View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by KnowledgeBank at OSU

α7 Nicotinic Receptor Modulation Alters Glutamate Release: Implications for Cognitive Deficits in Schizophrenia

Undergraduate Research Thesis

Presented in partial fulfillment of the requirements for graduation with honors research distinction in the undergraduate colleges of The Ohio State University

By

Brian A. Upton

The Ohio State University
April 2015

Project Advisor: Professor John P. Bruno, Department of Neuroscience

Contents

List of Figures 5

Abstract 7

Introduction 9

Research Overview 17

- Mesolimbic Stimulation Assay 17
- Aims 17
- Hypothesis 18
- Experimental Design 18

Methods 19

- Microelectrode Array: Second-by-Second Measurements of Glutamate 19
- Sensor Calibration: *in vitro* 20
- Experimental Subjects 21
- Implantation of Microelectrode and Infusion Cannulae 21
- *In vivo* Recordings and Drug Administration 22
- Histology 22
- Data Analysis 23

Results 25

- AVL-3288 Results 25
  - Effects of AVL-3288 on basal glutamate 25
  - Effects of AVL-3288 on evoked glutamate 25
- PNU-120596 Results 27
  - Effects of PNU-120596 on basal glutamate 27
  - Effects of PNU-120596 on evoked glutamate 28

Discussion 31

References 41

Figures 51

List of Figures

Figure 1: enzyme detection scheme. 51

Figure 2: calibration of the MEA. 51

Figure 3: sample placement of MEA and cannula. 53

Figure 4: sample tracing of low-dose NMDA after vehicle and low-dose AVL-3288 55

Figure 5: sample tracing of high-dose NMDA after vehicle and high-dose AVL-3288 55

Figure 6: AVL-3288 Group Data 57

Figure 7: sample tracing of low-dose NMDA after vehicle and high-dose PNU-120596 59

Figure 8: PNU-120596 Group Data. 59

Abstract

Cognitive deficits in schizophrenia are thought to be caused, in part, by disrupted prefrontal cholinergic and glutamatergic transmission. Activation of the α7 nicotinic acetylcholine receptor (α7nAChR) has been shown to increase prefrontal glutamate as well as rescue failing performance in cognitive tasks in rodents and primates. Intra-accumbens stimulation with NMDA dose-dependently increases prefrontal acetylcholine, which in turn increases prefrontal glutamate via α7nAChR activity. Using NMDA stimulation as an assay to examine the potentiation of glutamate release as a function of the amount of acetylcholine released in the PFC, the potentiation profiles of two α7nAChR positive allosteric modulators (PAMs), AVL-3288 and PNU-120596, were assessed at varying levels of PAM and stimulation. Second-by-second measurements with a glutamate-sensitive microelectrode in the PFC in awake rats reveal that only an appropriate combination of the dose of NMDA and dose of PAM consistently potentiates prefrontal glutamate release. However, at other concentrations of NMDA and PAM, the effect on mesolimbic stimulation varied greatly from significant potentiation to a reduction of glutamate release. Furthermore, the potentiation profile for the type I and type II PAMs differed significantly, possibly due to differences in receptor desensitization in the presence of these two drugs. Overall, these results demonstrate the importance of dose of α7nAChR PAMs in modulating neurotransmitter systems, and PAMs as a potential therapeutic in treating cognitive deficits of schizophrenia as effects were dependent on activity of the orthosteric ligand.

Introduction

Schizophrenia is a chronic brain disorder disrupting thought, perception, and behavior. Schizophrenia is prevalent in roughly 1% of the population and has an economic burden exceeding $62 billion/year in the United States alone (Perala et al., 2007; Wu et al., 2005). Diagnosis typically occurs after the first psychotic episode, occurring in late adolescence to early adulthood in men and throughout early adulthood in women, although prodromal symptoms are often present before this episode (Faraone et al. 1994; White et al., 2006). While the age of the first psychotic episode varies between men and women, both are affected in equal numbers (Saha et al., 2005). Not only does schizophrenia affect equal numbers across sex, culture, and socioeconomic status, the symptoms of the disorder also appear to be conserved across these demographic factors as well (Saha et al., 2006; Karagianis et al., 2009).This similar presentation is not to say that schizophrenia is a homogeneous disorder, but rather that schizophrenia is a worldwide problem with significant psychosocial and economic impact.

Schizophrenia is a heterogeneous disorder composed of symptoms from three symptom clusters: positive, negative, and cognitive symptoms. Positive symptoms include features of the disease that are present in patients that are not present in the general population. These symptoms include hallucinations, delusions, and disorganized thought and speech (American Psychiatric Association, 2013). Similarly, negative symptoms of schizophrenia include features that are absent in schizophrenia but present in the general population, including flattened affect, avolition, and anhedonia (American Psychiatric Association, 2013). Lastly, schizophrenia presents with cognitive deficits, or deficits in thought processes (Kerns et al., 2008). Aspects of executive functioning are impaired in schizophrenia, particularly in working memory, cognitive flexibility, and attentional processing. Current antipsychotic medications are effective at addressing some, but not all, of the symptoms associated with schizophrenia.

