previously).

14.5 Behavioural Procedures

Locomotor

Increased locomotor activity in an animal model of schizophrenia is representative of the positive symptoms of schizophrenia. In other words, the positive symptoms of schizophrenia are believed to manifest in rats as increased locomotor activity (hyper-locomotor activity). On Day 4, rats were habituated to AccuScan computerized locomotor activity chambers (AccuScan Instruments,

Columbus, OH) for 1 hour. On Day 5, baseline locomotor activity was collected over a period of 3 hours. An infrared photobeam laser on the floor measured the amount of horizontal movement and recorded it as distance travelled. The chambers were cleaned with 75% ethanol. Measurements were taken in 10-minute intervals for 18 intervals. For both cohorts, locomotor activity was tested Day 24 to determine if PAOPA had any preventative or reversal effects on PCP-induced and amphetamine-challenged hyperactivity. Before starting testing, rats in the saline, PCPa, and PCP/PAOPA treatment groups were injected with 2 mL/kg saline I.P., while rats in the PCPb treatment group were injected with 1 mg/kg (dissolved 0.5 mg/mL in saline) I.P. Rats were placed in locomotor activity chamber for 45 minutes to record baseline activity. After 45 minutes, all rats were challenged with 1 mg/kg amphetamine (dissolved 1 mg/mL in saline) I.P. The locomotor activity of the rats was recorded for another 2 hours and 15 minutes. Once complete, the rats were returned to their cages and the locomotor chambers cleaned with 75% ethanol. For only the second cohort, two additional locomotor tests were performed on Day 8 (after the first injection) and on Day 25 with a 5 mg/kg PCP—instead of amphetamine—challenge. For the test on Day 8, rats were treated with their respective drug (saline, $n=2$; PCPa, $n=6$; PCPb, $n=4$; PCP/PAOPA, $n=4$) and immediately placed into locomotor activity chambers and recorded for 3 hours. The procedure for testing on Day 25 with the PCP challenge was identical to the procedure described for testing on Day 24 with the amphetamine challenge—the

sole difference being a 5 mg/kg (2 mL/kg) PCP challenge given instead of a 1 mg/kg amphetamine challenge.

Pre-pulse Inhibition (PPI)

PPI is a measure of sensorimotor gating or how effectively rats can attenuate their response to startling stimuli when given a less startling stimuli. Compared to healthy controls, schizophrenia patients have been shown to be less able to attenuate their startled responses when presented with a startle-eliciting stimulus preceded by a pre-pulse (Jentsch and Roth, 1999). Using the SR-Lab Startle Response System (San Diego Instruments, San Diego, CA) rats were exposed first to 5 trials of startle pulses (120 dB) and then 65 trials of startle pulses (120 dB for 40 s) only, pre-pulses (either 68, 71, or 77 dB for 20 s) preceding startle pulses by 100 ms or presented alone, or no acoustic stimuli presented at random. Finally, rats were presented with 5 more trials of startle pulses. Trials ranged in length from 10 to 20 s, with an average of 15 s. A 65-dB background white noise was present throughout the session. The dependent variable in this task is the vibrational response of the rat to the acoustic stimuli. Rats were placed in snug Plexiglas cylindrical containers with little room to move. A sensor on the bottom of the container measured the amplitude of the vibration of the container. After the session was complete, the amplitudes of each type of trial were automatically averaged by the program and computed into the formula to yield percent PPI:

$$\text{Percent pre-pulse inhibition (\%PPI)} = \frac{S - PS}{S} \times 100\%$$

S denotes the mean amplitude of response to startle-only trials and PS denotes the mean amplitude of response to pre-pulse and startle trials. Rats were habituated to PPI containers on Day 6. PPI baseline was performed on Day 7, and testing was performed on Day 23 for both cohorts. On the day of testing, two pairs of rats first completed social interaction testing (see below), before starting PPI testing. Rats in the saline, PCPa, and PCP/PAOPA groups were administered 2 mL/kg saline I.P. 50 minutes before the start of testing. At the same time, rats in the PCPb group were given 1 mg/kg PAOPA (dissolved 0.5 mg/mL in saline) I.P. After completion of the test, the cylindrical containers were cleaned with 75% ethanol. The second cohort was also tested on PPI on Day 9 (after the second morning injection) and Day 14 (after the final evening injection). Rats were injected with their respective treatments (saline, $n=2$; PCPa, $n=6$; PCPb, $n=4$; PCP/PAOPA, $n=4$) 10 minutes prior to starting PPI.

Social Interaction

This test measures the social interaction of pairs of rats. A lack of social interaction is one of the negative symptoms of schizophrenia that is not fully treated with antipsychotics (Jentsch and Roth, 1999). Pairs of rats belonging to the same treatment group were placed in a large open arena-like chamber (100 by 100 by 40 cm) made of black PVC, with a video camera mounted over the center of the

chamber. One rat in each pair was coloured with non-toxic orange paint, while the other was coloured with non-toxic blue paint for identification purposes. Rats were left in the chamber for 10 minutes under dim lighting. After the 10 minutes, rats were removed from the chamber, placed into their respective cages and the arena cleaned with 75% ethanol. Rats were habituated, one by one, to the social interaction chamber on Days 20 and 21 and tested on Day 23. On the day of testing, two pairs of rats were consecutively tested for social interaction. After the second pair finished testing, the two pairs were tested on PPI. This order was repeated for the remaining rats. 40 minutes prior to testing, rats in the saline, PCPa, and PCP/PAOPA groups were administered 2 mL/kg saline I.P. and rats in the PCPb group were administered 1 mg/kg PAOPA (dissolved 0.5 mg/mL in saline) I.P.

Novel Object Recognition Task

This test measures rats' ability to differentiate between familiar and novel objects. Schizophrenia patients show impaired recognition memory (Grayson et al., 2007). We modified the 8-arm radial maze to make a V-shaped maze. By blocking arms 1, 2, 3, 5, 7, and 8, two arms remained open forming the shape of a V. Rats were habituated to the maze for 18-30 minutes on Days 18 to 21, one at a time. The maze was cleaned with 75% ethanol. Rats were tested on Day 22. A rat was put in the center of the maze and allowed to habituate for 3 minutes. Then, the rat was returned to its home cage for 1 minute. During this time the maze was cleaned with 75% ethanol. Two identical objects were placed in the two arms and were secured

to the floor of the maze using sticky-tack. After 1 minute, the rat was placed in the center of the maze, and allowed to explore for another 3 minutes. During this time, the amount of time spent interacting was scored by two experimenters, one experimenter looking at each arm. Interaction was defined strictly as having the nose within 1 cm of the object. Crawling past or touching the object were not scored as interactions if the nose was not within 1 cm of the object. At the end of 3 minutes, the rat was returned to its cage for 1 minute. The objects were removed and cleaned with 75% ethanol along with the maze. Two objects were placed in the arm once again: one of them was identical to the objects before, and the other was a novel object. The location of the familiar and the novel object (left versus right arm) was random and counterbalanced for each group. The rat was placed in the center of the maze after the 1 minute elapsed and allowed to explore for a final 3 minutes. The rat was scored on the amount of time spent interacting with the objects. After 3 minutes, the rat was returned to its cage, the maze and objects cleaned with 75% ethanol, and the next rat was tested. 30 minutes prior to testing, rats in the saline, PCPa, and PCP/PAOPA groups were administered I.P. 2 mL/kg saline and rats in the PCPb group were administered I.P. 1 mg/kg PAOPA (dissolved 0.5 mg/mL in saline).

14.6 Analyses

Locomotor

As described previously, locomotor activity data was recorded in 10-minute intervals for 18 intervals: the first 6 for baseline, the later 12 after the amphetamine challenge. Locomotor activity from the first hour was summed to calculate baseline performance; locomotor activity from the second two hours was summed to calculate the total distance travelled after an amphetamine challenge. The resulting data was analyzed using two-way analysis of variance (ANOVA) and Bonferroni post-hoc tests. The two factors were drug pre-treatment—that is, saline, PCP, and PCP/PAOPA—and locomotor challenge—baseline and amphetamine challenge. A one-way ANOVA with Tukey's multiple comparison tests was used to analyze baseline data. Alpha was set to 0.05 and significance was defined when $p < 0.05$.

PPI

PPI data was generated by SR-Lab Startle Response System (San Diego Instruments, San Diego, CA) and percent PPI was grouped into three categories based on the decibel of the pre-pulse: 68 dB, 71 dB, or 77 dB. The first and last five trials were excluded from analysis as they did not serve the purpose of determining percent PPI. The resulting data was analyzed using two-way ANOVA and Bonferroni post-hoc tests. The two factors were drug pre-treatment and pre-pulse decibel (68 dB, 71 dB, or 77 dB). Alpha was set to 0.05 and significance was defined when $p < 0.05$. For PPI analysis following acute drug administrations, the PCPa and PCPb groups were combined as they did not differ at this time in drug treatment.

Social Interaction

Experimenters blind to the treatment groups of the rats watched playback of the recorded social interaction videos. The video was started at the 45-second mark and analyzed for 5 minutes. Experimenters scored a rat on the number and duration of interactions. Interactions were defined as sniffing (nose was within 1 cm), climbing over or under, following, or showing aggressive behaviour towards another rat. The data for each rat was averaged between the experimenters. The number and duration of interactions were analyzed by separate one-way ANOVAs and Tukey's multiple comparison post-hoc tests. Alpha was set to 0.05 and significance was defined when $p < 0.05$.

Novel Object Recognition Task

The third trial of the novel object test was used to calculate the Discrimination Index (DI), using the formula:

$$DI = \frac{t_{Novel}}{t_{Novel} + t_{Familiar}}$$

t_{Novel} was the time spent exploring the novel object, $t_{Familiar}$ was the time spent exploring the familiar object, and $t_{Novel} + t_{Familiar}$ was the total time spent exploring objects. Data was analyzed using one-way ANOVA and Tukey's multiple comparison post-hoc tests. Alpha was set to 0.05 and significance was defined when $p < 0.05$.

15 RESULTS

15.1 Baseline

Rats were grouped into four treatment groups that did not differ in locomotor activity ($F=0.3658$, $p=0.7782$; Figure 15) or percent PPI ($p=0.2428$). Figure 16 shows PPI grouped by pre-pulse decibel and Figure 17 combines all the data for each group.

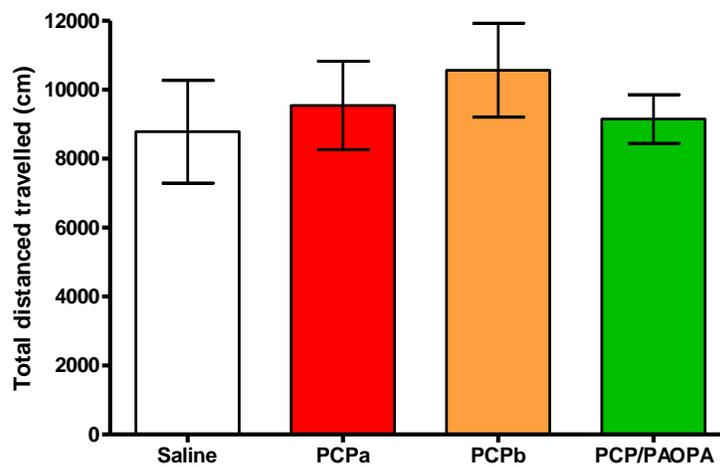


Figure 15. Baseline locomotor. After handling and before the start of injections, rats were grouped into treatment groups based on locomotor activity and PPI. One-way ANOVA showed no difference between treatment groups ($F=0.03529$,

$p=0.7782$) in locomotor activity. Data represents mean \pm standard error of mean.

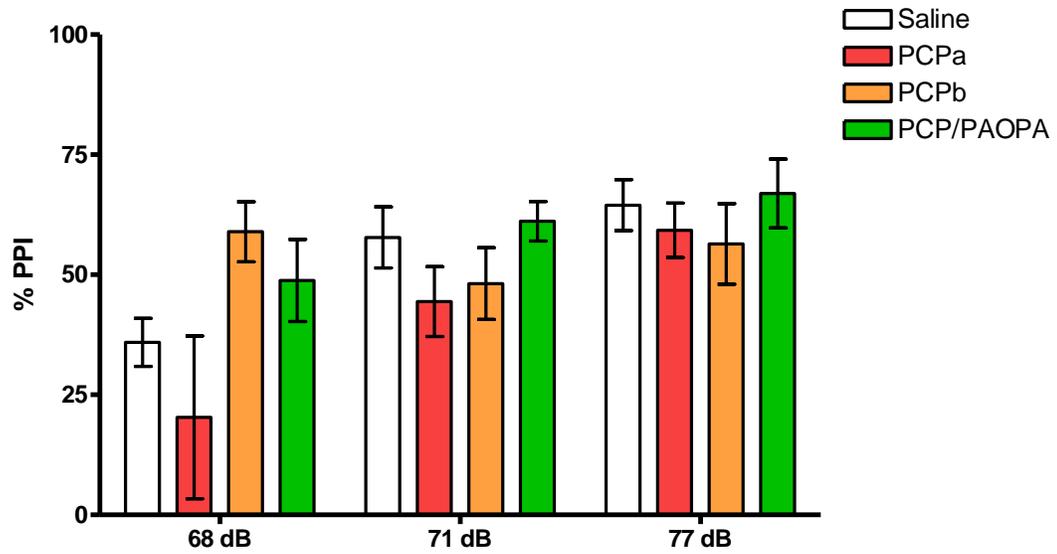


Figure 16. Baseline PPI grouped by pre-pulse decibel. After handling and before treatment, rats were divided into the four treatment groups based on locomotor activity and PPI. There was no main effect of treatment ($p=0.2428$). There was a main effect of decibel ($p=0.0008$) and subjects ($p=0.0033$).

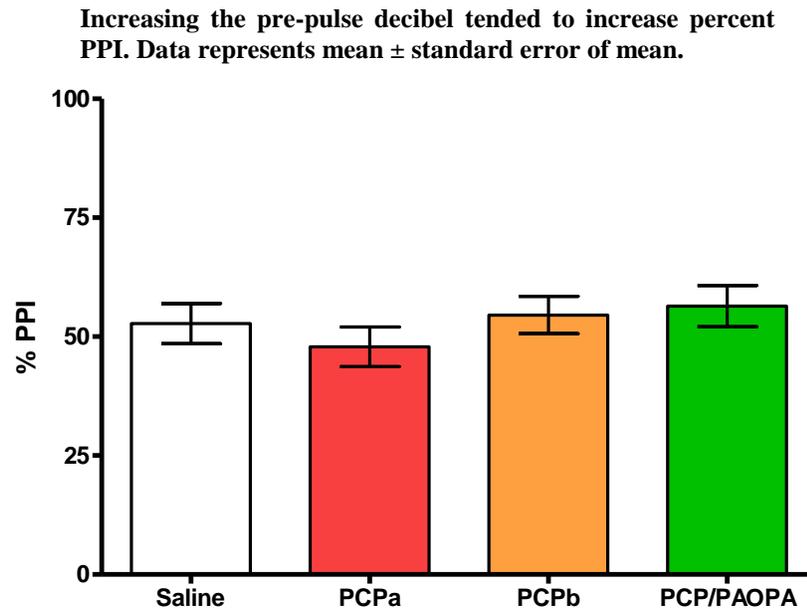


Figure 17. Baseline PPI with combined pre-pulse decibels. After handling and before treatment, rats were divided into the four treatment groups based on locomotor activity and PPI. When the percent PPI data were combined for each pre-pulse decibel, there was no significant difference between treatments groups as shown by one-way ANOVA ($F=0.8220$, $p=0.4849$). Data represents mean \pm standard error of mean.

15.2 Locomotor

An acute injection of 5 mg/kg PCP or concurrent 5 mg/kg PCP and 1 mg/kg PAOPA may have produced an increase in locomotor activity (Figure 18)—however this increase was not significant ($F=0.1450$, $p=0.3613$).

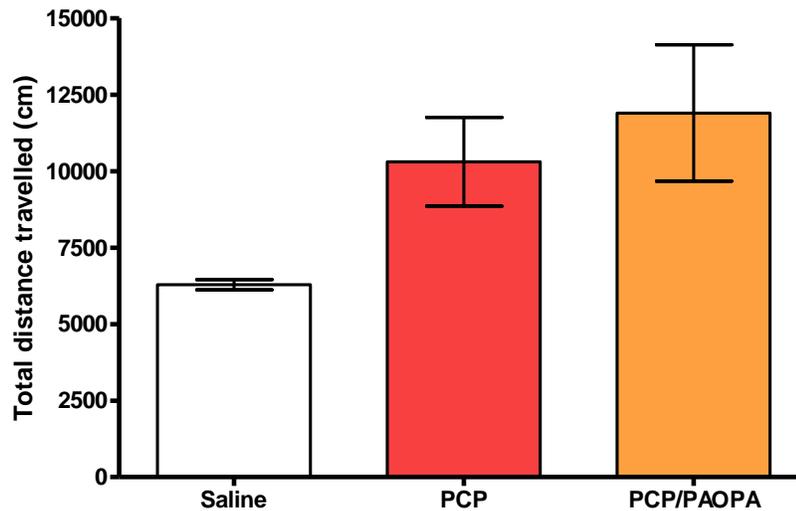


Figure 18. Locomotor activity following acute drug administration. After the first drug administration, rats were immediately recorded for locomotor activity for 3 hours. Since the PCPa and PCPb treatment groups received 5 mg/kg PCP, their data was combined. One-way ANOVA did not find any difference between treatment groups ($F=1.102$, $p=0.3613$), although it appeared that the PCP and PCP/PAOPA groups had greater locomotor activity than the saline group. Data represents mean \pm standard error of mean.

On Day 24, both cohorts were tested with a 1 mg/kg amphetamine challenge, with only the PCPb group administered a 1 mg/kg PAOPA dose prior to starting locomotor activity. There was no significant main effect of treatment ($p=0.1914$), however the amphetamine challenge produced a significant main effect ($p<0.0001$). Bonferroni post-tests revealed that the PCPa group had significantly lower locomotor activity than both the PCPb ($t=2.671$, $p<0.05$) and the PCP/PAOPA ($t=2.464$, $p<0.05$) groups, but not the saline group ($t=1.021$, $p>0.05$; Figure 19).

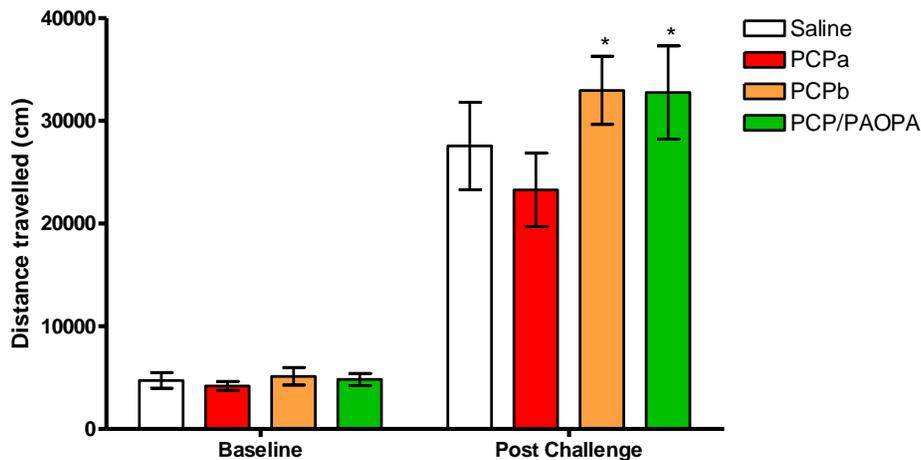


Figure 19. Locomotor activity with an amphetamine challenge. On Day 24, saline, PCPa, and PCP/PAOPA treatment groups were pretreated with 2 mL/kg saline, while the PCPb group was pretreated with 1 mg/kg PAOPA prior to the start of locomotor activity. Forty five minutes later, an injection of 1 mg/kg amphetamine was administered and locomotor activity recorded for two hours. Two-way ANOVA did not find a main effect of treatment ($p=0.1914$). There was a main effect of challenge ($p<0.0001$) as the amphetamine injection increased locomotor activity for all groups. Bonferroni post-tests showed significant differences for the PCPa group, which was significantly hypo-locomotive compared to the PCPb ($t=2.671$, $*p<0.05$) and PCP/PAOPA ($t=2.464$, $*p<0.05$) groups. Data represents mean \pm standard error of mean.