The first antipsychotic, chlorpromazine (trade name Thorazine), was originally developed as an anesthetic agent, yet successfully reduced many of the positive symptoms associated with schizophrenia (Ban, 2007). The success of chlorpromazine led to the development of similar drugs, known as first generation, or typical, antipsychotics. This class of antipsychotic medication exerts its antipsychotic effects as D2 antagonists (Kapur & Mamo, 2003). Additionally, these medications have activity on the cholinergic, histaminergic, and adrenergic systems, which caused a variety of side effects such as dry mouth, weight gain, and sedation. The most debilitating and possibly permanent side effects of typical antipsychotics are extrapyramidal side effects, which are caused by high D2 occupancy leading to a dopamine hypofunction in extrapyramidal pathways, similar to what is seen in Parkinson’s disease (Tuppurainen et al., 2010). Extrapyramidal side effects involve movement difficulties, including dystonia, tremors, akathisia, bradykinesia, and tardive dyskinesia. To reduce these side effects, second generation, or atypical, antipsychotics were developed that bind more transiently to the D2 receptor, allowing for more endogenous dopamine binding, and additionally serve as 5-HT_{2A} antagonists producing antipsychotic effects (Seeman 2002). While atypical antipsychotics had a smaller side effect profile than typical antipsychotics, they were no more efficacious than some typical antipsychotics in their antipsychotic properties (Lieberman & Stroup 2011). Both classes of drugs are capable at reducing many of the positive and negative symptoms; however, neither sufficiently address cognitive symptoms and subsequently, cognitive symptoms remain untreated (Keefe, 2007a; 2007b).

Cognitive deficits of schizophrenia result in a poorer quality of life and are more debilitating to daily function than positive or negative symptoms (Swartz et al., 2007). Cognitive function is often the best predictor of long-term functional outcome in the treatment of schizophrenia (Green et al., 2000). Presumably, improving cognitive functioning in patients with schizophrenia will improve their treatment outcome and quality of life. Poor cognitive function may predict a poor prognosis because poor cognitive function is a barrier to employment and a risk factor for the discontinuation of treatment (Rosenheck et al., 2006; Robinson et al. 2002). In addition to a personal decline in quality of life, cognitive symptoms contribute to the social and economic burden of the disease; therefore, it is imperative to improve treatments aimed at ameliorating the cognitive deficits associated with schizophrenia.

Many aspects of cognition are mediated via a distributed neural network comprised of cortical and subcortical structures. The prefrontal cortex (PFC), serving as a source of top-down control, is important in all aspects of higher-order cognition (Miller, 2000). The PFC plays important roles in goal-directed behavior, personality expression, error detection, and problem solving. Disruptions in prefrontal activity can lead to deficits in tasks designed to measure working memory, cognitive flexibility and attentional processing (Demakis, 2004). It should serve as no surprise that the prefrontal cortices of individuals with schizophrenia appear disrupted. The brains of patients with schizophrenia display hypofrontality, or decreased prefrontal activity, both at rest and during cognitive task performance (Berman et al., 1992). Individuals with schizophrenia also display deficits in tasks dependent on prefrontal activity, such as the Stroop task and Wisconsin card sorting test (Barch et al., 2004; Haut et al., 1996). In longitudinal studies, patients who are later diagnosed with schizophrenia have reduced prefrontal gray matter at the time of their first episode (Hirayasu et al., 2001; Molina et al., 2006). Thus, prefrontal irregularities may be involved in the cognitive deficits present in schizophrenia as similar deficits are seen in both patients with schizophrenia as well as frontal lobe damage.

Glutamate, the primary excitatory neurotransmitter in the CNS, is important for cognitive performance. Disrupted prefrontal glutamate transmission can lead to impaired performance in a variety of cognitive tasks, including tasks designed to measure working memory, cognitive flexibility, attentional processing, and sensory gating (Umbrecht et al. 2000). Similarly, enhancing glutamate transmission in the PFC may lead to increased cognitive performance. Genetic risk factors in schizophrenia, including altered expression of catechol-o-methyl transferase (COMT), dysbindin, neuregulin, disrupted in schizophrenia 1 (DISC1), regulator of G-protein signaling 4 (RGS4), metabotropic glutamate receptor-3 (GRM3), and G72, have been linked to disruptions in glutamate transmission (Harrison & Weinberger 2004). Additionally, transcript expression for vesicular glutamate transporter-1 (VGLUT1) is down-regulated in the prefrontal cortex of individuals with schizophrenia, likely negatively affecting glutamate transport and release (Eastwood & Harrison, 2004). Furthermore, the activity of excitatory amino acid transporters (EAATs) appears disrupted in schizophrenia. Glial EAATs (i.e. EAAT1 and EAAT2) are down-regulated, possibly leading to an increase in autoreceptor activation and compensatory up-regulation of neuronal EAATs (i.e. EAAT3), which together, lead to a decrease in synaptic glutamate and increase in extrasynaptic glutamate (Bauer et al. 2008). Accordingly, group II metabotropic glutamate receptor expression is increased in the prefrontal cortex in schizophrenia (Gupta et al. 2005). Altered expression of the NMDA receptor, a glutamate receptor important in learning and memory, has been noted in the dorsolateral PFC and anterior cingulate cortex in schizophrenic brains (Kristiansen et al., 2006). Antagonism of the NMDAR is capable of producing psychotic symptoms in non-diseased individuals and exacerbates psychotic symptoms in schizophrenic individuals (Krystal et al. 1994; Lahti et al., 1995). Additionally, NMDA blockade produces cognitive deficits in humans and animals and is frequently used as an animal model for cognitive deficits in schizophrenia (Krystal et al., 1994; Blot et al., 2013). Taken together, disruptions of glutamate transmission in the PFC may lead to glutamatergic hypofunction and underlie cognitive deficits seen in the disorder; therefore, methods of enhancing prefrontal glutamate may have pro-cognitive effects and serve to ameliorate the cognitive deficits seen in schizophrenia.

Prefrontal glutamate can be modulated bi-directionally via the α7 nicotinic acetylcholine receptor (α7nAChR; Konradsson-Geuken et al., 2010). The α7nAChR is a homopentameric heteroreceptor, located pre- and postsynaptically, that gates cations (i.e. calcium, sodium, potassium)(Dajas-Bailador & Wonnacott, 2004). Calcium ion influx through the α7nAChR initiates a signal cascade known as the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway (also known as the mitogen-activated protein kinase (MAPK) pathway), which is a transduction mechanism that has implications for learning and memory (Gubbins et al., 2010). Thus, presynaptic α7nAChR activity enhances neurotransmitter release both by causing depolarization at the axon terminal as well as affecting gene expression. In this manner, acetylcholine and other direct agonists of the α7nAChR are able to enhance glutamate transmission, while antagonism of the α7nAChR decreases glutamate transmission (Bortz et al., 2013; Wu et al., 2010). Similarly, drugs that increase α7nAChR activity in the PFC have pro-cognitive effects in rodents and result in an increased performance in a variety of cognitive tasks, while drugs that reduce α7nAChR activity can produce cognitive deficits (Redrobe et al., 2009; Thomsen et al., 2009; Castner et al., 2011; Alexander et al., 2012). Therefore, increasing α7nAChR activity may serve as a target for cognitive-enhancing therapeutics in schizophrenia by enhancing several neurotransmitter systems, including glutamate.

In schizophrenia, α7nAChR activity appears disrupted. Single nucleotide polymorphisms in the promoter region of the CHRNA7 gene, the gene that encodes the α7 protein, are a risk factor in the development of schizophrenia (Leonard et al., 2002). In individuals who develop schizophrenia, a decrease in both α7 protein, and in some populations, decreases in mRNA expression have been reported (Guan et al., 1999; Matthew et al., 2007). Additionally, kynurenic acid, a tryptophan metabolite, is an endogenous antagonist to the α7nAChR and has been found to be elevated in cerebrospinal fluid and postmortem brains of patients with schizophrenia (Hilmas et al., 2001; Linderholm et al., 2012; Schwarcz et al., 2001). Given the importance of α7nAChR activity in cognition, decreased α7nAChR activity may contribute to the cognitive deficits and glutamate hypofunction seen in schizophrenia.

Nicotine, the primary drug found in tobacco products, is a direct agonist of the α7nAChR and has limited pro-cognitive effects. Interestingly, between 60 and 90% of patients with schizophrenia smoke cigarettes (Leonard et al., 2001). This staggering correlation has led some to believe that increased nicotine consumption is a form of self-medication to alleviate cognitive deficits that are not managed with current treatment regimens (Leonard et al., 2007). Unfortunately, nicotine is not an ideal therapeutic for treating cognitive deficits for a variety of reasons: the pro-cognitive effects of nicotine are short-lived, as the α7nAChR receptor is easily desensitized; nicotine is not selective to the α7nAChR, leading to many side effects; and many negative health effects of nicotine products (Papke et al. 2009). As a result, direct α7nAChR agonists have been developed which can reduce cognitive symptoms in animal models of schizophrenia (Hauser et al., 2009). Additionally, pro-cognitive effects α7nAChR agonists have been demonstrated in human populations (Olincy et al., 2006). The tendency for the α7nAChR receptor to desensitize has prompted investigation into low-dose agonists, which have shown benefits in animal models of cognitive deficits (Werkheiser et al., 2011). While selective α7nAChR agonists, particularly at low doses, may have advantages over the use of nicotine, they may still be limited to characteristics inherent to direct agonists.

There are two primary fundamental limitations of direct agonists as pharmacotherapeutics. First, direct agonists bind to the receptor and cause a response when present in a system. This indiscriminant activation occurs with or without afferent activity, leading to the tendency to cause false alarms at the receptor. Second, direct agonist activity does not preserve the temporal integrity of endogenous neurotransmission, which may be important to many neural processes underlying cognitive performance. In other words, neural connections lose the degree-of-freedom afforded by the ability to selectively activate and deactivate the receptor as is biologically-relevant. Instead, direct agonists reduce this complexity to an “always on” signal while the agonist is present in the brain. Due to the disruptions of native spatial and temporal signaling caused by direct agonism, a more subtle method of enhancing receptor activity is needed that maintains the complexity of the neural system.