On Day 25, the second cohort was tested with a 5 mg/kg PCP challenge, again with only the PCPb group given a 1 mg/kg PAOPA dose prior to testing. Again, there was no main effect of treatment ($p=0.1424$), and again the challenge produced a main effect ($p=0.0003$). However, Bonferroni post-tests revealed that the PCPa group was significantly higher in locomotor activity than the saline group ($t=2.857$, $p<0.05$), as was the PCP/PAOPA group ($t=3.634$, $p<0.01$). The PCPb group may have had greater and lower locomotor activity than the saline and PCPa

groups, respectively—however, the difference did not achieve statistical significance (Figure 20).

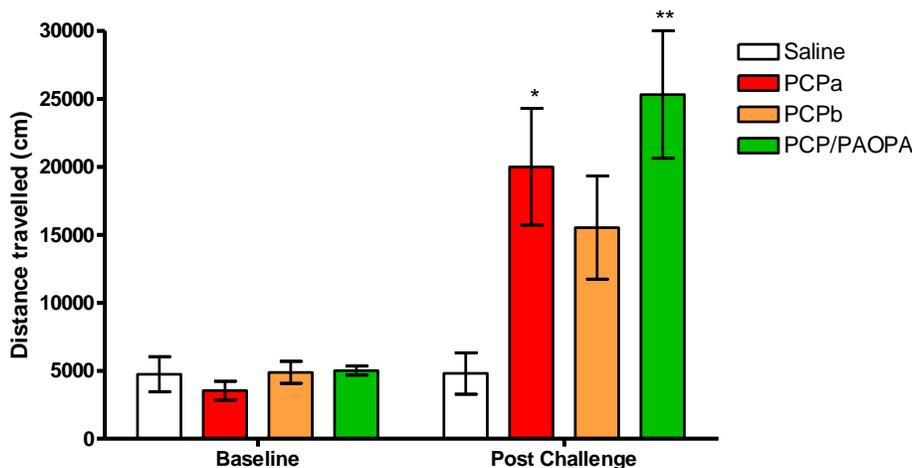


Figure 20. Locomotor activity with a PCP challenge. On Day 25, in the second cohort, saline (n=2), PCPa (n=6), and PCP/PAOPA (n=4) treatment groups were pretreated with 2 mL/kg saline, while the PCPb group (n=4) was pretreated with 1 mg/kg PAOPA prior to the start of locomotor activity. Forty five minutes later, an injection of 5 mg/kg PCP was administered and locomotor activity recorded for two hours. Two-way ANOVA did not identify a main effect of treatment ($p=0.1424$). There was a main effect of challenge ($p=0.0003$) as the PCP challenge increased locomotor activity for all groups. Bonferroni post-tests revealed that the saline group was lower in locomotor activity than the PCPa ($t=2.857$, $*p<0.05$) and the PCP/PAOPA ($t=3.634$, $**p<0.01$) groups. Data represents mean \pm standard error of mean.

15.3 PPI

On the second day of injections, PPI was assessed 10 minutes after the morning administration. Acute injections of drugs did not elicit any significant main effects of treatment ($p=0.5236$) and Bonferroni post-tests did not reveal any differences between groups, although it may seem that with a 77 dB pre-pulse the

PCP-treated rats (PCPa and PCPb) were less able to attenuate their startle response than the saline-treated rats (Figure 21). When the data was combined for each pre-pulse decibel (Figure 22), the PCP-treated rats may have had reduced percent PPI than saline, and PCP/PAOPA-treated rats may have had the lowest percent PPI, but these differences—if real—were not detected by one-way ANOVA ($F=1.219$, $p=0.3051$).

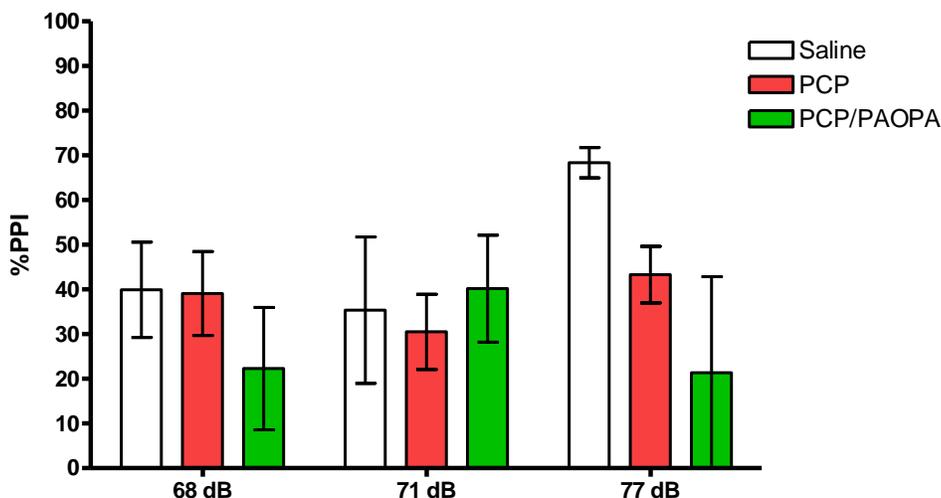


Figure 21. PPI on the second day of injections grouped by pre-pulse decibel. For the second cohort (saline, $n=2$; PCP, $n=10$; PCP/PAOPA, $n=4$), after the second morning injection, rats were assessed for PPI 10 minutes following drug administration. As the PCPa and PCPb groups received the same dose of the same drug, their data was combined. Repeated measures two-way ANOVA revealed no main effect of treatment ($p=0.5236$) nor did pre-pulse decibel ($p=0.4849$). Subjects had a main ($p=0.01255$) effect on percent PPI. Bonferroni post-tests did not reveal any significant differences

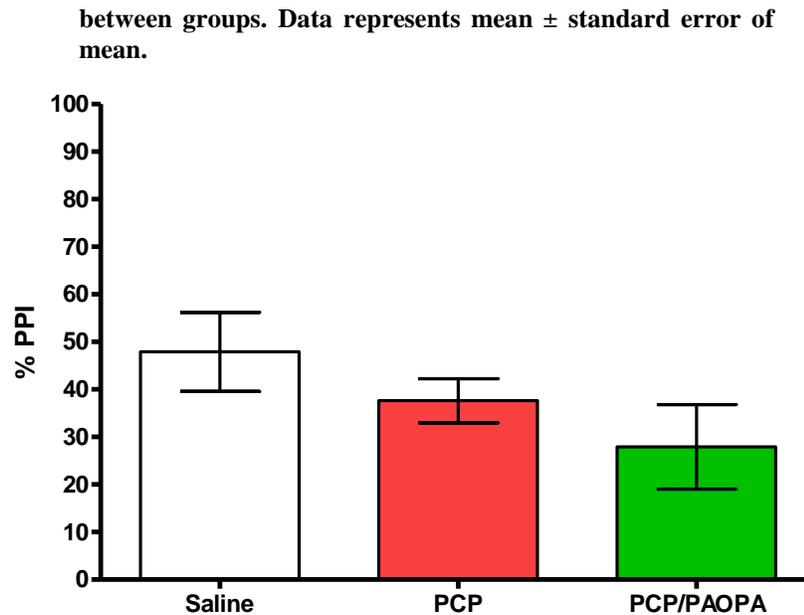


Figure 22. PPI on the second day of injections with combined pre-pulse decibels. For the second cohort (saline, n=2; PCP, n=10; PCP/PAOPA, n=4), after the second morning injection, rats were assessed for PPI 10 minutes following drug administration. As the PCPa and PCPb groups received the same dose of the same drug, their data was combined. The percent PPI data was combined for each pre-pulse decibel. One-way ANOVA showed no differences between treatments ($F=1.219$, $p=0.3051$). Data represents mean \pm standard error of mean.

On the final day of injections, PPI was again assessed 10 minutes after the evening administration. Yet again, there was no main effect of treatment ($p=0.2171$) and Bonferroni post-tests did not reveal any significant differences, even though the PCP-treated rats appeared to have lower percent PPI than saline-treated rats at all three pre-pulse decibels (Figure 23). One-way ANOVA of combined data for each pre-pulse also did not reveal any significant differences between groups

($F=2.646$, $p=0.0819$), although the p value approached the 0.05 threshold (Figure 24).

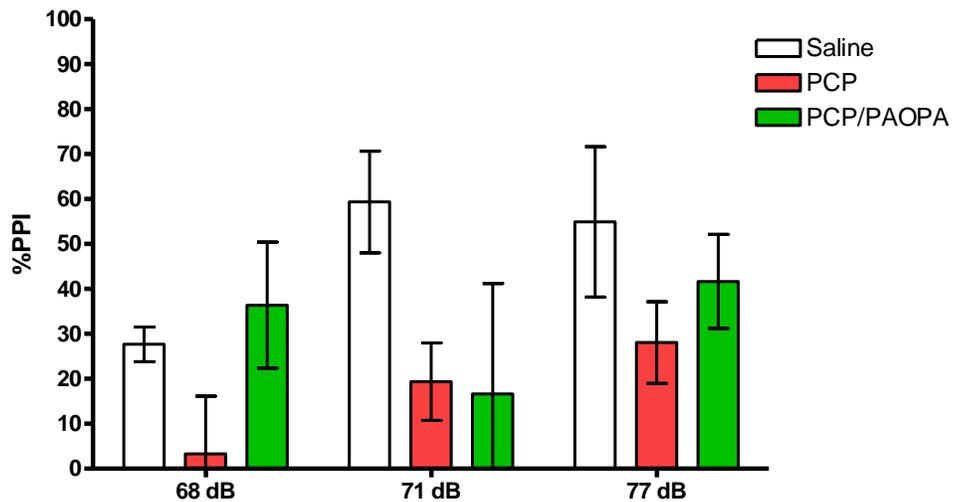


Figure 23. PPI on the final day of injections, grouped by pre-pulse decibel. For the second cohort (saline, $n=2$; PCP, $n=10$; PCP/PAOPA, $n=4$), after the final evening injection, rats were assessed for PPI 10 minutes following drug administration. As the PCPa and PCPb groups received the same dose of the same drug, their data was combined. Repeated measures two-way ANOVA showed no effect of treatment ($p=0.2171$), pre-pulse decibel ($p=0.3020$), or subjects ($p=0.0573$). Bonferroni post-

tests also did not show any differences between groups. Data represents mean \pm standard error of mean.

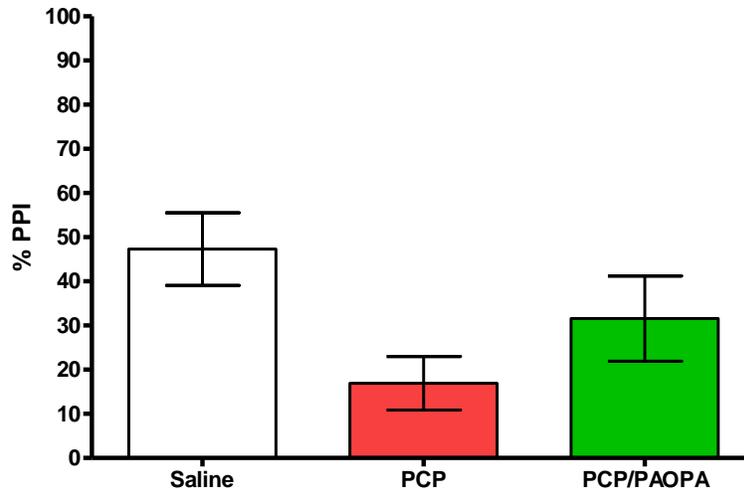


Figure 24. PPI on the final day of injections with combined pre-pulse decibels. For the second cohort (saline, n=2; PCP, n=10; PCP/PAOPA, n=4), after the final evening injection, rats were assessed for PPI 10 minutes following drug administration. As the PCPa and PCPb groups received the same dose of the same drug, their data was combined. Percent PPI data was combined for each pre-pulse decibel. One-way ANOVA yielded a p value that approached significance ($F=2.646$, $p=0.0819$). Data represents mean \pm standard error of mean.

On Day 23, after approximately a week of drug washout, PPI was assessed to determine the effects of drug treatment as a result of lasting neurochemical changes. No significant main effects of treatment or significant Bonferroni post-tests were observed ($p=0.8057$; Figure 25) using two-way ANOVA. Analysis of combined data also did not identify any significant differences ($F=0.8968$, $p=0.4122$; Figure 26).

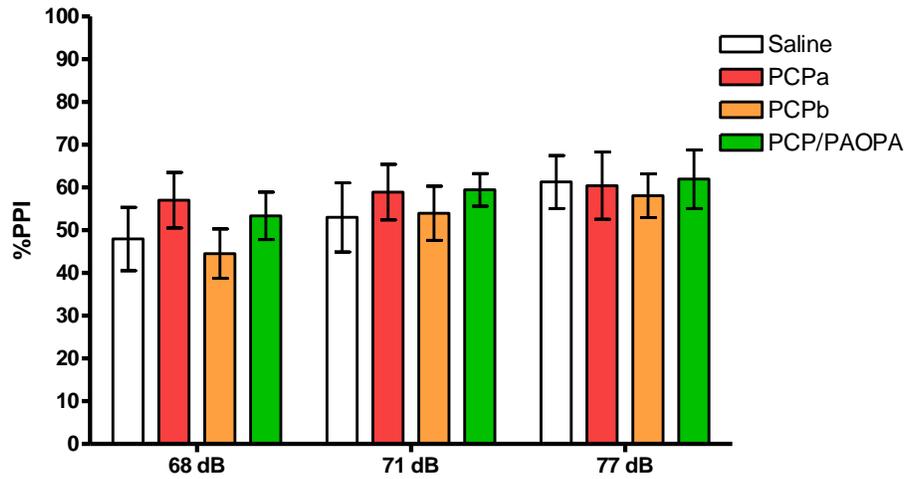


Figure 25. PPI after a 1-week drug washout grouped by pre-pulse decibel. On Day 23, PPI was assessed for both cohorts. Repeated measures two-way ANOVA revealed a main effect of pre-pulse decibel ($p=0.0009$) and subjects ($p<0.0001$), but not treatment ($p=0.8057$). Bonferroni post-tests also did not show any significant results for any treatment group. Data represents mean \pm standard error of mean.

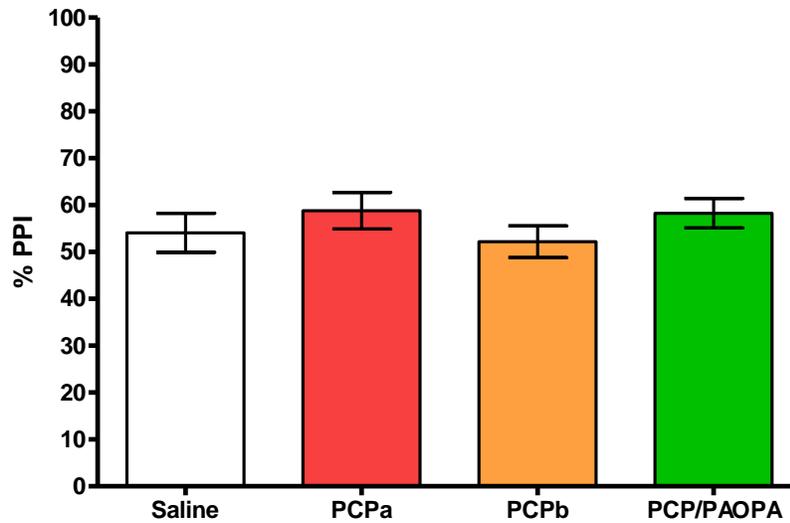


Figure 26. PPI after a 1-week drug washout with combined pre-pulse decibels. On Day 23, PPI was assessed for both cohorts. The percent PPI data was combined for each pre-pulse decibel. One-way ANOVA did not reveal any significant

differences between treatment groups ($F=0.8968$, $p=0.4122$).
Data represents mean \pm standard error of mean.

15.4 Social Interaction

On Day 23, the same day as PPI testing, social interaction was assessed prior to PPI. Time analysis of videos with loose criteria for social interaction yielded no significant results ($F=0.2091$, $p=0.8893$; Figure 27).

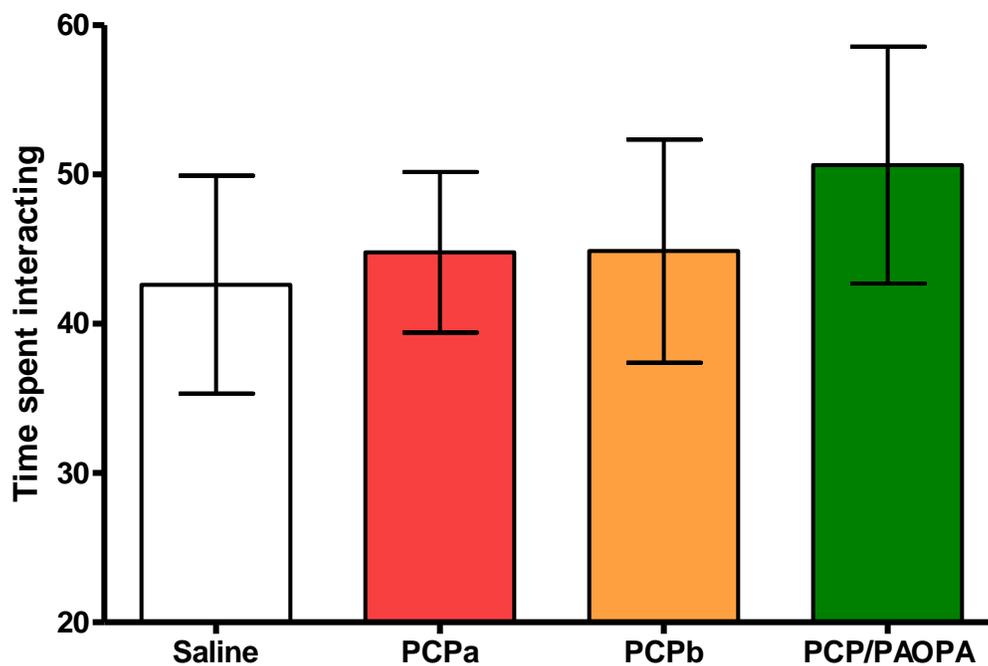


Figure 27. Time (seconds) analysis of social interaction 1 week after drug cessation. Social interaction videos were analyzed for time spent interacting (seconds). With loose criteria for social interactions, one-way ANOVA did not reveal any differences between groups in time spent interacting ($F=0.2091$, $p=0.8893$). Data represents mean \pm standard error of mean.

When the criteria for social interaction were constrained to specific interactions (sniffing, following, climbing, aggression) and the number of interactions were scored, two-way ANOVA revealed a main effect of treatment ($p=0.0285$), as well as type of social interaction ($p<0.0001$), and an interaction between the two factors ($p<0.0001$). While following, climbing, and aggressive behaviour did not show differences in number of interactions, the PCPa group showed fewer interactions than the saline ($t=6.552$, $p<0.001$; Figure 28), and PCP/PAOPA groups ($t=4.908$, $p<0.001$). The PCPb group had significantly fewer sniffing interactions than both the saline ($t=4.535$, $p<0.001$) and the PCP/PAOPA ($t=3.271$, $p<0.01$) groups. The PCP/PAOPA group was not significantly different from the saline group.

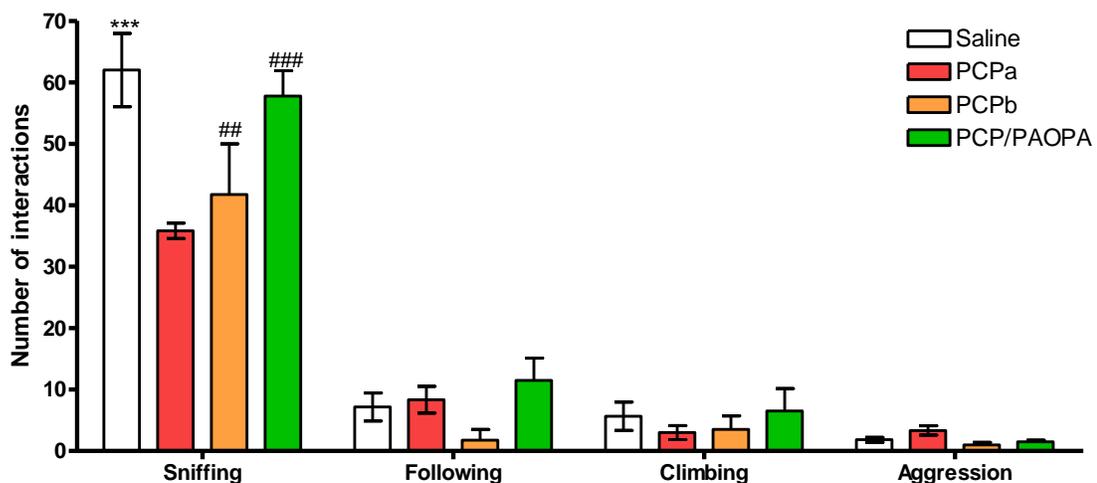


Figure 28. Number of specific social interactions 1 week after drug cessation. Social interaction videos were analyzed for number of specific interactions. Repeated measures two-way ANOVA revealed a main effect of treatment ($p=0.0285$), type of social interaction ($p<0.0001$), subjects ($p=0.0201$), and interaction between the number and type of interactions

($p < 0.0001$). Bonferroni post-tests revealed that only sniffing showed a significant result between saline and PCPa rats ($t = 6.552$, $*p < 0.001$), saline and PCPb rats ($t = 4.535$, $***p < 0.001$), PCPa and PCP/PAOPA rats ($t = 4.908$, $###p < 0.001$), and PCPb and PCP/PAOPA rats ($t = 3.271$, $##p < 0.01$). Data represents mean \pm standard error of mean.**

15.5 Novel Object Recognition Task

Approximately a week after drug cessation, the ability of PAOPA to prevent and/or reverse PCP-induced deficits in novel object recognition were assessed on Day 22. One-way analysis of variance of the discrimination index showed a significant difference between groups ($F = 3.181$, $p = 0.0354$). Tukey's multiple comparison test revealed that the difference came from the PCPa group, which had a significantly lower discrimination index than only the saline-treated group ($p < 0.05$). The PCPb and PCP/PAOPA groups were each not different from either the saline or PCPa groups (Figure 29).

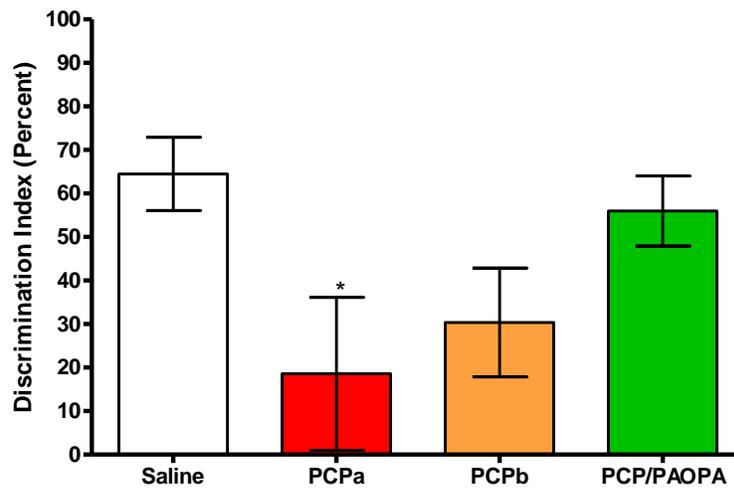


Figure 29. Novel object recognition assessed 1 week after drug cessation. One-way ANOVA revealed a significant difference between groups ($F=3.181$, $p=0.0354$) for discrimination index. Tukey's multiple comparison test revealed that the PCPa group had a significantly lower discrimination index ($*p<0.05$) than only the saline group. No other differences were observed. Data represents mean \pm standard error of mean.

16 DISCUSSION

This study examined the preventative effects of PAOPA by treating eight rats with concurrent 5 mg/kg PCP and 1 mg/kg PAOPA and comparing their results to saline- and PCP-treated rats. The reversal effects of PAOPA was also examined by including another treatment group ($n=10$) that received 5 mg/kg PCP during drug administration and a 1 mg/kg dose prior to behavioural testing. Because not all animals could be tested in one day for each test, the animals were divided into two cohorts. Drug administration on the second cohort (saline, $n=2$; PCPa, $n=6$; PCPb, $n=4$; PCP/PAOPA, $n=4$) only started after the first cohort (saline, $n=4$; PCPa, $n=4$; PCPb, $n=6$; PCP/PAOPA, $n=4$) had been sacrificed.

Rats were divided into treatment groups that did not differ in locomotor activity (Figure 15) or PPI (Figure 16 and Figure 17). When the PPI data was grouped by pre-pulse decibel, Figure 16 may show a (non-significant) decrease in PPI by the PCPa group. However, PPI with a 77 dB did not show any differences between treatment groups and furthermore, when the pre-pulse decibels were combined for each treatment group, there were no observable differences, indicating that no differences existed between groups before the start of treatment.

Locomotor activity was assessed acutely (after the first drug administration; Figure 18) and sub-chronically (approximately 1 week after 7 days of drug treatment; Figure 19 and Figure 20). Figure 18 shows that an acute PCP administration to the PCPa and PCPb groups and PCP and PAOPA administration to the

PCP/PAOPA group may have increased their locomotor activity with respect to the saline group. Although this difference was not significant ($p=0.3613$), the trend supports a general increase in locomotor activity following acute NMDA receptor antagonism (Jentsch and Roth, 1999). It is thought to occur by the blockade of NMDA receptors on GABAergic interneurons that tonically inhibit excitatory glutamatergic neurons in the prefrontal and ventral tegmental area (Jentsch and Roth, 1999). Thus, the disinhibited excitatory transmission would increase excitatory motor output leading to increased locomotion. Sub-chronically, the effects of an amphetamine challenge were assessed in both cohorts of animals 10 days after drug cessation (Figure 19), and the effects of a PCP challenge were assessed in only the second cohort 11 days after drug cessation (Figure 20). In the amphetamine challenged test, only Bonferroni post-tests revealed differences: the PCPa group was significantly lower in locomotor activity than the PCPb ($p<0.05$) and PCP/PAOPA ($p<0.05$) groups when challenged with 1 mg/kg amphetamine; the PCPa group did not have significantly enhanced locomotor activity relative to the saline group, suggesting that rats treated only with PCP were not sensitized to the amphetamine challenge. This is in contrast to the findings of Jentsch et al. (1998). That study performed the same regimen of PCP administration as the present study: a week of 5 mg/kg PCP injections twice daily followed by a week of withdrawal. Jentsch et al. (1998) tested locomotor activity with a 1 mg/kg amphetamine challenge exactly a week after the last drug administration and reported enhanced locomotor activity by the PCP-treated animals. They also found

reduced basal dopamine usage (ratio of a dopamine metabolite concentration to dopamine concentration) in the prefrontal cortex 3 weeks after drug cessation and no change in basal dopamine usage in the nucleus accumbens one week after drug cessation. When the same group used osmotic pumps to deliver PCP for two weeks and did not give a withdrawal, they found that amphetamine-induced enhanced locomotor activity correlated with an amphetamine-induced increase in prefrontal dopamine release—dopamine release was not changed in the nucleus accumbens after an amphetamine challenge (Balla et al., 2003). Interestingly, when they gave a 4 day-withdrawal, they found that after an amphetamine challenge, dopamine release was the same as controls and lower than when no withdrawal was given (Balla et al., 2003). Another group tested sub-chronic and chronic regimens of 2.6 mg/kg PCP and did not find amphetamine-induced enhancement of locomotor activity, although the amphetamine challenge did not seem to increase locomotor activity in controls either (Egerton et al., 2008). In the present study, locomotor activity was tested 3 days later than when Jentsch et al. (1998) tested, and it is unlikely that this delay could wane the effects of an amphetamine challenge. It should also be noted that in the present study, the novel object, social interaction, and PPI tests were conducted before locomotor activity. The simple performance of these tests, albeit also unlikely, may have masked the effects of an amphetamine challenge by providing new environments—especially in novel object and social interaction—to rats. The discrepancy between the present results and those of Jentsch et al. (1998) remain puzzling and it may be worthwhile to repeat the present

experiment to confirm these results. However, it should be noted that results of Balla et al. (2003) and Egerton et al. (2008) are also inconsistent with those of Jentsch et al. (1998).

When a 5 mg/kg PCP challenge was given the next day, the locomotor activity of the PCP-treated group spiked compared to the saline group ($p < 0.05$). Unlike the amphetamine challenge that increased locomotor activity in all treatment groups, the PCP challenge did not increase locomotor activity in the only group that had not been administered PCP during drug administration—saline—which does not support the acute results where PCP seemed to increase locomotor activity and general findings in the literature (Jentsch and Roth, 1999). PAOPA treatment did not prevent the hyper-locomotor activity induced by PCP, as the PCP/PAOPA group was significantly higher in locomotor activity than the saline group ($p < 0.01$) but not the PCPa group. The PCPb group seemed intermediate between the PCPa and PCP/PAOPA groups on the one hand and the saline group on the other. These results suggest that concurrent PAOPA administration does not prevent the increased sensitivity to a PCP challenge, brought about by a week of PCP treatment. Yet Beyaert et al. (2013) found that concurrent PAOPA treatment with amphetamine prevented amphetamine-induced enhanced locomotor activity. Collectively, these results suggest that PCP and amphetamine change different neural systems controlling locomotor but elicit the same hyper-locomotor activity when a challenge of the same drug is given—Vandershuren and Kalivas (2000) have reviewed and reported that different psychostimulants act at different primary

neural sites, but have common distal outputs. Furthermore, PAOPA is better able to prevent neural changes caused by amphetamine than it is by PCP, which may be due to PAOPA's selectivity for dopamine D₂ receptors, which are predominantly found in the striatum.

The results also suggest that PAOPA's chronic effects are not the same as its acute effects: a one-time PAOPA administration seemed to partially reverse the PCP-induced hyper-locomotion in PCP-treated rats (present study) and amphetamine-induced hyper-locomotion in amphetamine-sensitized rats (Beyaert et al., 2013). There is evidence that chronic PAOPA treatment may downregulate the expression of D₂Rs by internalization (increased expression of GPCR-regulatory proteins have been found; see Study Three), whereas acutely it acts more like an agonist (Verma et al., 2005). The observed results could be explained by changes to the indirect pathway of the basal ganglia. The indirect pathway “starts” in the striatum and projects inhibitory GABA neurons to the external segment of the globus pallidus (GP_e). The GP_e also sends inhibitory GABAergic projections to the internal segment of GP (GP_i) and tonically to the subthalamic nuclei, which projects excitatory axons to the GP_i. GP_i is the final structure of the basal ganglia that tonically projects inhibitory GABAergic axons to the thalamus, which projects to the frontal cortex. Activation of the inhibitory pathway results in strengthened inhibitory output to the thalamus, decreasing locomotion, whereas inhibition of the inhibitory pathway disinhibits the thalamus, increasing locomotion (Kandel et al., 2013b; Purves et al., 2008). The results of the present and previous (Beyaert et al.,

2013) support of mechanism of PAOPA where acutely it facilitates dopamine binding to the presynaptic D₂ short autoreceptor (D_{2s}R)—which is more predominant than the postsynaptic D₂ long receptor (D_{2l}R)—in the striatum and chronically promotes the internalization of D_{2l}R. Postsynaptic D_{2l}Rs hyperpolarize and inhibit striatal neurons by increasing outward potassium conductance (Lacey et al., 1987, 1988; Lüscher and Slesinger, 2010). If PAOPA acutely decreases dopamine release onto D_{2l}Rs, this would have the effect of activating the indirect pathway and decreasing locomotion. If PAOPA chronically decreases the expression of D_{2l}Rs, this would have the same effect of decreasing locomotion.

PPI was tested on the second day of drug administration (Figure 21 and Figure 22), on the last day of drug administration (Figure 23 and Figure 24), and 9 days after drug cessation (Figure 25 and Figure 26). PPI was tested acutely on the first and final day of drug administration in the second cohort only. On the first day, there were no effects of treatment or significant Bonferroni post-tests. At a 71-dB pre-pulse there did not seem to be any observable changes and PCP/PAOPA may have reduced PPI at the 68-dB pre-pulse. At the 77-dB pre-pulse, the PCP only treated rats seemed to have reduced PPI compared to saline, and PCP/PAOPA seemed to be even further reduced in PPI, although this group had a very high variability (Figure 21). When the PPI data for each pre-pulse was combined by treatment group, the same trend as with the 77-dB pre-pulse persisted. Yet, one-way ANOVA did not reveal any significant changes (Figure 22), although acute PCP administration has produced PPI deficits in other studies (Egerton et al., 2008;

Ehrhardt et al., 1999; Martinez et al., 1999; Tenn et al., 2005). On the final day of drug administration, there were more observable yet non-significant differences between treatment groups across all pre-pulse decibels (Figure 23). The rats receiving only PCP had lower PPI than saline at all pre-pulse decibels, and the PCP/PAOPA had the same (68 dB), or lower (71 and 77 dB) mean PPI as the saline group. When the PPI data for each pre-pulse was combined by treatment groups (Figure 24), the saline group had a PPI at approximately 49%, PCP at approximately 15%, and the PCP/PAOPA group at approximately 30%. One-way ANOVA determined a p value of 0.0819, which could be interpreted as approaching significance. This is likely again a result of a low sample size, and suggests that repeated and an acute PCP injection disrupted sensorimotor gating, which on the first injection was not prevented by PAOPA but with repeated injections is partially ameliorated. Nine days after drug cessation, there were no observable or significant differences between treatment groups in PPI using grouped (Figure 25) or combined (Figure 26) analysis, which supports previous studies showing PPI is not affected when tested a few days after drug cessation (Egerton et al., 2008; Ehrhardt et al., 1999; Martinez et al., 1999). These findings also suggest that repeated PCP administration does not produce lasting neural changes in the basal ganglia, which has been shown to influence PPI (Kodsi and Swerdlow, 1997a, b; Wan and Swerdlow, 1996). This parallels the work of Jentsch et al. (1998) who showed that the same sub-chronic PCP regimen used in the present study did not change basal dopamine usage in the nucleus accumbens one week after drug cessation. As

described previously, acutely PAOPA could bind to the more predominant striatal D_{2S} autoreceptor and decrease the inhibition of the indirect pathway. This would theoretically result in increased inhibitory output from the basal ganglia, allowing for greater attenuation of the startle response: increased PPI. Such a mechanism could explain how PAOPA partially attenuated an acute PCP-induced decrease in PPI/sensorimotor gating. As PPI deficits have been observed in schizophrenia patients (Jentsch and Roth, 1999), these findings are clinically relevant.

Social interaction was also tested sub-chronically on the same day as PPI, 9 days after drug cessation. The videos were analyzed for number of interactions using stringent definitions of social interaction: either sniffing, intentionally following, climbing over or under, or displaying aggressive behaviour towards the other rat. When grouped by type of interaction, there was a significant main effect of treatment ($p=0.0306$), type of social interaction ($p<0.0001$), and interaction between the two factors ($p<0.0001$). Bonferroni post-tests revealed a difference between saline and PCP groups in the number of sniffing interactions ($p<0.001$; Figure 28). The PCPa group also had significantly fewer sniffing interactions than the PCP/PAOPA group ($p<0.001$) and was not different from the PCPb group, which had fewer interactions than the PCP/PAOPA group ($p<0.01$). These results suggest that PAOPA was able to prevent but not reverse deficits in PCP-induced social interaction.

It is believed that a reduced activation of dopaminergic and glutamatergic neurons in the frontal cortex contributes to the negative symptoms of schizophrenia

(Howes and Kapur, 2009; Jentsch and Roth, 1999; Neill et al., 2010). Similar to the present study, others have also reported social withdrawal, a common finding in PCP models (Jentsch and Roth, 1999; Neill et al., 2010), following NMDA receptor antagonist administration (Daya et al., 2014; Dyck et al., 2011a; Sams-Dodd, 1995, 1998).

The novel object recognition task was the final test of this study and was performed 8 days after drug cessation. The time spent exploring the familiar and novel objects were transformed into a discrimination index and expressed as a percent. The discrimination index takes into account the variability between rats in the amount of time spent examining an object, by taking the difference of time spent exploring the novel and familiar object and expressing it as a percentage of total time spent exploring both objects. One-way ANOVA revealed a significant difference between the treatment groups ($p=0.0354$) and Tukey's multiple comparison test determined that it originated from the PCPa group, which had a lower discrimination index than only the saline group ($p<0.05$; Figure 29). This establishes that a sub-chronic PCP regimen induced an impairment in the ability of treated rats to recognize familiar objects and concurs with other studies (Popik et al., 2015; Pyndt Jørgensen et al., 2015) and the broader literature (Neill et al., 2010). The PCPb and PCP/PAOPA groups were not different from either the saline or PCPa groups. Looking at Figure 29, the PCPb group seems to have a slightly higher discrimination index than the PCPa group, and PCP/PAOPA seemed to be higher still. Not showing significant difference from either the saline or the PCPa groups—

which were significantly different to each other—might suggest that a one-time PAOPA dose prior to testing slightly attenuated PCP-induced deficits in recognition memory, but PAOPA was better able to ameliorate the deficit when administered concurrently with PCP.

PAOPA's ameliorating effects seem to parallel those seen by atypical antipsychotics, and not typical antipsychotic haloperidol, which does not attenuate deficits (Neill et al., 2010). Grayson et al. (2007) observed that clozapine and risperidone but not haloperidol attenuated deficits in the novel object test using a sub-chronic PCP regimen. Using microdialysis, vehicle-treated rats showed an increase in dopamine concentration in the prefrontal cortex during the test, whereas PCP-treated rats did not (Neill et al., 2010), suggesting that prefrontal dopamine contributes to performance in the novel object recognition task.