Positive allosteric modulators (PAMs) are chemicals that enhance receptor activity in the presence of an orthosteric ligand, while in the absence of an orthosteric ligand, have no intrinsic receptor activity. Unlike orthosteric agonists, which bind to the same location as the endogenous neurotransmitter, positive allosteric modulators bind at a different site, termed allosteric site. Binding at the allosteric site allows PAMs to alter the receptors configuration and, therefore, activity at the orthosteric site. In this manner, PAMs are able to enhance receptor activity in the presence of an orthosteric ligand; however, binding to the allosteric site in the absence of orthosteric activity does not result in a receptor response. In the case of α7nAChRs, PAMs do not cause cation influx in the absence of orthosteric activity.

α7nAChR PAMs are now being investigated as pro-cognitive therapeutics with potential in ameliorating deficits in diseases such as schizophrenia. Several α7nAChR PAMs have been used either alone or in conjunction with drugs that increase α7nAChR orthosteric activity to ameliorate cognitive deficits and sensory gating deficits in several animal models of schizophrenia (Hurst et al., 2005; Ng et al., 2007; Thomsen et al., 2011; McLean et al., 2012; Callahan et al., 2013). Additionally, α7nAChR PAMs have been shown to potentiate evoked excitatory amino acid release *in vitro* and evoked prefrontal dopamine release *in vivo* (Zwart et al., 2002; Livingstone et al., 2010). α7nAChR PAMs fall into two categories: type I and type II. The PAM types differ in that type I PAMs affect peak current, whereas type II PAMs affect both peak current and current decay (Grønlien et al., 2007). That is, both types increase the peak amount of cation influx, but while type I PAMs allow the receptor to naturally desensitize into a closed configuration, type II PAMs prevent the receptor from entering this desensitized state and allow the receptor to remain open with continued cation influx (Williams et al., 2011a). Furthermore, only type II PAMs have been shown to reactivate desensitized α7nAChRs (Hurst et al., 2005; Grønlien et al., 2007). While interest has been growing in the use of α7 PAMs for a variety of neurological disorders, limited research has been published characterizing effects on neurotransmitter systems *in vivo*.

Research Overview

Mesolimbic Stimulation Assay

In order to investigate the effect of PAMs, the drug must be applied in the presence of the neurotransmitter that is modulated, as PAMs have no effect on receptor activity in the absence of orthosteric activity. Both task- and chemical-induced activity in the nucleus accumbens (NAc) have been previously shown to cause increases in both prefrontal cholinergic and glutamatergic activity (Neigh et al., 2004; Zmarowski et al., 2007; Bortz et al., 2014). Similarly, NMDA infusion into the anterior shell of the nucleus accumbens dose-dependently increases prefrontal choline, a selective α7nAChR agonist, and glutamate (Alkondon et al., 1997; Bortz et al., unpublished data). NMDA-evoked glutamate release is dependent on α7nAChR activity, as antagonism of the α7nAChR abolishes this evoked release (Bortz et al., unpublished data). Therefore, differing concentrations of NMDA infused into the accumbens cause different concentrations of acetylcholine to be released in the prefrontal cortex. These varying concentrations of acetylcholine activate the α7nAChR, facilitating glutamate release dose-dependently as a function of the concentration of acetylcholine. Thus, with this assay, the effects of α7nAChR PAM can be assessed in the presence of endogenously released acetylcholine without disrupting the integrity of cholinergic transmission in the PFC. Additionally, this assay is a model of top-down cognitive processing and is capable of rescuing failing performance during the distractor portion of sustained attention tasks (St. Peters et al., 2011).

Aims

The goal of this experiment is to assess the ability of different doses of both type I and type II α7nAChR PAMs, AVL-3288 and PNU-120596 respectively, in potentiating glutamate release *in vivo* as a function of choline release in awake, freely moving rats.

Hypothesis

If cholinergic activity is required for PAMs to enhance activity at the orthosteric site of the α7nAChR, then each PAM will potentiate prefrontal glutamate release as a result of mesolimbic stimulation, but not under basal conditions.

Experimental Design

Animals were tested on three consecutive test days, receiving the same concentration of NMDA stimulation on each day. Prior to NMDA stimulation, animals were systemically injected with either AVL-3288 or PNU-120596. Dose of PAM was manipulated between test days so that the effect of either high-dose PAM, low-dose PAM, or vehicle on a constant level of stimulation could be measured. Each subject only received one type of PAM for experiments. The effects of each dose of PAM were compared to the glutamate release on the vehicle test day. Using this set-up, an increase or decrease in glutamate release from the vehicle test day in the presence of PAM could be observed as a potentiation or attenuation, respectively, within subject.