Overall, this study has revealed that PAOPA is not able to prevent PCP-induced enhancement of locomotor activity, but—strangely—a may be able to reverse it. The low sample size may have prevented any significant results from being shown, but PAOPA seemed to have a partial ameliorative effect with repeated administrations on acute PCP-induced PPI deficits. In addition, PAOPA prevented but did not reverse PCP-induced deficits in the number of social interactions. Finally, PAOPA seemed to be able to partially prevent and reverse PCP-induced deficits in novel object recognition memory. It is important to note that none of these observations were found to be significant, although in some cases PAOPA had an intermediate effect that was not significantly different from saline or PCP

groups. Nonetheless, these interpretations should be treated with caution, and the promising results merit further confirmation.

17 Study Three – An investigation of the mechanism of action of PAOPA

17.1 Brief Summary

The purpose of this study was to investigate the molecular effects of PAOPA on GPCR regulatory machinery (GRK2 and arrestin-3 protein expression), downstream signalling (ERK1 and 2 protein expression) and synaptic vesicular control (synapsin II protein expression). To investigate this, PAOPA or saline was administered to a cohort of rats and concentrations of these proteins measured in the mPFC, striatum, nucleus accumbens, and cerebellum using western immunoblots. The concentration of GRK2, arrestin-3, and ERK1 and 2 was found increased in the striatum but not the cerebellum of chronically PAOPA-treated rats. The protein concentration of synapsin IIa and not IIb was increased in all regions of the brain examined except for the cerebellum.

18 MATERIALS AND METHODS

18.1 Animals

Twelve male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and kept at McMaster University Central Animal Facility. The room temperature and humidity were constant and the 12-hour light

cycle reversed. Rat had unrestricted access to food and water. The use of animals in this study was approved by the McMaster University Animal Research Ethics Board and all procedures were compliant to the Canadian Council on Animal Care (animal utilization protocol 10-08-59).

18.2 Drugs

PAOPA was synthesized by Dr. Rodney Johnson at the University of Minnesota (MN) as described previously (Yu et al., 1988). Fresh solutions of PAOPA dissolved in saline (0.9% NaCl) at a concentration of 1 mg/mL were prepared daily and injected for 45 days. Rats were weighed daily and injected via the intraperitoneal route 1 mg/kg of PAOPA (n=6) or saline (n=6) in a volume of 1 mL/kg at approximately 11 am.

18.3 Sacrifice

On the last day of drug administration, 1 hour after injection of PAOPA or saline rats were sacrificed. Each rat was placed in an anaesthetic container connected to an oxygen tank and a delivery system feeding isoflurane into the container. Rats took on average 2 minutes to anaesthetize and were immediately decapitated by a guillotine. The brains were quickly and carefully extracted from the skull and transferred to a brain mold kept on ice. The brains were sliced using thin blades and the slices placed on a large plate kept on ice. The medial prefrontal

cortex, striatum, nucleus accumbens, and cerebellum were extracted and kept in microcentrifuge tubes. The microcentrifuge tubes were deposited in a closed container with dry ice and later stored in a -70°C freezer.

18.4 Western Immunoblotting

To determine whether PAOPA induced any changes in the cerebral concentration of GRK2, arrestin-3, ERK 1 or 2, and synapsin IIa or IIb, western immunoblotting was employed. Tissue samples were removed from the freezer and allowed to thaw. Samples were homogenized to lyse cells using a pestle in phosphate-buffered saline with Complete Mini, EDTA-free protease inhibitor tablet (Hoffmann La-Roche, Mississauga, ON) and PhosStop phosphatase inhibitor cocktail tablet (Hoffmann La-Roche, Mississauga, ON) to prevent catalysis of proteins and phosphate groups. To ensure cell lysis, the homogenate was sonicated on ice for 10 seconds thrice. A Bradford protein assay was used to determine the concentration of protein. A blank solution tube was created by adding 795 μL of phosphate-buffered saline and 200 μL of Bio-Rad 1X dye reagent (Bio-Rad). The protein solution tube was created by adding 5 μL of the protein sample, 795 μL of phosphate-buffered saline, and 200 μL of Bio-Rad 1X dye reagent (Bio-Rad). The tubes were allowed to incubate for 10 minutes at room temperature. After 10 minutes, samples were transferred to disposable cuvettes and placed in a Beckman Coulter DU® 640 spectrophotometer set to 595 nm wavelength. Samples were read

in triplicates with the blank first followed by the protein sample. The triplicate concentrations were averaged to give the mean concentration of protein. 15 µg of protein was resuspended using sodium dodecyl sulfate buffer and boiled for 10 minutes. The protein sample was separated by electrophoresis using 10% acrylamide gels for approximately 1 hour until the loading dye was close to the bottom of the gel. The resulting bands were blotted onto a 0.45 µM polyvinylidene fluoride membrane using transfer buffer (12 mM Tris, 96 mM glycine, 10% methanol). To prevent/reduce non-specific binding of the primary antibody, the membrane was blocked for 1 hour with diluted skim milk (5% skim milk, 50 mM Tris, 150 mM NaCl, 0.2% Tween-20, pH 8.5) at room temperature. Subsequently, the membrane was incubated overnight with the primary antibody diluted in Tris-buffered saline-Tween-20 (TBS-T; 50 mM Tris, 150 mM NaCl, 0.2% Tween-20, pH 8.5) at 4°C on a shaker. The primary antibodies used to detect GRK2, arrestin-3, phosphorylated ERK (1/2), synapsin II (both isoforms), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which was used as a reference, were respectively: monoclonal anti-GRK2 antibody produced in rabbit (1:2000; Abcam, Cambridge, MA), monoclonal anti-ARRB2 antibody produced in mouse (1:1000; Sigma, St. Louis, MO), polyclonal phosphor-specific anti-p44/42 MAPK (Thr202 for ERK 1 and Tyr404 for ERK 2) produced in rabbit (1:2000; Cell Signaling, Boston, MA), polyclonal synapsin II antibody produced in rabbit (1:2500; Enzo Life Sciences, Ann Arbor, MI), and anti-GAPDH antibody produced in mouse (1:10000; Millipore, Billerica, MA). The next day, unbound primary antibodies on

the membrane were washed in TBS-T for 5 minutes, three times on a shaker at room temperature. Next, the membrane was exposed to the secondary antibody for 1.5 hours at room temperature on a shaker. The primary antibodies produced in rabbit were incubated with anti-rabbit IgG horseradish peroxidase (HRP)-linked whole secondary antibody from donkey (1:5000; Sigma Aldrich, St. Louis, MO); the primary antibodies produced in mouse, anti-mouse IgG HRP-linked whole secondary antibody from sheep (1:5000; Sigma Aldrich, St. Louis, MO). Unbound secondary antibodies were washed with TBS-T for 5 minutes, three times on a shaker at room temperature. The labeled secondary antibody, which was bound to the primary antibody bound to the target protein, was developed using enhanced chemiluminescence reagents and then exposed to a Kodak X-OMAT film (Eastman Kodak Co., NY) in a dark room.

18.5 Quantitation

The intensity of the protein bands was quantified using ImageJ 1.43M software (National Institutes of Health). All protein values were normalized to GAPDH. GraphPad Prism 4 was used for statistical analysis. The student's t-test was chosen to compare the concentration of each protein in each brain region between rats treated with PAOPA and saline. The alpha level was 0.05 and any *p* values less than 0.05 were deemed significant.

19 RESULTS

19.1 GRK2, arrestin-3, and ERK 1/2 in the striatum and cerebellum

The concentration of GRK2, arrestin-3, and phosphorylated ERK 1 and 2 were examined in the striatum and cerebellum of rat brains. The concentration of all proteins were increased after PAOPA treatment compared to saline treatment in the striatum (Figure 30). The concentrations of GRK2, arrestin-3, and phosphorylated ERK 1 and 2 increased by 41% ($p<0.05$), 34% ($p<0.05$), 51% ($p<0.01$), and 36% ($p<0.05$), respectively. There was no difference in the concentration of GAPDH in the striatum between PAOPA- and saline-treated animals. In the cerebellum, there was no difference in the concentration of GRK2, arrestin-3, or phosphorylated ERK 1 and 2 between PAOPA- and saline-treated animals ($p>0.05$; Figure 31). There was also no difference in GAPDH concentration in the cerebellum between PAOPA- and saline-treated animals ($p>0.05$).

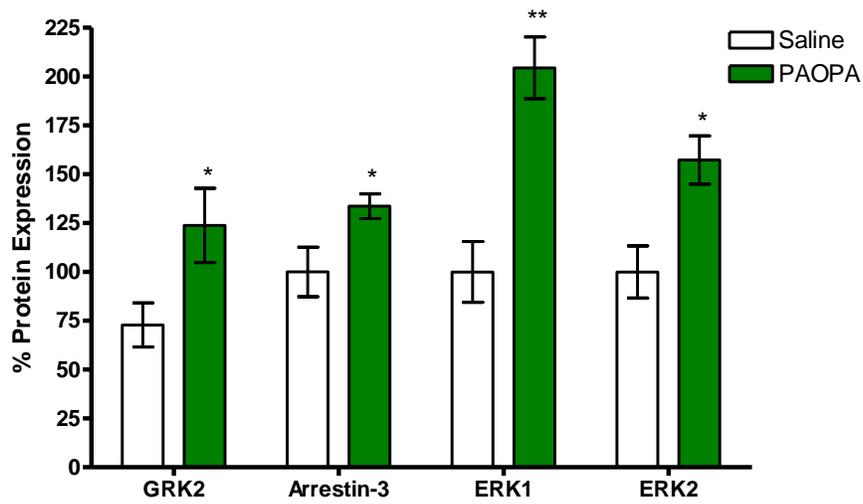


Figure 30. The relative protein expression of proteins of interest in the striatum. Student's t-tests revealed that PAOPA significantly increased the protein concentration of all proteins of interest compared to saline. GRK2 was increased by 41% (* $p < 0.05$), Arrestin-3 was increased by 34% (* $p < 0.05$), ERK1 was increased by 51% (** $p < 0.01$), and ERK2 was increased by 36% ($p < 0.05$). Data represents mean \pm standard error of mean.

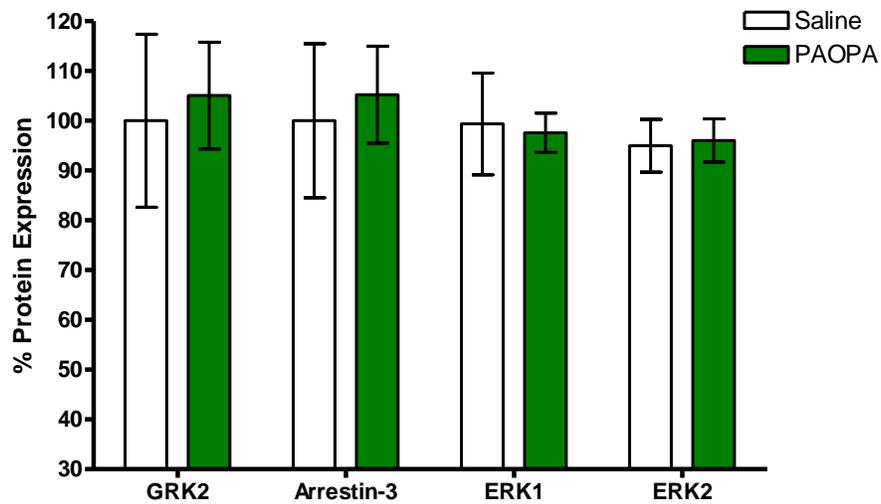


Figure 31. The relative protein expression of proteins of interest in the cerebellum. Student's t-tests showed no

significant difference in protein concentration of GRK2, Arrestin-3, ERK1, or ERK2 in the cerebellum. Data represents mean \pm standard error of mean.

19.2 Synapsin IIa and IIb in the mPFC, striatum, nucleus accumbens, and cerebellum

The concentration of synapsin IIb in the mPFC, striatum, nucleus accumbens, or cerebellum was not significantly different ($p>0.05$) between PAOPA- and saline-treated animals (Figure 33). The concentration of synapsin IIa was significantly increased in the striatum by 48% ($p=0.0126$), in the nucleus accumbens by 119% ($p=0.0103$), and in the mPFC by 30% ($p=0.0296$) after PAOPA treatment compared to after saline treatment (Figure 32). The concentration of synapsin IIa in the cerebellum was not significantly different ($p>0.05$) between PAOPA- and saline-treated animals. The concentration of GAPDH was not significantly different in any of the brain regions after PAOPA or saline treatment.

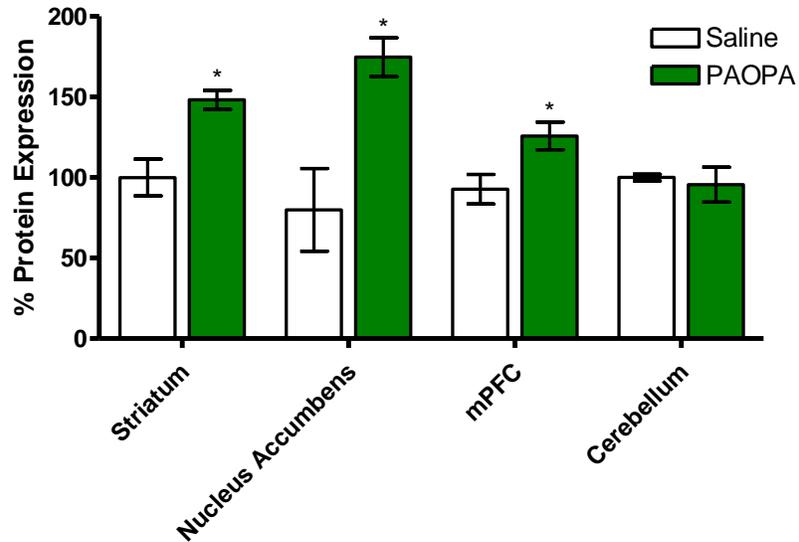


Figure 32. Relative protein concentration of synapsin IIa in various brain regions. Student's t-tests revealed that PAOPA increased the protein concentration of synapsin IIa in the striatum by 48% (* $p < 0.05$), in the nucleus accumbens by 119% (* $p < 0.05$), and in the mPFC by 30% (* $p < 0.05$) compared to saline-treated animals. Synapsin IIa concentration in the cerebellum was not different between treatment groups ($p > 0.05$). Data represents mean \pm standard error of mean.

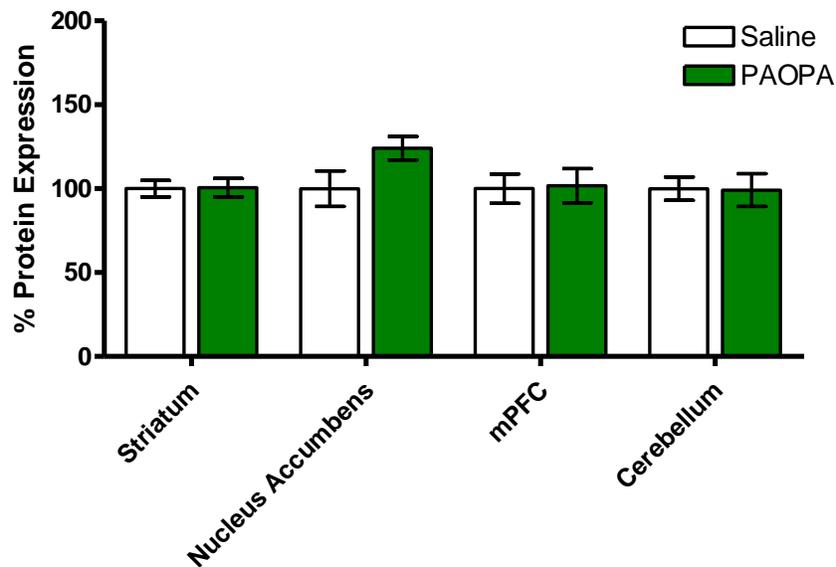


Figure 33. Relative protein concentration of synapsin IIb in various brain regions. Student's t-tests revealed that the concentration of synapsin IIb was not different in any of the brain regions studied ($p>0.05$). Data represents mean \pm standard error of mean.

20 DISCUSSION

In the final study of this thesis, PAOPA ($n=6$) or saline ($n=6$) were administered to twelve rats for 45 days. The concentrations of key proteins involved in cellular receptor trafficking (G protein-coupled receptor kinase 2, GRK2, arrestin-3; Krupnick and Benovic, 1998), downstream signalling (ERK1 and 2; Beom et al., 2004), and neuronal signalling (synapsin IIa and IIb; Cesca et al., 2010) were measured in brain regions implicated in schizophrenia (mPFC, striatum, nucleus accumbens) and the cerebellum.

Administration of PAOPA to rats for 45 days increased the concentration of GRK2 ($p < 0.05$) and arrestin-3 ($p < 0.05$) in the striatum (Figure 30) but not the cerebellum ($p > 0.05$; Figure 31). PAOPA also increased the concentration of phosphorylated ERK 1 ($p < 0.01$) and 2 ($p < 0.05$), in the striatum (Figure 30) and not the cerebellum (Figure 31).

Although the effects of agonists can be modified at different levels, the primary control occurs by GRKs and arrestins (Krupnick and Benovic, 1998). Most of the family of GRKs are ubiquitous and have a prominent role in receptor desensitization of G protein-coupled receptors (GPCRs), such as the dopamine D₂ receptor. This is mediated by two methods: a rapid receptor-G protein uncoupling and a slower receptor down-regulation (Krupnick and Benovic, 1998). In receptor-G protein uncoupling, GRK binds to the C-terminal of the GPCR and prevents the binding of the G protein, thereby preventing the normal downstream effects of receptor activation. Continued agonist-induced receptor activation leads to the phosphorylation of specific serine and threonine residues in the C-terminal. This phosphorylated receptor state is stabilized by the recruitment and binding of another family of proteins known as arrestins (Krupnick and Benovic, 1998). One of the arrestins, arrestin-3, is expressed ubiquitously, has broad receptor specificity, and also acts as a un-coupler of the GPCR and its G protein. Arrestin acts like an adapter protein, associating the phosphorylated GPCR with clathrin and thus targeting it to clathrin-coated pits for endocytosis. Although internalization of the GPCR would

prevent it from interacting with agonists and thereby preventing its normal function, Krupnick and Benovic's (1998) also suggest that internalization may additionally play a role in GPCR re-sensitization after the agonist has been removed.

The increase in GRK2 and arrestin-3 by PAOPA administration in the present study suggests that PAOPA promotes the uncoupling of the dopamine D₂ receptor to its G_{ai} protein and also perhaps promotes internalization of the receptor. Studies from the laboratory support an internalization mechanism of PAOPA: TREx-293 cells expressing GRK2, arrestin-3, and the dopamine D₂ receptor fused with an enhanced yellow fluorescent protein (eYFP) were treated with DMEM (control), a dopamine D₂ receptor agonist (quinpirole), PAOPA, and a combination of quinpirole and PAOPA. Live cell imaging showed no internalization of the receptor in control cells, slight presence of receptors in the cytosol of cells treated with only quinpirole, and an enhanced presence of receptors in the cytosol of cells treated with both quinpirole and PAOPA (Basu et al., 2013). Furthermore, receptor binding studies were performed on these cells using the dopamine D₂ receptor-like antagonist sulpiride, which can only bind to membrane receptors owing to its high hydrophilicity. This experiment found that quinpirole treatment reduced the membrane expression of the dopamine D₂ receptor by approximately 56%, the addition of PAOPA to quinpirole treatment reduced it by 89%, and PAOPA alone did not significantly alter expression from control although a slight decrement was observable (Basu et al., 2013). Thus, with these experiments in mind, the increased

protein expression of GRK2 and arrestin-3 adds good support to the hypothesis that PAOPA promotes agonist-induced internalization of the receptor via the classical GRK/arrestin-mediated pathway.