Methods

Microelectrode Array: Second-by-Second Measurements of Glutamate

Self-referencing, ceramic-based, multisite microelectrode arrays (MEA) were used for the detection of *in vivo* glutamate release in awake, freely-moving rats (Quanteon, LLC, Nicholasville, KY)(see figure 1). Each MEA consists of two pairs of platinum recording channels (15 x 333µm each) separated 100µm vertically and 30µm horizontally. The highly-selective enzyme, glutamate oxidase (GluOx, 2%, 1 unit/µL, 100nL) was cross-linked to the first pair of recording channels using glutaraldehyde (0.125%) and bovine serum albumin (BSA; 1%). GluOx oxidizes glutamate, producing α-ketoglutarate, ammonia, and hydrogen peroxide (H_2O_2). H_2O_2, which is electrically active at the applied voltage, serves as the reporting molecule for glutamate. H_2O_2 is oxidized at the platinum recording site due to an electric potential of +0.7V relative to a Ag|AgCl reference electrode, placed distant from the recording site. The oxidation of H_2O_2 produces a current, which is amplified and recorded using the FAST-16 mkII recording system (Quanteon, LLC, Nicholasville, KY). This amperometric design allows for sub-second temporal resolution of the MEA. By calibrating each MEA *in vitro* prior to implantation, the *in vivo* concentration of glutamate can be derived from the recorded current. The second pair of recording channels, coated in only glutaraldehye and BSA but lack GluOX, is not glutamate-sensitive and serves as a background, or sentinel, channel, recording signals that are not glutamate-driven. The glutamate-sensitive channels are self-referenced with the sentinel channels, isolating the signal derived exclusively from glutamate (Burmeister & Gerhardt, 2001; Day et al., 2006; Rutherford et al., 2007). In order to minimize the signal from oxidizable interferents, such as dopamine or ascorbic acid, m-phenylenediamine dihydrochloride (mPD) was electroplated onto the recording sites prior to sensor calibration and implantation. mPD serves as a physical barrier, preventing larger molecules, such as catecholamines and ascorbic acid, from interacting with the recording site, while smaller molecules, such as H_2O_2, are not excluded (Wahono et al., 2012). The exclusion barrier in conjunction with the self-referencing technique allow each MEA to be highly selective for glutamate with a limit of detection less than 0.5µM. MEAs can remain selective and sensitive to glutamate for up to seven days of chronic recording (Rutherford et al., 2007).

Sensor Calibration: *in vitro*

Immediately prior to sensor implantation, the microelectrodes were calibrated *in vitro* in a solution of PBS (0.05 M, 40 mL; pH 7.4; 37°C; +0.7 V). After baseline was established, channel signals were recorded at a rate of 1 Hz following the addition of ascorbic acid (250 µM), glutamate (3 x 20 µM), dopamine (2 mM), and hydrogen peroxide (8.8 µM) (see fig. 2). The effectiveness of the mPD barrier was assessed by observing no change in measurement on any of the four channels during the addition of ascorbic acid or dopamine, both of which can be found in high concentrations *in vivo* and are electrically active at the sensor's applied voltage (Lowry & O'Neill, 1992). When glutamate was added, an increase in current was observed on the two GluOx channels. The slope (nA/µM glutamate) was determined from these three glutamate additions in order to relate the measured current to the glutamate concentration when used *in vivo*. The final addition of hydrogen peroxide created a current observable on all four channels to ensure all channels are equally sensitive to the reporting molecule. In order to qualify for use, every sensor must have met the following criteria: (i) no difference greater than 20 pA during background current between all four channels, (ii) linear response to increase in glutamate concentration with a coefficient of determination (r^2) value greater than 0.998, (iii) high sensitivity, determined by a slope greater than -0.003 nA/µM glutamate, (iv) a limit of detection

<0.5 µM, (v) high selectivity of glutamate to ascorbic acid and dopamine (>50:1), and (vi) greater than 80% but less than 125% similarity to hydrogen peroxide for channel pairings.

Experimental Subjects

All measurements were done in adult male Wistar rats (body weight 280-420 g; Charles River Laboratory: Wilmington, MA). Animals were housed in individual plastic cages with corn cob bedding (Harlan Teklad, Madison, WI) in a temperature (72°) and humidity (30-70%) controlled room on a 12:12 light:dark cycle. All animals had access to food and water *ad libitum*. All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals. As such, all efforts were made to minimize animal suffering, to reduce the number of animals used, and to consider alternatives to *in vivo* techniques.

Implantation of Microelectrode and Infusion Cannulae

Animals were anesthetized using isoflorane gas (2%, 0.6 L/min) and positioned with a stereotax. An MEA was placed unilaterally between infralimbic and prelimbic cortices of the medial prefrontal cortex (in mm from bregma: AP +2.7, ML ±0.65, DV -4.0; hemispheres counterbalanced; see fig. 3A and 3B). A steel guide cannula used for intracoritcal infusions of NMDA was placed in the anterior shell of the nucleus accumbens, ipsilateral to the MEA (in mm from bregma: AP +0.4, ML ±0.70, DV -6.4; see fig. 3C and 3D). The infusion cannula extended 1.0 mm farther than the guide cannula and when not in place, a dummy cannula was inserted, extending 0.7 mm past the tip of the guide cannula. The Ag|AgCl reference electrode was placed in the contralateral parietal lobe, distant from the MEA. The atlas of Paxinos and Watson (1998) was used to determine all coordinates. The sensor was secured using three stainless steel screws mounted to the skull and dental acrylic (DuraLay Inlay Pattern Resin). Animals were not tested until the second day after surgery to allow time to recover.

In vivo Recordings and Drug Administration

The day following implantation surgery, the rats habituated to their testing environment, a wooden test box (16" width, 16" length, 24" height) with corn cob bedding inside a Faraday cage, but were not connected to the sensor's preamplifier. On the following days, the animals were placed into the testing box and connected to the preamplifier and a baseline recording was established for a minimum of two hours before the infusion of any drugs. Prior to NMDA infusion, rats were pretreated with an intraperitoneal injection of AVL-3288 (1 or 3 mg/kg) or PNU-120596 (3 or 9 mg/kg) or vehicle (dimethyl sulfoxide (DMSO), 5% by volume; Solutol®, 8% by weight). Thirty minutes after PAM pretreatment, artificial cerebrospinal fluid (aCSF; pH 7.1-7.4) was infused into the NAc to serve as a positive control as well as to clear the cannula and guide cannula prior to drug administration. Ten minutes after aCSF infusion, NMDA (0.05 or 0.30 µg/0.5 µL aCSF) was infused into the NAc. A Hamilton PB600-1 manual dispenser was used to deliver aCSF and NMDA through the cannula over two seconds. Dose of PAM was manipulated within subjects between test days, while the dose of NMDA was consistent between test days and was only manipulated between subjects. The dose order of PAMs was counterbalanced among animals.

Histology

All MEA and cannula placement was verified post mortem. Following the third test day, rats were given an overdose of pentobarbital sodium and transcardially perfused with heparinized saline (0.2%) followed by formalin (10%). Brains were removed and were placed in formalin for a minimum of three days at 4°C. One day prior to sectioning, the brains were moved to a sucrose solution (30%) and maintained at 4°C. The sucrose solution serves as a cryoprotectant to prevent artifacts as a result of freezing the tissue samples. Brains were sectioned into coronal slices (50µm) using a cryostat (-25°C), mounted on gelatin-coated slides, and stained using cresyl violet. Slices were examined under a light microscope to determine sensor and cannula placement. If MEA placement into the mPFC or cannula placement into the anterior NAcSh could not be verified, the data from the animal was excluded from analysis (see figure 3 for sample MEA and cannula placement).

Data Analysis

Measurements derived from the MEA recording and the FAST-16 data file included: (i) basal glutamate levels, (ii) maximum peak concentration (uM) of glutamate, (iii) the latency (second) of onset of the effect from the time of drug infusion, and (iv) T_{80}, the time in seconds from maximum peak amplitude to 80% decay of signal (a measure of glutamate clearance). The signal derived exclusively by the oxidation of glutamate was isolated using a self-referencing procedure between the glutamate-sensitive recording channel and the adjacent background sentinel channel (Burmeister and Gerhardt, 2001; Day et al., 2006; Rutherford et al., 2007). All dependent measures will be analyzed by analysis of variance (ANOVA) using the SPSS 18 statistics program (V19, IBM Corporations, Armonk, NY). In all ANOVAs, the Huynh-Feldt correction will be utilized to reduce type I errors associated with repeated measure ANOVAs (Vasey and Thayer, 1987). Statistical significance was defined as $P < 0.05$.

Results

AVL-3288 Results

Effects of AVL-3288 on basal glutamate

Basal glutamate, measured after IP injection but before aCSF infusion, did not vary between AVL-3288 dose ($F_{2,28}$=3.134, p=0.068) or NMDA dose ($F_{1,9}$=1.031, p=0.395) and there was no interaction between the dose combinations ($F_{4,18}$=0.321, p=0.860). These results indicate that systemic injection of AVL-3288 does not affect basal glutamate release, i.e. in the absence of stimulation.

Effects of AVL-3288 on evoked glutamate

The type I α7nAChR PAM, AVL-3288, was injected systemically 40 minutes prior to mesolimbic-stimulation with NMDA. The dose of NMDA (0.05µg and 0.30µg) was held constant across test days and was administered in the presence of vehicle, low-dose, and high-dose of AVL-3288. With this setup, effects of dose of AVL-3288 will be presented as a percent change from the vehicle test day. In this manner, a positive percent change signifies that more glutamate was released in the presence of AVL-3288 with the same level of stimulation (i.e. concentration of NMDA), while a negative percent change signifies that less glutamate was released in the presence of AVL-3288 than on the vehicle-treated day.