The classical mechanism by which extracellular signal-regulated kinase is activated starts with the G protein Ras, which is induced to replace its GDP with GTP, thereby activating it. Ras recruits and activates Raf, which in turn phosphorylates MAP (mitogen activated protein)/ERK kinase (MEK). MEK subsequently activates ERK by phosphorylation. ERK/MAPK are involved in a variety of cell functions such as cell proliferation, survival, differentiation, apoptosis, motility and differentiation; they have more than 70 substrates, including nuclear transcription factors (Egan and Weinberg, 1993; Kolch, 2005). The activation of ERK via the dopamine D₂ receptor has been studied but the precise mechanisms observed are not consistent between studies and therefore remain to be resolved. Beom et al. (2004) studied activation of ERK in HEK-293 by the dopamine D₂ receptor. The presence of dopamine increased the concentration of phosphorylated ERK1/2, which was abolished by pre-treatment with the antagonists haloperidol or sulpiride. This phosphorylation was mediated by the G α_i subunit and not by internalization of the dopamine D₂ receptor did not change ERK activation. However, the authors noted that other studies using different cell types did not necessarily observe the same results (Beom et al., 2004). Supporting these results, Bruins Slot et al. (2006) showed that in CHO cell lines expressing the short

dopamine D₂ receptor (D_{2S}R), dopamine increased the expression of phosphorylated ERK and raclopride, haloperidol, olanzapine, ziprasidone, and clozapine inhibited this dopamine-induced increase in phospho-ERK. Unlike those antipsychotics, the novel antipsychotic aripiprazole increased ERK phosphorylation. But where Beom et al. (2004) and Bruins Slot et al. (2006) found that antagonist pre-treatment abolished dopamine-induced activation of ERK via D₂R, Pozzi et al. (2003) and Bertran-Gonzalez et al. (2008) observed that mice acutely administered D₂R antagonists, such as haloperidol and eticlopride, had increased ERK1 and 2 activation in the dorsal striatum (Pozzi et al., 2003) or in D₂R neurons (Bertran-Gonzalez et al., 2008) compared to controls. Corroborating the *in vitro* data, the atypical antipsychotic clozapine decreased ERK phosphorylation (Pozzi et al., 2003). Haloperidol and eticlopride produced a transient increase in phosphorylation of the transcription factor cyclic adenosine monophosphate (cAMP) response element binding (CREB) protein, whereas clozapine produced a long-lasting (>60 min) decrease (Pozzi et al., 2003). The authors postulated that the disinhibition of adenylyl cyclase and subsequent phosphorylation of protein kinase A (PKA) was the mechanism responsible for the activation of the various downstream proteins and transcription factors (Pozzi et al., 2003). These *in vivo* results are diametrically opposite to the *in vitro* results of Beom et al. (2004) and Bruins Slot et al. (2006). They likely reflect an increasing complexity of regulatory machinery inside the striatum compared to cell cultures, not to mention the influence of other receptors and neurons. For example, Pereira

et al. (2014) found that quetiapine increased ERK concentration in the striatum in an EGFR (epidermal growth factor receptor)-dependent manner.

The concentration of all proteins was not increased in the cerebellum. The cerebellum is a region often used as reference in imaging studies because it has a low density of dopamine receptors (Millet et al., 2012; Pilowsky et al., 1997; Shalgunov et al., 2015; Suhara et al., 1999). Without any dopamine D₂ receptors present, PAOPA would be expected to have no effect on the concentration of GRK2, arrestin-3, and ERK1 and 2 in the cerebellum.

PAOPA increased the concentration of the synapsin IIa (Figure 32) and not the synapsin IIb isoform (Figure 33) in the mPFC ($p < 0.05$), striatum ($p < 0.05$), and nucleus accumbens ($p < 0.05$), but not the cerebellum ($p > 0.05$). Synapsins are a family of synaptic vesicle phosphoproteins that are expressed in vertebrates and invertebrates. They are most notably known for maintenance of the reserve pool of synaptic vesicles inside the pre-synaptic terminal, but are also involved in the basic mechanisms of synapse formation and plasticity (Cesca et al., 2010; Shin, 2014; Sudhof, 2004). There are three isoforms of synapsin (1 – 3), each with multiple variants. Synapsins I and II are widely expressed in synapses but not III (Sudhof, 2004). In their stable state, synapsins bind to actin and the synaptic vesicle membrane, tethering the synaptic vesicle inside the pre-synaptic terminal. The A and C domains of synapsin are able to bind to the synaptic vesicle membrane, but it is phosphorylation of the A domain—by PKA or Ca²⁺/calmodulin-dependent

protein kinase I, which are activated by Ca^{2+} from depolarization—that induces the dissociation of synapsin from the synaptic vesicle (Kandel et al., 2013c; Shin, 2014). Dissociation of synapsin from the synaptic vesicle increases the size of the releasable pool of synaptic vesicles (Shin, 2014).

Synapsin has been implicated in schizophrenia, with evidence pointing towards synapsin II in particular (Cesca et al., 2010). Mirnics et al. (2000) found that of the various proteins regulating presynaptic function, synapsin II in the prefrontal cortex was decreased in patients with schizophrenia compared to healthy controls. A recent study by Tan et al. (2014) also found decreased synapsin II in the dorsolateral prefrontal cortex of schizophrenia patients compared to healthy controls. Case-control (Chen et al., 2004) as well as family-based (Chen et al., 2004) gene association studies by the same group revealed the increased frequency of specific gene markers in schizophrenics versus controls. These studies of correlation were not able to clarify whether decreased synapsin II concentration is involved in the cause of schizophrenia or if it is an un-related by-product of the disease. If a decrease in synapsin protein contributes to the disease, it would be encouraging to identify potential therapeutics that can increase and restore its concentration. In the present study, chronic PAOPA treatment increased the protein expression of synapsin IIa in various brain regions. The increase induced by PAOPA parallels the ability of antipsychotics to also increase synapsin's concentration.

Chronic administration of typical antipsychotic haloperidol to rats increased the mRNA and protein expression of synapsin IIa and IIb, but not synapsin I, in the mPFC, nucleus accumbens, and striatum (Chong et al., 2006; Chong et al., 2002). Chong et al. (2006) also tested the effects of a D₁R antagonist (SCH23390) and agonist (SKF38393) on synapsin protein expression in mouse primary midbrain cells and found that the protein concentration of synapsin II increased with D₁R agonist, and not antagonist treatment. As D₁Rs promote and D₂Rs inhibit adenylyl cyclase function, their discoveries led Chong et al. (2006) to propose that the regulation of synapsin II by D_{1/2}Rs occurred via cAMP and downstream transcription factors such as activating protein 2 α (AP-2 α), which was also increased by haloperidol and not SCH23390 treatment, and early growth response factor 1 (EGR-1). Later Skoblenick et al. (2010) confirmed these results (Chong et al., 2006; Chong et al., 2002). Furthermore, Skoblenick et al. (2010) additionally found that inhibition of PKA or AP-2 α , and not EGR-1, reduced—but not abolished—the protein expression of synapsin II in mouse primary midbrain neurons and was unaffected by SCH38393 (D₁R agonist) or haloperidol (D₂R antagonist) treatment. These studies provide support for the involvement of PKA and AP-2 α in increasing synapsin II protein concentration and indicate the mechanism by which PAOPA exerted its effects on synapsin IIa in the present study. In contrast to Chong et al. (2006; 2002) and Skoblenick et al.'s (2010) finding that haloperidol increases synapsin II concentration in rats and cell cultures, when Guest et al. (2010) measured the protein concentration of synapsin IIa and IIb from

post-mortem prefrontal brain samples, both isoforms of synapsin II were found to be comparable between patients who took typical antipsychotics and controls, whereas patients who took atypical antipsychotics had increased concentrations of the two isoforms. In a recent study examining the lifetime intake of antipsychotics with synapsin II expression, Tan et al. (2014) discovered that antipsychotic use positively correlated with increased synapsin IIa expression. In a series of experiments, knockout or knockdown of the synapsin II gene or mRNA in the prefrontal cortex of mice or rats led to the observations of schizophrenia-like phenotypes: decreased PPI, decreased social interaction, and increased locomotor activity compared to controls (Dyck et al., 2011b; Dyck et al., 2007, 2009; Dyck et al., 2012). The schizophrenia-like phenotypes were reversed by olanzapine and the concentration of glutamate (VGLUT1 and 2), GABA (VGAT), but not dopamine (VMAT2) vesicular neurotransmitter transporter proteins were found decreased (Dyck et al., 2011b). Thus there is some groundwork of evidence to suggest that synapsin II may be one of the targets of pharmacological treatment for schizophrenia. The present observation that PAOPA increased synapsin II expression is encouraging since it also parallels most of the described studies that found an association between antipsychotic treatment and synapsin II increase. The next step would be to examine established animal models of schizophrenia, such as the amphetamine, PCP, and MK-801 pharmacological models, to see whether synapsin II is reduced and whether PAOPA can prevent and restore its concentration. A few studies have been conducted on PCP and amphetamine-

sensitized models and expression of various proteins: PCP administration increased synapsin I in mPFC of rats (Pickering et al., 2013); amphetamine sensitization in rats either did not affect concentration of synapsin I in the nucleus accumbens and ventral tegmental area (Subramaniam et al., 2001) or increased the concentration of phosphorylated synapsin I in the striatum of rats (Iwata et al., 1996; Iwata et al., 1997); methamphetamine sensitization in rats increased the concentration of synapsin IIb in the striatum (Iwazaki et al., 2007). Not all (Iwata et al., 1996; Iwata et al., 1997; Subramaniam et al., 2001) of these studies specifically tested for changes in synapsin II protein concentration and those that did (Iwazaki et al., 2007; Pickering et al., 2013) screened for all types of synaptic proteins using proteomics and likely had a low threshold for protein detection. Consequently, it may be useful to specifically test for alterations in synapsin IIa and IIb protein concentrations in various brain regions using animals models, such as the ones in the present study.

21 LIMITATIONS

The first and most obvious limitations of the present thesis was the lack of sample size for the first two studies. The small sample size might have prevented some expected significant results and prohibited valid interpretations and firm conclusions being made. However, these studies have served an investigative role, and have been useful in revealing promising results for PAOPA's ameliorative effects in the amphetamine and PCP models of various behaviours and for guiding future research with a sufficient sample size to either reject or confirm present results.

The use of the 5-choice serial reaction time task (5-CSRTT) for assessing attention has been criticized (Young et al., 2012). Challenging animals with variable stimulus durations and inter-trial intervals (ITI) has been used to reveal deficits in accuracy between the amphetamine-treated animals and saline-treated animals. The impairment of amphetamine-sensitized animals has been interpreted as decreased attention, but Young et al. (2012) have pointed out that an impairment in learning and memory to adapt to the changing stimulus durations and ITI might also account for the impairment. That is to say, the amphetamine-sensitization may have induced animals to “forget” correct performance on the task, which was emphasized by the variable stimulus duration and ITI. However, such a hypothesis might also predict that as the animals are treated with amphetamine, there is a progressive, albeit perhaps small, decline in accurate performance under baseline

or standard conditions. This was not observed during the drug administration or withdrawal period (Figure 34) and supports attentional impairment as an explanation for the (non-significant) decrease in accuracy by the amphetamine-treated group.

Young et al. (2012) have also criticized the use of the novel object recognition task for assessing recognition memory. They make the valid argument that the task relies on the spontaneous curiosity of animals, not any type of motivation. Thus pharmacological manipulations may in fact affect this spontaneous curiosity to explore objects, and not the ability to remember previously explored objects. However, if spontaneous exploration has been affected, then a lack of exploration should be seen for both objects, resulting in a discrimination index of zero. This was not observed in Study Two. Young et al. (2012) also argue that neither MATRICS nor CNTRICS (Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia) have identified this task as an acceptable assay for evaluating one of the cognitive domains. This point cannot be argued against; but perhaps it should be noted that the novel object recognition task provides a very rapid assay for filtering putatively effective candidate drugs from ineffective ones. This is one of the main advantages that has given it much popularity in research.

Finally, the main interpretation from Study Three, drawn from the observation of increased GRK2 and arrestin-3, was that the necessary machinery existed and was increased for the internalization of D₂Rs by chronic PAOPA treatment. However, this does not necessarily mean that the receptors are

degraded—they might be recycled back to the membrane. Furthermore, allosteric modulators have been theorized to not have any effects on their own. The fact that sole treatment with PAOPA changed *anything* raises interesting questions about its function as an allosteric modulator. However, as this study was not conducted in an animal model and no behaviours were measured, the changes in protein expression seen may not necessarily translate into behavioural changes.

22 CONCLUSIONS

The present three studies have investigated the ability of PAOPA to ameliorate amphetamine-induced impairments in attention, PCP-induced impairments in locomotor activity, PPI/sensorimotor gating, social interaction and novel object recognition memory, and the molecular mechanisms by which effects of PAOPA are mediated.

The first two studies were limited by a small sample size and therefore any interpretations must be treated with caution. At best, preliminary evidence *suggesting* an ameliorative effect of PAOPA in preventing and/or reversing amphetamine-induced impairments in attention, PCP-induced impairment in sensorimotor gating, social interaction, and novel object has been presented. Furthermore, a new method of pre-training animals to the 5-CSRTT task has been developed, reducing time and manual labour. It has been shown that a single session of variable stimulus can produce the same results as manipulating stimulus duration

over the course of two-and-a-half weeks, greatly reducing experimental time and allowing for testing of other behaviours. A limit on the length of amphetamine-induced impairments has been suggested and PAOPA did not seem able to prevent PCP-induced hyper-locomotor activity, suggesting it acts primarily at the dopaminergic site in the striatum. The final study showed a significant increase in proteins that regulate GPCRs, transcription factors, and neurotransmitter release. These results suggest that chronic PAOPA treatment promotes internalization of dopamine D₂ receptors, and increases the expression of synapsin IIa. These molecular changes may be the neurochemical changes that mediate changes in behaviour by PAOPA treatment.

Neurons are made of an intricate and complex system of molecules. There are still many proteins to investigate and the precise mechanism by which PAOPA increases GRK2, arrestin-3, ERK1 and 2, and synapsin IIa remain to be discovered, as well how they influence behavioural changes. Perhaps the next great leap in understanding the actions of PAOPA will come through the use of microdialysis to gain parallel insights into how changes in neurotransmitters change behaviour in the desired direction and for the treatment of schizophrenia.

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24 APPENDIX A: FIGURES

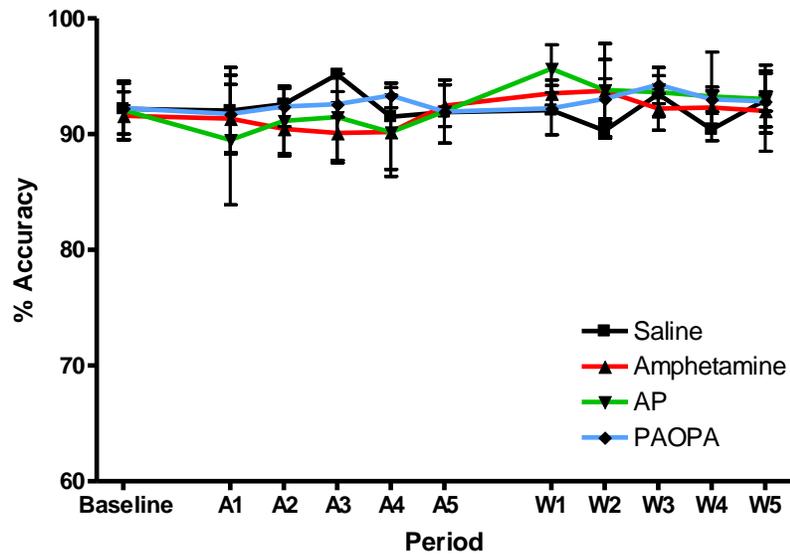


Figure 34. Percent accuracy during the baseline, drug administration, and withdrawal periods. There was a significant main effect of subject matching ($p < 0.0001$). AP indicates rats treated with amphetamine and PAOPA concurrently; A1 – A5 indicates drug administration weeks 1

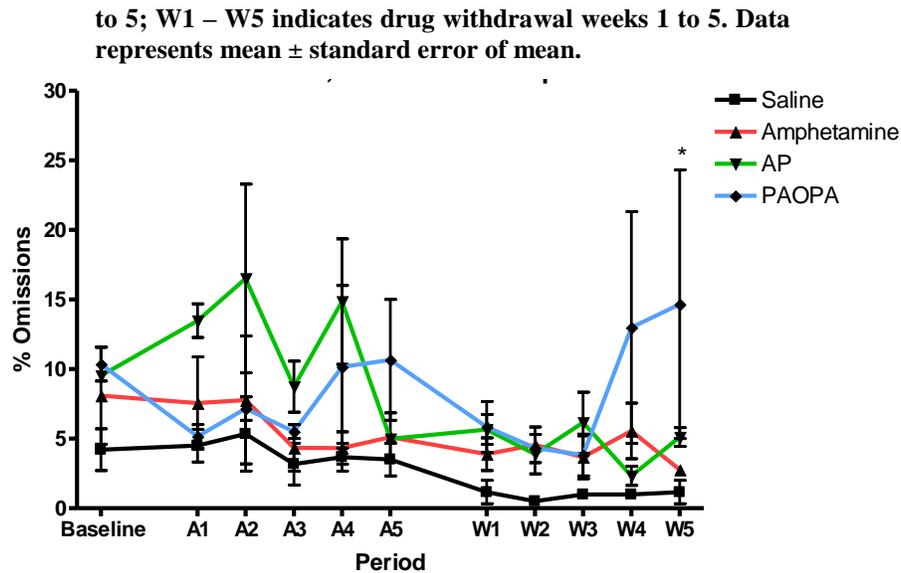


Figure 35. Percent omissions during the baseline, drug administration, and withdrawal periods. There was a main effect of time period ($p=0.0258$), indicating that percent omissions declined with time, and subject matching ($p=0.0058$). Bonferroni post-tests revealed that PAOPA treated rats had greater percent omissions than PAOPA treated rats in the final week of withdrawal ($t=3.008$, $*p<0.05$). AP indicates rats treated with amphetamine and PAOPA concurrently; A1 – A5 indicates drug administration weeks 1

to 5; W1 – W5 indicates drug withdrawal weeks 1 to 5. Data represents mean ± standard error of mean.