Two-way (AVL dose x NMDA dose) omnibus ANOVA revealed a significant effect on the percent change in glutamate release between AVL-3288 dose ($F_{2,28}$=9.128, p=0.001) and an AVL dose-NMDA dose interaction ($F_{4,28}$=5.008, p=0.04). Subsequent one-way ANOVA on dose of AVL-3288 (vehicle, 1 mg/kg, 3 mg/kg) revealed a significant difference between AVL doses within the 0.30 NMDA group ($F_{2,14}$=11.873, p=0.001), but not within the 0.05 NMDA group ($F_{2,17}$=0.494, p=0.620). Within the high-dose of NMDA group, percent change in glutamate release was significantly potentiated by 1 mg/kg AVL-3288 compared to the vehicle response ($F_{1,9}$=5.932, p=0.41) with an average percent change of 84.70 ± 34.78%. Conversely, the percent change in glutamate release was significantly attenuated by 3 mg/kg AVL-3288 with respect to vehicle response ($F_{1,9}$=20.575, p=0.002) with an average percent change of -64.24 ± 14.16%. The percent change of glutamate release also varied between 1 mg/kg and 3 mg/kg AVL-3288 ($F_{1,9}$=15.732, p=0.004). An additional one-way ANOVA on dose of NMDA (aCSF, 0.05µg, 0.30µg) revealed an interaction within the 1mg/kg AVL-3288 treated group ($F_{2,16}$=5.661, p=0.016) but not within the 3mg/kg AVL-3288 treated group ($F_{2,16}$=1.953, p=0.179). 1 mg/kg AVL-3288 potentiated glutamate release more after 0.05µg compared to aCSF ($F_{1,11}$=17.471, p=0.002) and more after 0.30µg compared to aCSF ($F_{1,10}$=7.280, p=0.024). Thus, 1 mg/kg AVL-3288 produced a percent change of 24.12 ± 5.77% after 0.05µg stimulation. Lastly, additional two-way (AVL dose x NMDA dose) ANOVAs did not reveal any variation among latency to peak onset, T50, T80, and peak duration (all p-values >0.1).

Fig. 4 demonstrates representative line tracings for an animal receiving 0.05µg/0.5 µL NMDA in the presence of vehicle and 1 mg/kg AVL3288. Time is represented on the X-axis and glutamate concentration is represented on the Y-axis. Infusions of aCSF or NMDA into the nucleus accumbens are denoted with arrows. The top line tracing is the signal produced from the glutamate-sensitive channels, whereas the line tracing beneath is from the sentinel channels, which lack GluOx. The bottom tracing is the self-referenced tracing, which represents the signal derived solely from the oxidation of glutamate and is obtained by subtracting the sentinel signal from the glutamate signal. This sample tracing from an individual animal demonstrates an increase of 2.83µM glutamate from low-dose NMDA stimulation during the vehicle test day. However, when pretreated with 1 mg/kg AVL-3288, the low-dose NMDA produced a potentiated response of 3.46 µM, a percent change of 22%.

Fig. 5 is a representative tracing from an animal receiving high-dose NMDA stimulation and vehicle and high-dose AVL-3288 injections. For simplicity, only the self-reference tracing is shown. After vehicle injection, the high-dose of NMDA produced a 9.44 µM increase in glutamate. When pretreated with the high-dose of AVL-3288, the high dose NMDA response was attenuate and only 7.16µM was evoked, a percent change of -24%.

Overall, AVL-3288 differentially modulates NMDA-evoked glutamate release in the PFC as a function of dose of NMDA. At the low-dose of NMDA, the low-dose of AVL-3288 significantly potentiates glutamate release, but at the high-dose of AVL-3288, glutamate was not potentiated. At the high-dose of NMDA, glutamate release was significantly potentiated with the low-dose of AVL-3288, but significantly attenuated at the high-dose of AVL-3288. Group data for the dose combinations is presented in Fig. 6 as a percent change from vehicle test days. Absolute increases of glutamate from aCSF, low-dose NMDA, and high-dose NMDA on vehicle test days are presented in the inset graph for reference to percent change.

PNU-120596 Results

Effects of PNU-120596 on basal glutamate

Basal glutamate, measured after IP injection but before aCSF infusion, did not vary between PNU-120596 dose ($F_{2,30}$=1.704, p=0.207) or NMDA dose ($F_{1,15}$=0.580, p=0.572) and there was not an interaction between the dose combinations ($F_{2,30}$=0.332, p=0.804). These results indicate that systemic injection of PNU-120596 does not affect basal glutamate release, i.e. in the absence of stimulation.

Effects of PNU-120596 on evoked glutamate

The type II α7nAChR PAM, PNU-120596, was injected systemically 40 minutes prior to mesolimbic-stimulation with NMDA. Similar to AVL-3288 experiments, dose of NMDA (0.05μg or 0.30μg) was held constant between test days in the presence of vehicle, 3 mg/kg, and 9 mg/kg PNU-120596. Data will be presented as a percent change from NMDA-evoked glutamate release after vehicle treatment.