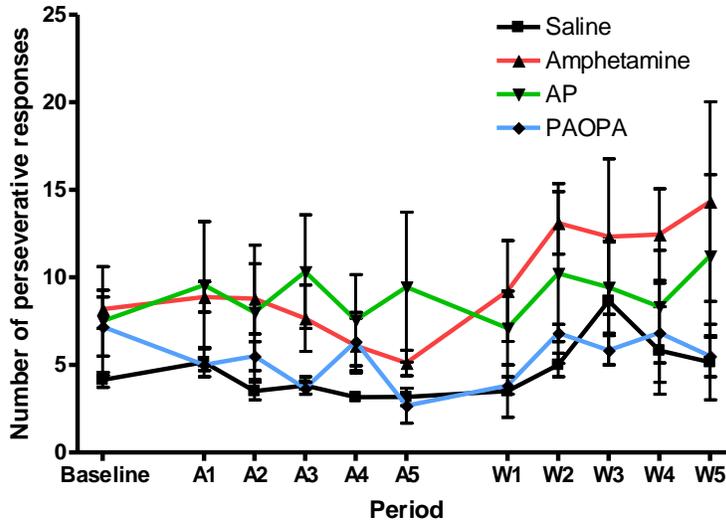


Figure 36. Perseverative responses during the baseline, drug administration, and withdrawal periods. There was a main effect of subject matching ($p < 0.0001$). AP indicates rats treated with amphetamine and PAOPA concurrently; A1 – A5 indicates drug administration weeks 1 to 5; W1 – W5 indicates drug withdrawal weeks 1 to 5. Data represents mean ± standard error of mean.

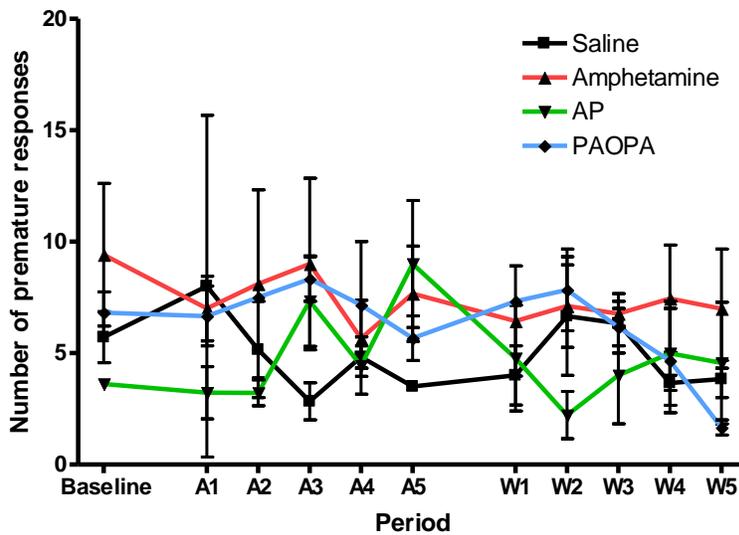


Figure 37. Premature responses during the baseline, drug administration, and withdrawal periods. There was a main

effect of subject matching ($p < 0.0001$). AP indicates rats treated with amphetamine and PAOPA concurrently; A1 – A5 indicates drug administration weeks 1 to 5; W1 – W5 indicates drug withdrawal weeks 1 to 5. Data represents mean \pm standard error of mean.

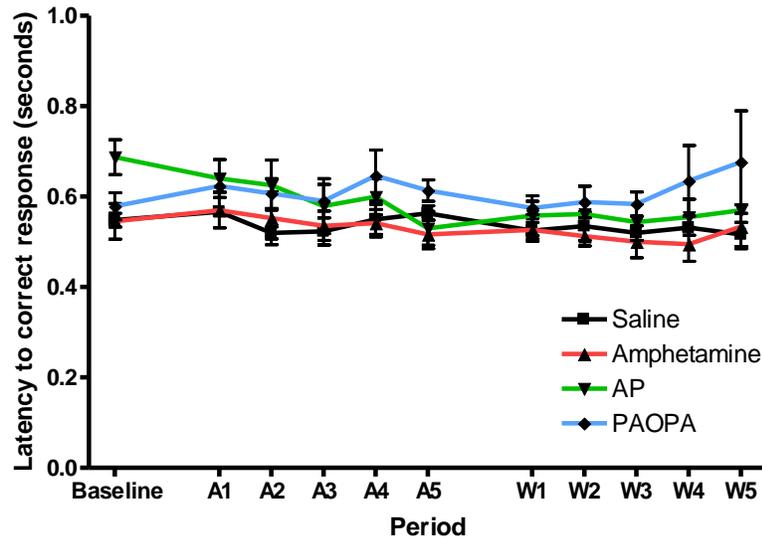


Figure 38. Correct response latency during the baseline, drug administration, and withdrawal periods. There was main effect of subject matching ($p < 0.0001$) and time period ($p = 0.0304$) indicating that response latency generally tended to decrease with time. AP indicates rats treated with amphetamine and PAOPA concurrently; A1 – A5 indicates drug administration weeks 1 to 5; W1 – W5 indicates drug

withdrawal weeks 1 to 5. Data represents mean \pm standard error of mean.

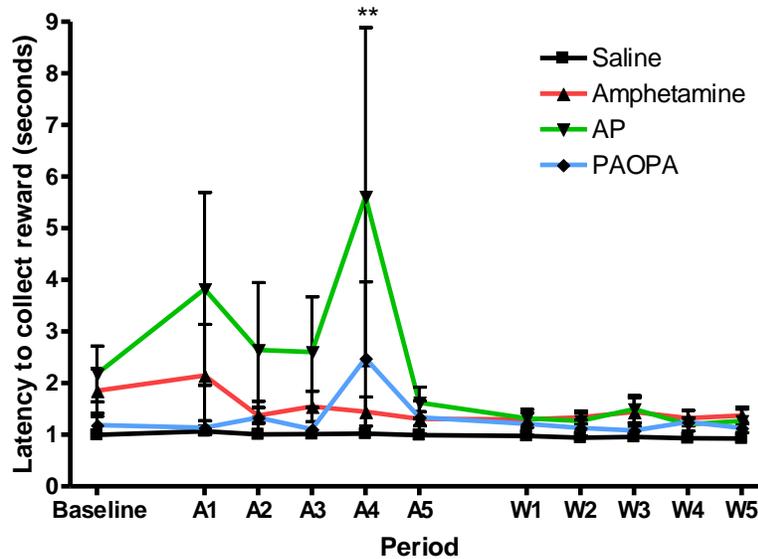


Figure 39. Reward latency during the baseline, drug administration, and withdrawal periods. There was a main effect of subject matching ($p=0.02317$) and none for treatment ($p=0.2112$); however, Bonferroni post-tests revealed a significant difference between AP and saline ($t=3.730$, $**p<0.01$), and AP and amphetamine ($t=3.781$, $**p<0.01$), where AP took greater time to collect the food reward than both saline and amphetamine rats. AP indicates rats treated with amphetamine and PAOPA concurrently; A1 – A5 indicates drug administration weeks 1 to 5; W1 – W5 indicates

drug withdrawal weeks 1 to 5. Data represents mean \pm standard error of mean.

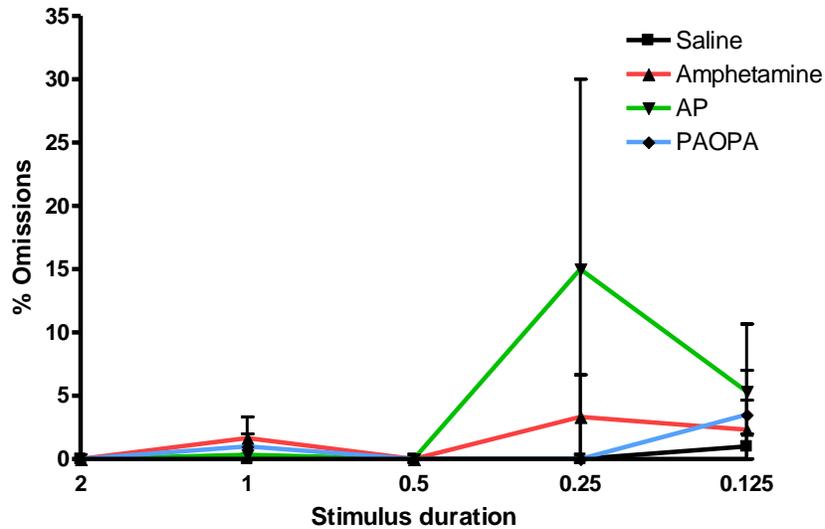


Figure 40. The effect of stimulus duration manipulation on percent omissions. Stimulus duration was varied across five sessions, one session at a time, using a Latin square design. There were no main effects of treatment ($p=0.4079$) or stimulus duration ($p=0.6242$). Bonferroni post-tests did not reveal any significant differences between groups for any

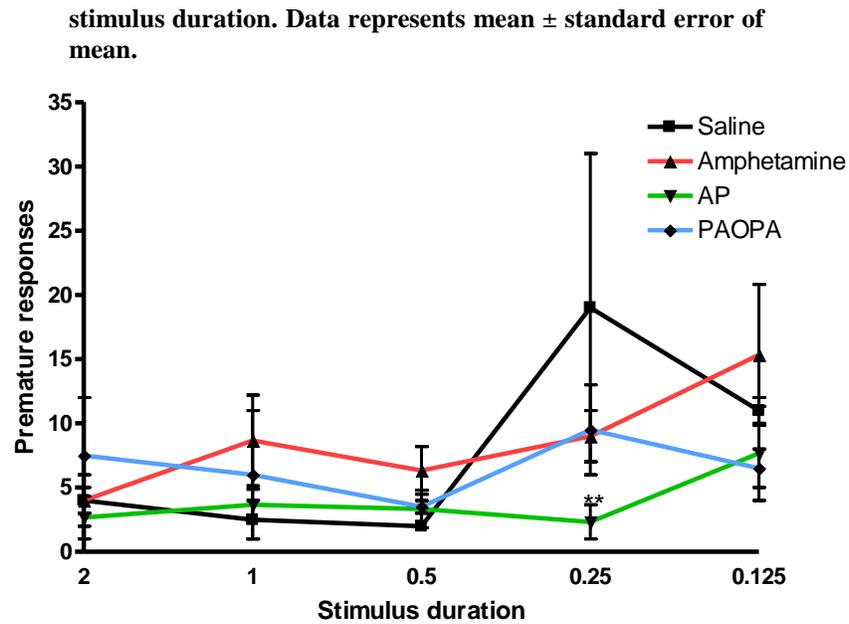


Figure 41. The effect of stimulus duration manipulation on premature responses. Stimulus duration was varied across five sessions, one session at a time, using a Latin square design. There was a main effect of treatment ($p=0.0138$) on premature responses. Bonferroni post-tests revealed a significant difference between saline treated and AP rats at 0.25 s

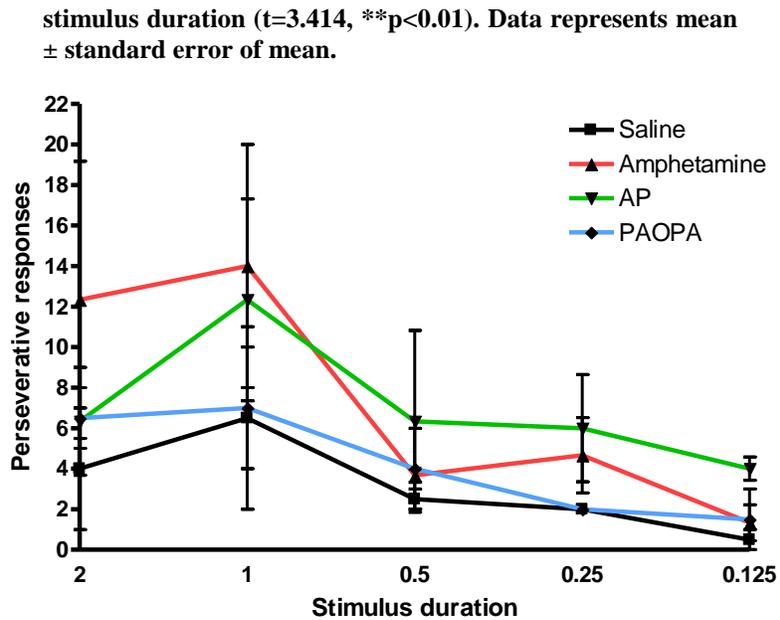


Figure 42. The effect of stimulus duration manipulation on perseverative responses. Stimulus duration was varied across five sessions, one session at a time, using a Latin square design. There was a main effect of stimulus duration ($p=0.0066$), indicating that perseverative responses decreased with decreasing stimulus duration, and subjects ($p=0.0130$). Bonferroni post-tests did not reveal significant differences

between groups for any stimulus duration. Data represents mean \pm standard error of mean.

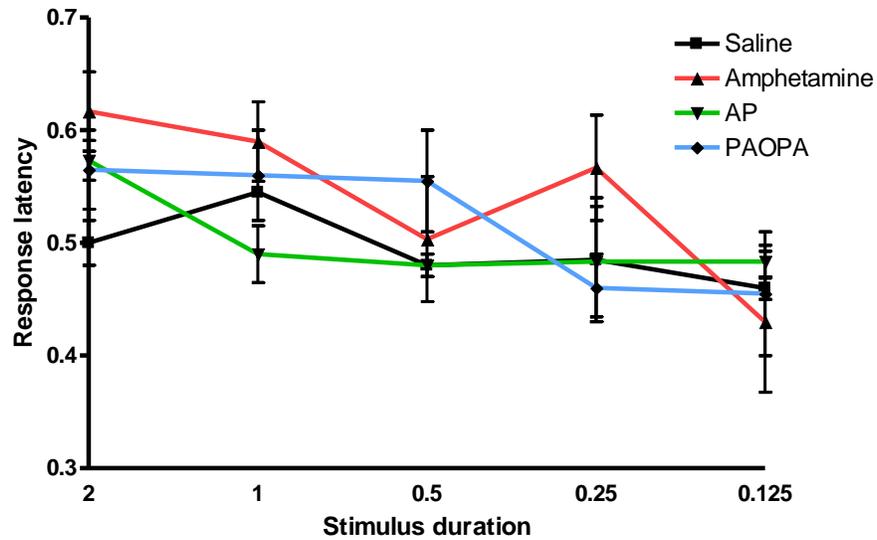


Figure 43. The effect of stimulus duration manipulation on correct response latency. Stimulus duration was varied across five sessions, one session at a time, using a Latin square design. There was a main effect of stimulus duration ($p=0.0040$) on correct response latency where decreasing stimulus duration tended to decrease response latency. Bonferroni post-tests did

not reveal any significant differences between groups. Data represents mean \pm standard error of mean.

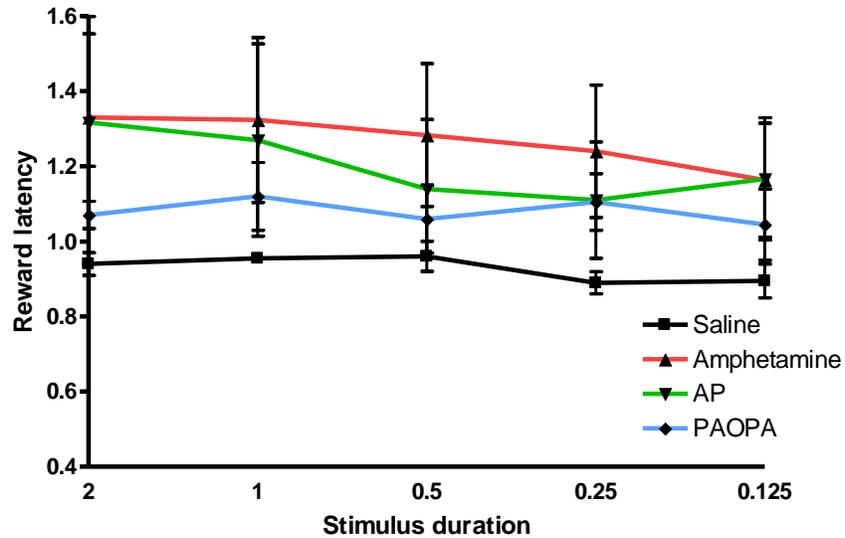


Figure 44. The effect of stimulus duration manipulation on reward latency. Stimulus duration was varied across five sessions, one session at a time, using a Latin square design. There was a main effect of stimulus duration ($p=0.0322$) indicating that reward latency tended to decrease with decreasing stimulus duration, and subject matching ($p<0.0001$). Bonferroni post-tests did not reveal any

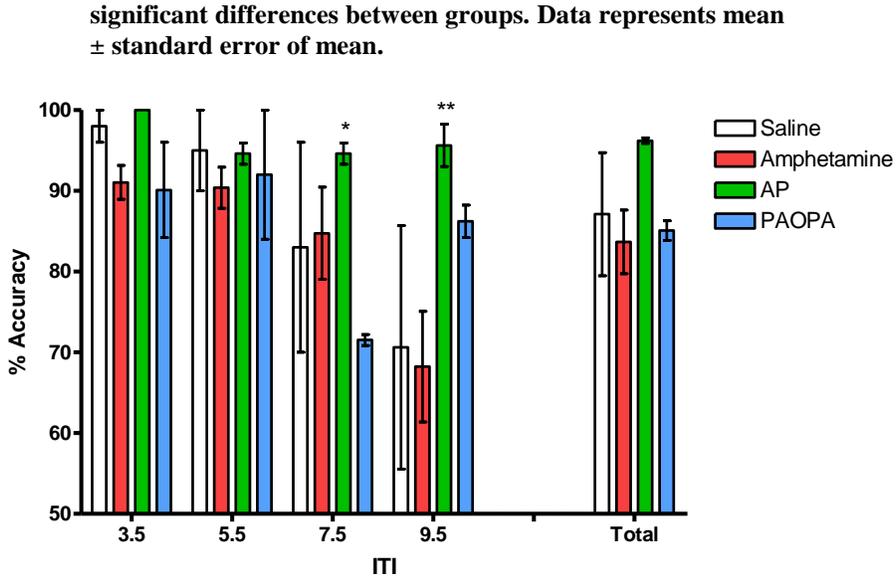


Figure 45. The effects of variable ITI on percent accuracy. ITI was varied between 3.5, 5.5, 7.5, and 9.5 s within a single session. There were significant main effects of interaction ($p=0.0134$), ITI ($p<0.0001$), and subjects ($p=0.0012$). Bonferroni post-tests revealed that AP treated rats had higher percent accuracy than saline (9.5 s, $t=3.464$, $**p<0.01$), amphetamine (9.5 s, $t=4.247$, $**p<0.01$), and PAOPA (7.5 s, $t=3.198$, $*p<0.05$) treated rats at specific ITIs. Data represents mean \pm standard error of mean.

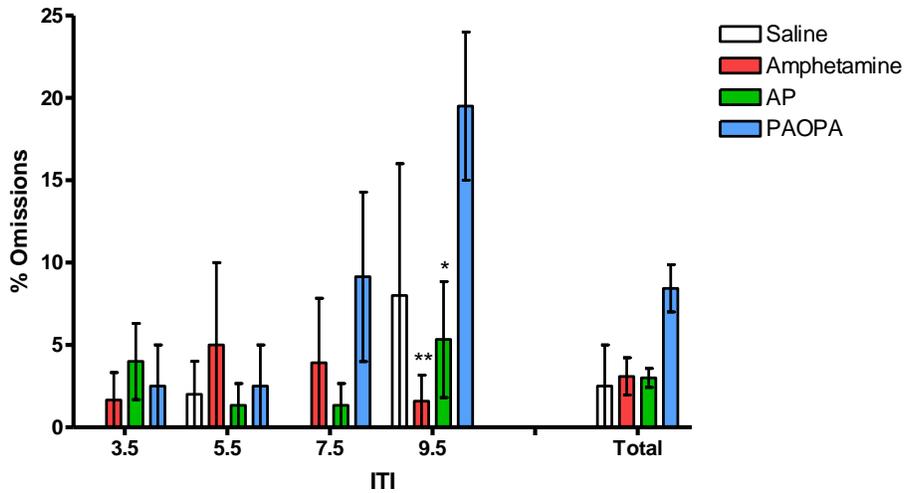


Figure 46. The effects of variable ITI on percent omissions. ITI was varied between 3.5, 5.5, 7.5, and 9.5 s within a single

session. There were no main effects of treatment, ITI, or subjects. Bonferroni post-tests revealed that PAOPA treated rats had significantly greater percent omissions than amphetamine ($t=4.113$, $**p<0.01$) and AP ($t=3.253$, $*p<0.05$) treated rats at an ITI of 9.5 s. Data represents mean \pm standard error of mean.

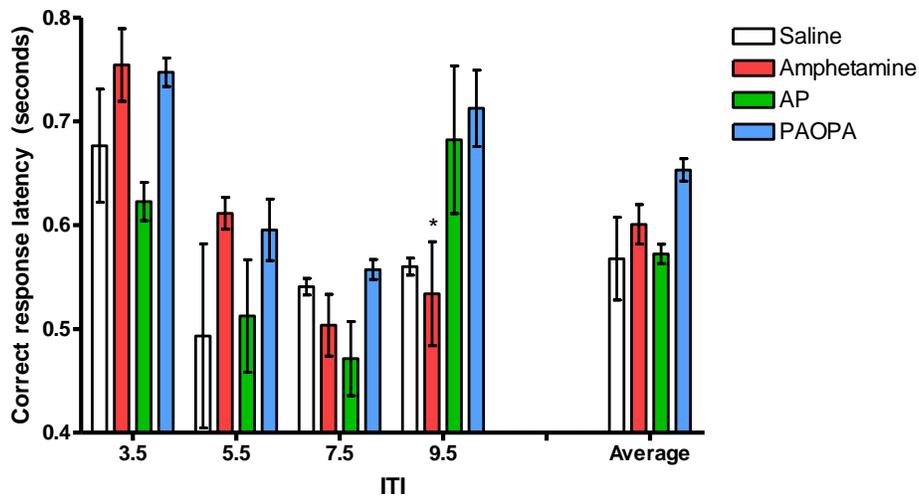


Figure 47. The effects of variable ITI on correct response latency. ITI was varied between 3.5, 5.5, 7.5, and 9.5 s within a single session. There was a main effect of ITI on premature responses ($p<0.0001$). Bonferroni post-tests revealed that amphetamine treated rats had reduced correct response latency compared to AP ($t=2.875$, $*p<0.05$) and PAOPA

($t=3.095$, $*p<0.05$) treated rats at a 9.5-s ITI. Data represents mean \pm standard error of mean.

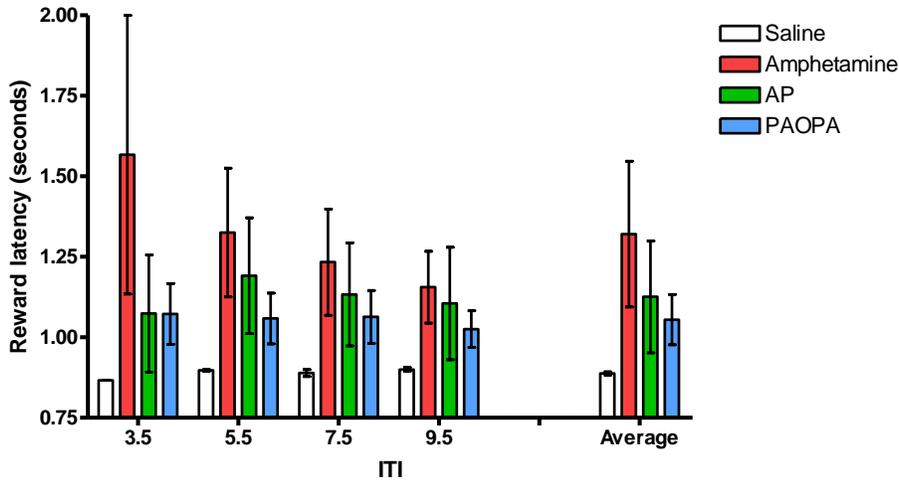


Figure 48. The effects of variable ITI on reward latency. ITI was varied between 3.5, 5.5, 7.5, and 9.5 s within a single session. There was a main effect of subjects ($p<0.0001$) on reward latency. Bonferroni post-tests did not reveal any significant differences between groups. Data represents mean \pm standard error of mean.

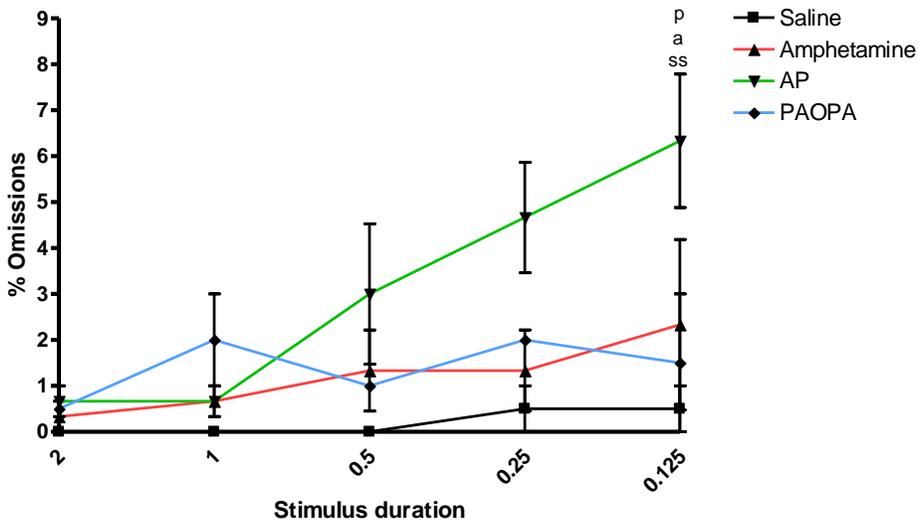


Figure 49. The effect of variable stimulus duration on percent omissions. Stimulus duration was varied within a single session (0.125, 0.25, 0.5, 1, and 2 s). There was a significant main effect of stimulus duration ($p=0.0092$) and subjects ($p=0.0496$) on percent omissions. Bonferroni post-tests revealed that AP rats had higher percent omissions than saline

($t=4.015$, $^{ss}p<0.01$), amphetamine ($t=3.078$, $^ap<0.05$), and PAOPA ($t=3.327$, $^pp<0.05$) treated rats. Data represents mean \pm standard error of mean.

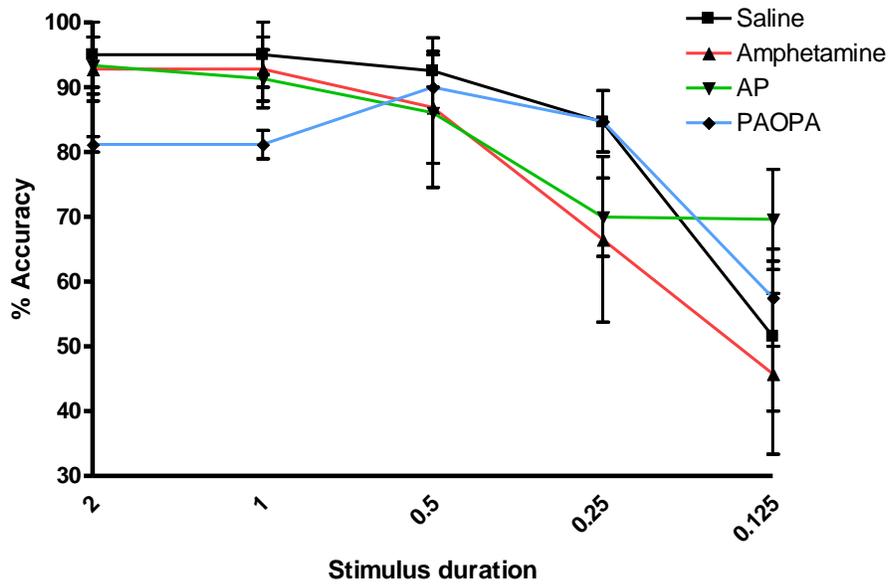


Figure 50. The effect of variable stimulus duration on percent accuracy, 10 weeks after drug cessation. Stimulus duration was varied within a single session (0.125, 0.25, 0.5, 1, and 2 s). There was a main effect of subjects ($p=0.0027$), and stimulus duration ($p<0.0001$), which indicates that decreasing stimulus

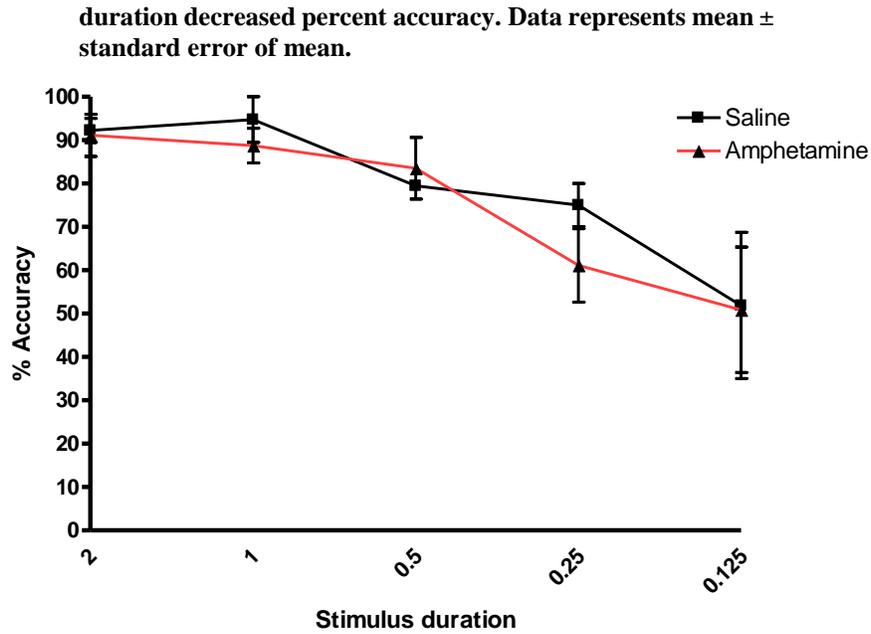


Figure 51. The effect of variable stimulus duration on percent accuracy, 13 weeks after drug cessation. Stimulus duration was varied within a single session (0.125, 0.25, 0.5, 1, and 2 s). There was a main effect of stimulus duration ($p=0.0007$), indicating that decreasing stimulus duration decreased percent accuracy. No main effect of treatment on percent accuracy was observed. Data represents mean \pm standard error of mean.

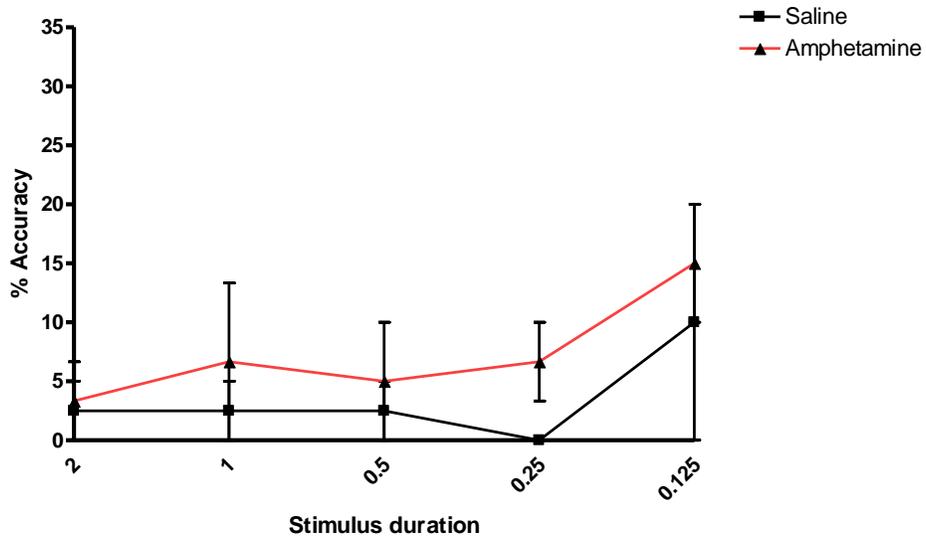


Figure 52. The effects of variable stimulus duration on percent omissions, 13 weeks after drug cessation. Stimulus duration

was varied within a single session (0.125, 0.25, 0.5, 1, and 2 s). There were no main effects of treatment or stimulus duration—only subjects ($p=0.0166$) on percent omissions. Data represents mean \pm standard error of mean.

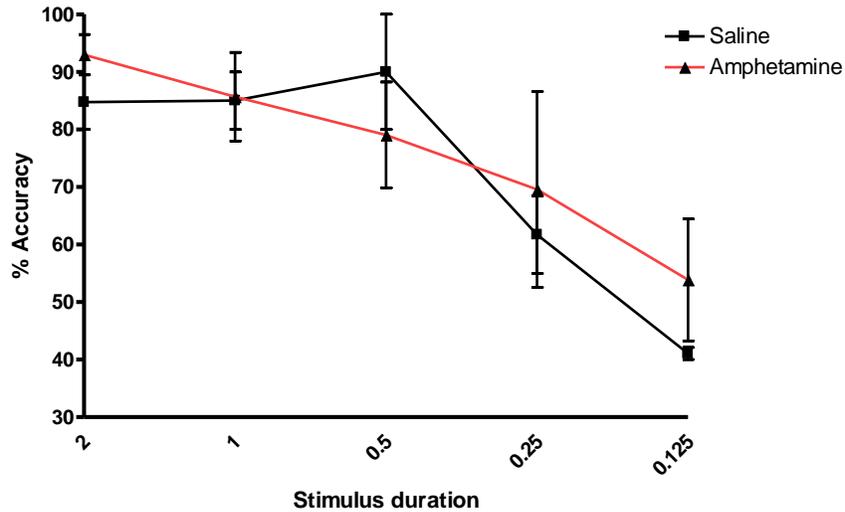


Figure 53. The effects of variable stimulus duration on percent accuracy, 14 weeks after drug cessation. Stimulus duration was varied within a single session (0.125, 0.25, 0.5, 1, and 2 s). There was a main effect of stimulus duration ($p=0.0005$)—indicating that decreasing stimulus duration decrease percent accuracy—and subjects ($p=0.0121$). Data represents mean \pm standard error of mean.

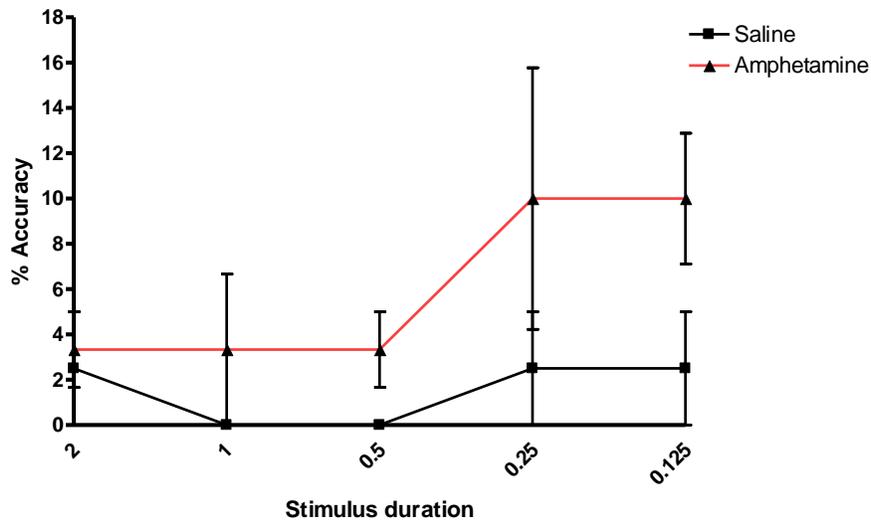


Figure 54. The effect of variable stimulus duration on percent omissions, 14 weeks after drug cessation. Stimulus duration

was varied within a single session (0.125, 0.25, 0.5, 1, and 2 s). There were no main effects of treatment or stimulus duration on percent omissions. Data represents mean \pm standard error of mean.

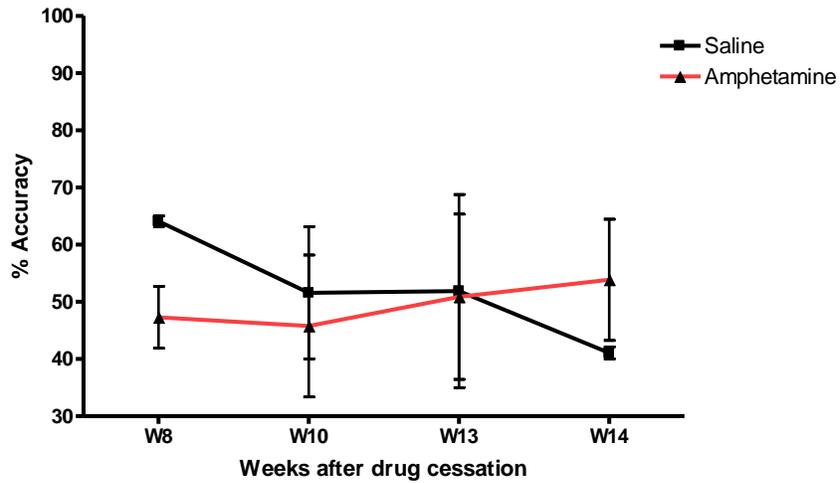


Figure 55. The duration of amphetamine-induced impairments in percent accuracy. Shown above are percent accuracies on 0.125 s trials across the four variable stimulus duration tests performed 8, 10, 13, and 14 weeks after drug cessation. There were no significant main effects of time, nor of treatment, on percent accuracy. This would suggest that percent accuracy did not change with subsequent variable stimulus duration testing. Data represents mean \pm standard error of mean.

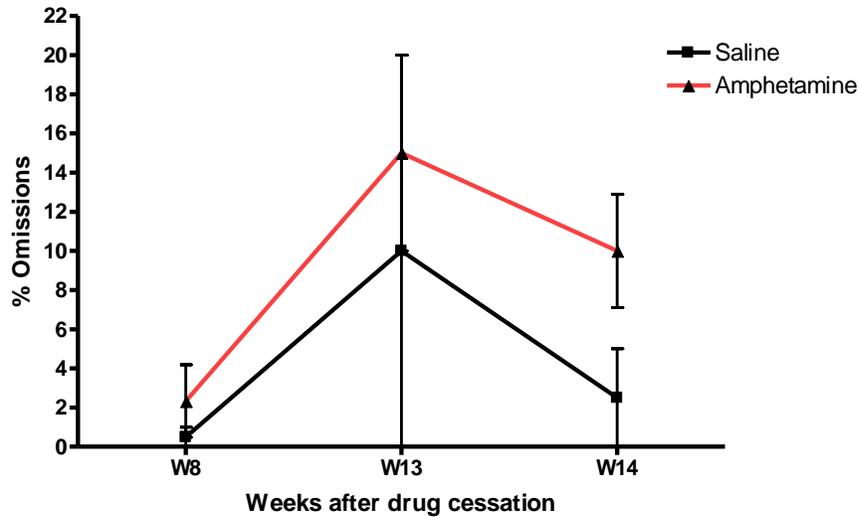


Figure 56. The duration of amphetamine-induced impairments in percent omissions. There was a significant

main effect of time ($p=0.0400$), indicating that percent omissions tended to increase with subsequent variable stimulus duration sessions. Data represents mean \pm standard error of mean.

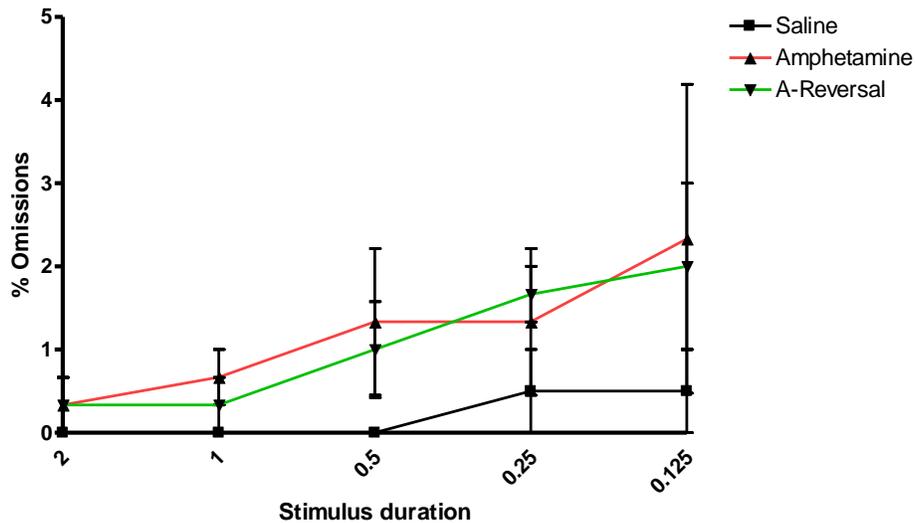


Figure 57. The effect of a one-time PAOPA administration on amphetamine-induced impairments in percent omissions. 1 mg/kg PAOPA was administered i.p. to amphetamine-treated rats (A-Reversal, green line). Testing was performed 9 weeks after drug cessation. The performance was compared to the previous performance, which took place 8 weeks after drug cessation, of amphetamine (red line) and saline (black line) treated rats. The stimulus duration was varied within a single session (2, 1, 0.5, 0.25, and 0.125 s). There were no main effects of treatment or stimulus duration. There was a significant main effect of subjects ($p=0.0230$) on percent omissions. Bonferroni post-tests did not reveal any significant differences

between treatment groups. Data represents mean \pm standard error of mean.

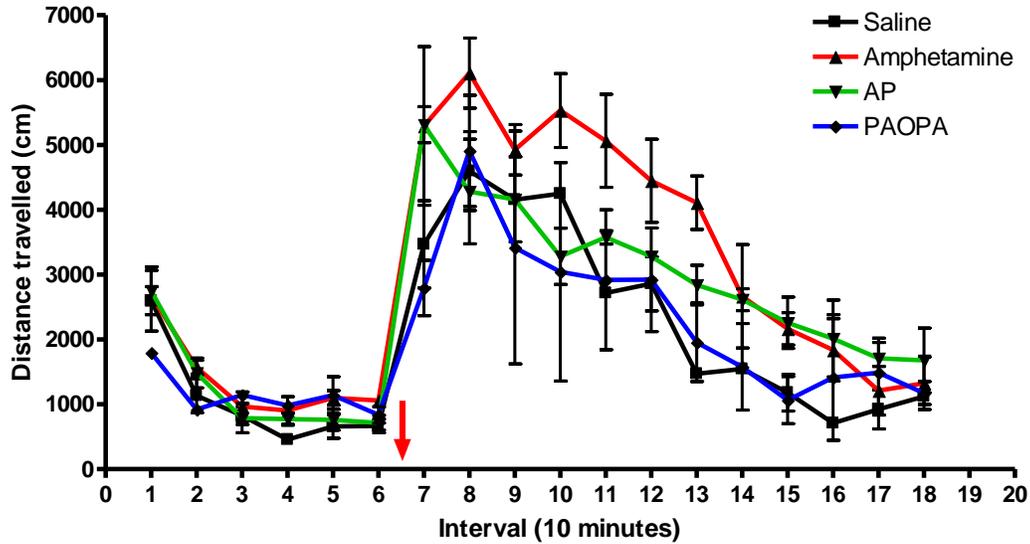


Figure 58. Confirmation of amphetamine sensitization, 6 weeks after drug cessation. Illustrated above is locomotor activity during recording of the first test of confirmation. Locomotor activity was recorded for 1 hour for baseline values. A 1 mg/kg amphetamine injection was given i.p. and locomotor activity recorded for two more hours. Red arrow indicates 1 mg/kg amphetamine injection. Data represents mean \pm standard error of mean.

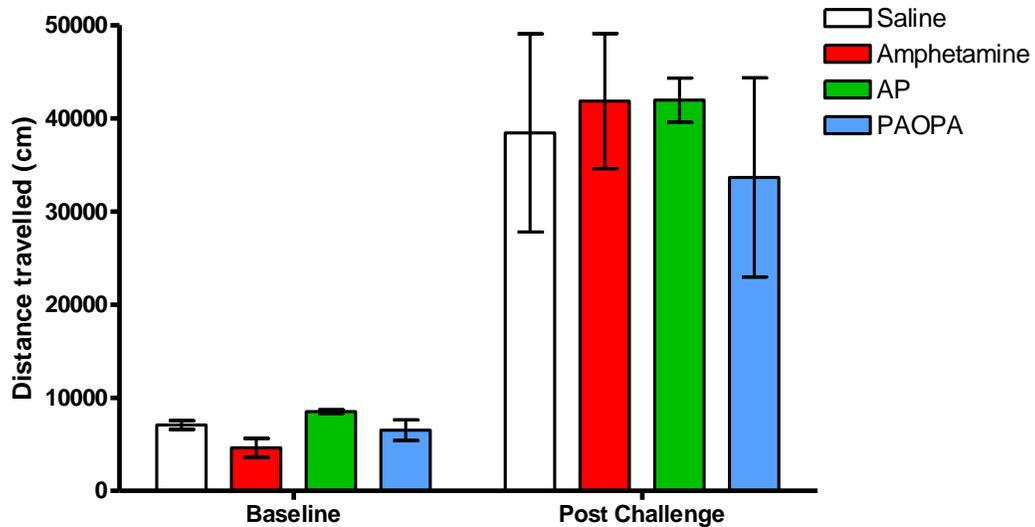


Figure 59. Confirmation of amphetamine sensitization, 14 weeks after drug cessation. Baseline activity was recorded for

one hour prior to a 1 mg/kg amphetamine i.p. injection, which was followed by two hours of recording. Shown above is the second test of confirmation. There was no significant main effect of treatment ($p=0.8303$) on distance travelled. There was a main effect of amphetamine challenge ($p=0.0001$), indicating that the amphetamine challenge increased the locomotor activity of all rats. Data represents mean \pm standard error of mean.

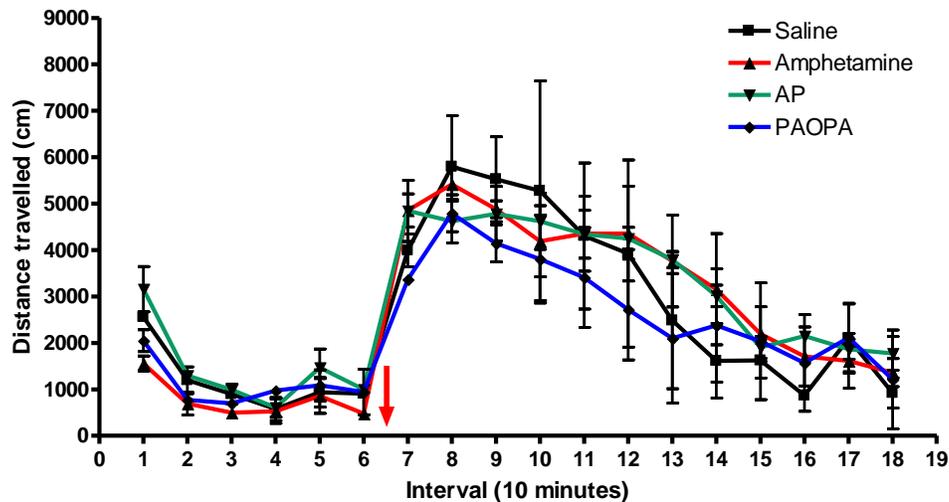


Figure 60. Confirmation of amphetamine sensitization, 14 weeks after drug cessation. Illustrated above is locomotor activity during recording of the second test of confirmation. Locomotor activity was recorded for 1 hour for baseline values. A 1 mg/kg amphetamine injection was given i.p. and locomotor activity recorded for two more hours. Red arrow

indicates 1 mg/kg amphetamine injection. Data represents mean \pm standard error of mean.

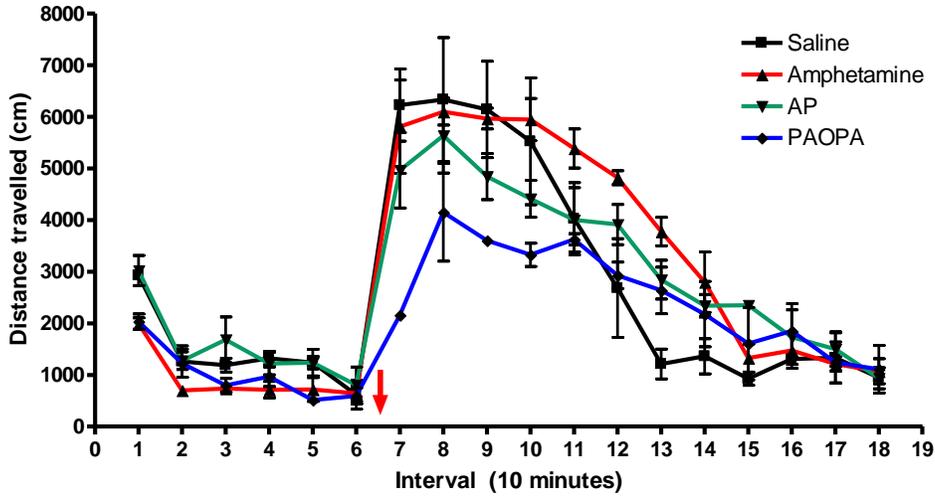


Figure 61. Confirmation of amphetamine sensitization, 14 weeks after drug cessation. Illustrated above is locomotor activity during recording of the third test of confirmation. Locomotor activity was recorded for 1 hour for baseline values. A 1 mg/kg amphetamine injection was given i.p. and locomotor activity recorded for two more hours. Red arrow indicates 1 mg/kg amphetamine injection. Data represents mean \pm standard error of mean.

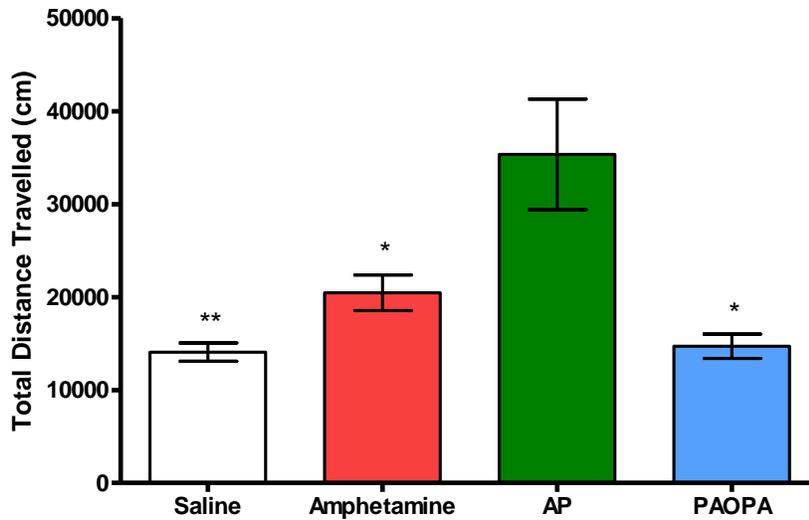


Figure 62. Locomotor activity after final drug administration. After the final drug administration, rats were recorded for

three hours for locomotor activity. Shown above is total distance travelled. One-way ANOVA revealed a significant difference between treatment groups ($F=7.960$, $p=0.0004$). Tukey's multiple comparison test revealed that only the AP treatment group differed from all others (saline, $**p<0.01$; amphetamine, $*p<0.05$; PAOPA, $*p<0.05$). Data represents mean \pm standard error of mean.

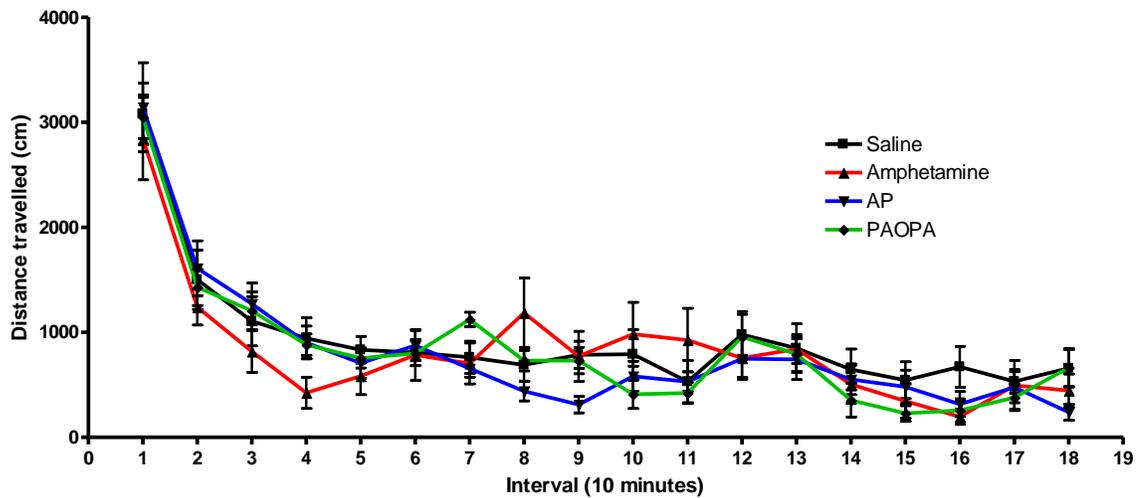


Figure 63. Locomotor activity in the first withdrawal week. In the first week of withdrawal, locomotor activity was recorded

over three hours. Data represents mean \pm standard error of mean.

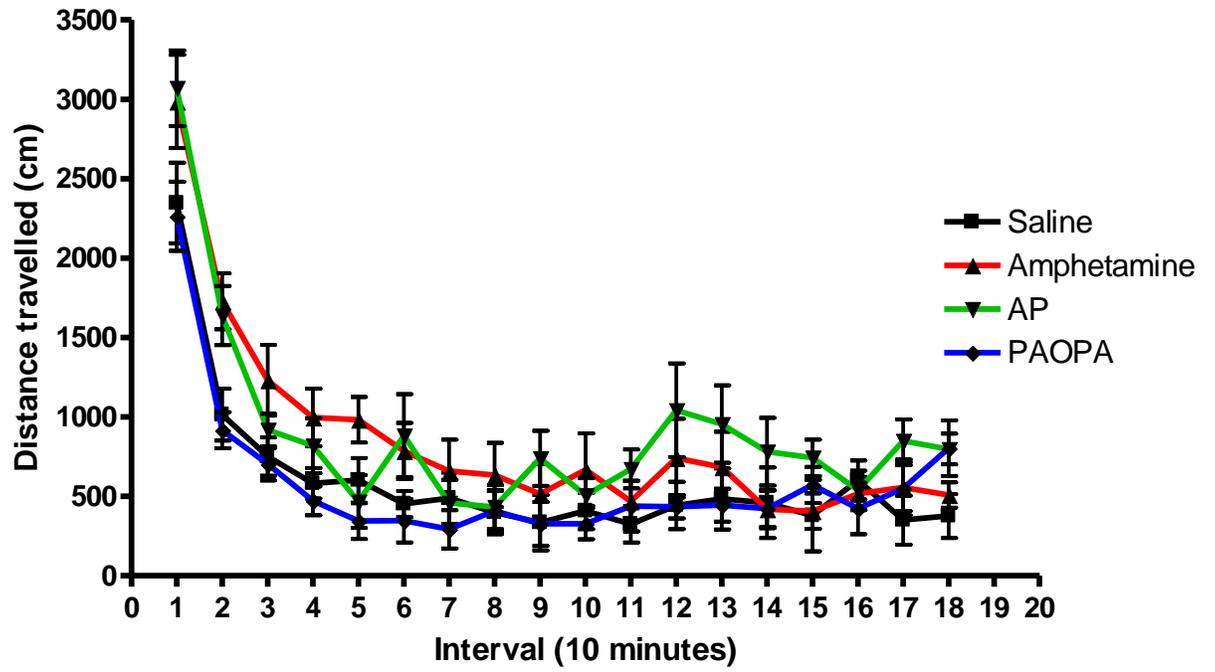


Figure 64. Locomotor activity five weeks after withdrawal. After five weeks of withdrawal, locomotor activity was

recorded for three hours without any amphetamine challenge.
 Data represents mean \pm standard error of mean.

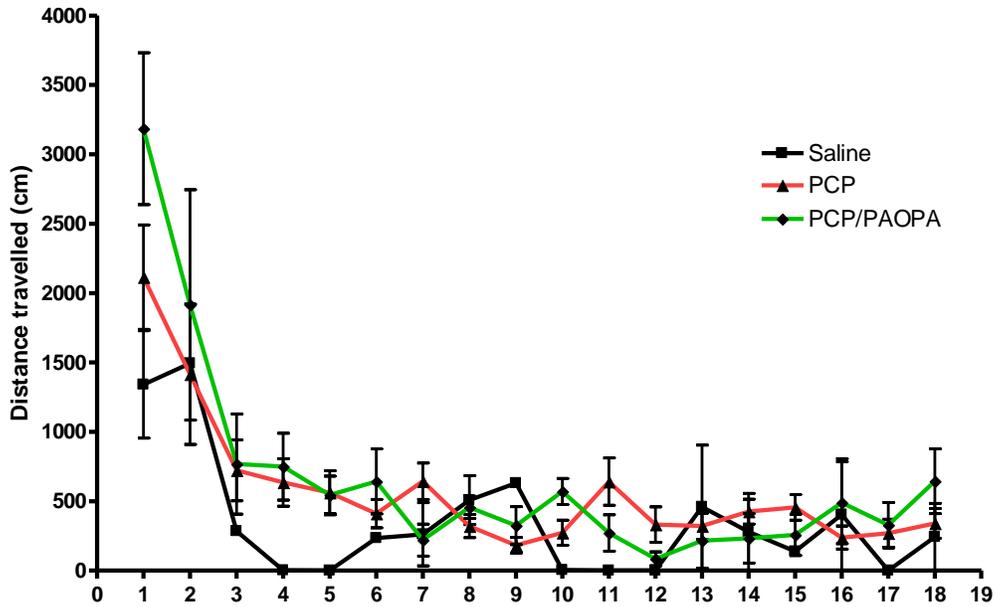


Figure 65. Interval locomotor activity after the first injection. Rats were injected with either saline (n=2), PCP (n=10), or PCP and PAOPA (n=4) and immediately recorded for locomotor activity for 3 hours. Data represents mean \pm standard error of mean.