Two-way (PNU dose x NMDA dose) omnibus ANOVA revealed a significant effect on the percent change in glutamate release between PNU-120596 dose ($F_{2,30}$=11.43, p<0.001) and NMDA dose ($F_{1,15}$=7.260, p=0.006) and an AVL dose-NMDA dose interaction ($F_{4,30}$=7.020, p<0.001). Subsequent one-way ANOVA on dose of PNU-120596 (vehicle, 3 mg/kg, 9 mg/kg) revealed a significant difference between PNU doses within the 0.05 NMDA group ($F_{2,17}$=9.128, p=0.003), but not within the 0.30 NMDA group ($F_{2,17}$=0.822, p=0.458). Within the low-dose NMDA group, 9 mg/kg PNU-120596 significantly potentiated glutamate release more than both vehicle ($F_{1,11}$=17.404, p=0,002) and 3 mg/kg PNU-120596 ($F_{1,11}$=6.958, p=0.025); however, 3 mg/kg PNU-120596 did not significantly potentiate glutamate release over vehicle ($F_{1,11}$=1.153, p=0.308). 9 mg/kg PNU-120596 potentiated 0.05μg by 211.95 ± 50.80% when compared to glutamate evoked by 0.05μg after vehicle pretreatment. An additional one-way ANOVA on dose of NMDA (aCSF, 0.05μg, and 0.30μg) revealed an effect of the 9 mg/kg PNU-120596 ($F_{2,17}$=9.827, p=0.002), but not 3 mg/kg PNU-120596 ($F_{2,17}$=0.917, p=0.421) on percent change of evoked glutamate release. 9 mg/kg PNU-120596 significantly potentiated glutamate release after 0.05μg NMDA more than after aCSF ($F_{1,11}$=17.404, p=0.002) and 0.30μg NMDA

($F_{1,11}$=7.788, p=0.019). Lastly, two-way (PNU dose x NMDA dose) ANOVAs did not reveal any variation among latency to peak onset, T50, T80, and peak duration (all p-values >0.7).

Fig. 7 demonstrates a representative self-tracing for low-dose NMDA stimulation after the pretreatment of vehicle and 9 mg/kg PNU-120596. The glutamate response of 1.72µM on the vehicle test day was potentiated to 4.27µM on the high-dose PNU-120596 test day, a percent change of 148%.

Overall, PNU-120596 differentially modulates NMDA-evoked glutamate release in the PFC as a function of dose of NMDA. At the low-dose of NMDA, the high-dose of PNU-120596 significantly potentiates glutamate release, whereas at the low-dose of PNU-120596, glutamate was not potentiated. Interestingly, at the high-dose of NMDA, glutamate release was not potentiated with the low-dose of PNU-120596 or high-dose of PNU-120596 nor was it attenuated at either dose. Group data for the dose combinations is presented in Fig. 8 as a percent change from vehicle test days. Absolute increases of glutamate from aCSF, low-dose NMDA, and high-dose NMDA on vehicle test days are presented in the inset graph for reference to percent change.

Discussion

Several lines of evidence indicate that the signal measured by the MEA is glutamate-derived. Reducing the applied potential from +0.7V to +0.2V eliminates the sensor's ability to record glutamate as the reporting molecule, H_2O_2, is not electrically active at this lower potential (Pomerleau et al., 2003; Day et al., 2006). In this circumstance, glutamate is being oxidized, but the H_2O_2 produced is unable to be reduced to generate a signal. While such experiments establish that the potential must be at large enough to reduce H_2O_2 in order to measure glutamate, they do not establish that the H_2O_2 is produced from the oxidation of glutamate. Although GluOx is a highly selective enzyme, further determination of the signal as glutamate-derived can be accomplished by altering glutamate kinetics. Application of a glutamate uptake inhibitor, TBOA, causes an increase in glutamate signal and clearance time (Pomerleau et al., 2003; Bortz et al., 2013). These increases in signal and clearance time reflect extracellular glutamate persisting due to the inhibition of glutamate transporters. Similarly, enhancing glutamate transporters with ceftriaxone leads to a decrease in clearance time as glutamate is cleared more rapidly (Bortz et al., 2013). Furthermore, stimulating mGluR2/3, a glutamate autoreceptor, decreases glutamate signal as less glutamate is released and antagonism of the mGluR2/3 results in an increase of glutamate signal because more glutamate is released (Hascup et al., 2010). Together, this evidence suggests that the MEA used in the experiments for this study was capable of measuring glutamate release *in vivo*.

In addition to measuring glutamate *in vivo*, the MEA measures glutamate that is neuronally-derived. Local application of ω-conotoxin (MCVIIC) blocks the influx of calcium through calcium channels, inhibiting exocytocic release of neurotransmitters at the axon terminal and decreasing basal glutamate measured with the MEA (Hascup et al., 2010). Unlike glutamate measured via microdialysis, glutamate measured with the MEA is tetrodotoxin (TTX)-sensitive (Day et al., 2006; van der Zeyden et al., 2008; Hascup et al., 2010). TTX blocks sodium channels, preventing action potentials; so, insensitivity to TTX indicates that the signal is not neuronally-derived, whereas a signal that is neuronally-derived would be affected by this inhibition. This discrepancy between microdialysis and the MEA TTX-sensitivity is likely due to differing amount of damage caused by either structure. The microdialysis probe causes more tissue damage and gliosis than the MEA, leading to more microglial and astrocytic activation (van der Zeyden et al., 2008; Hascup et al., 2009). Blocking the cysteine-glutamate exchanger, an antiporter located primarily on glia, with (S)-4-carboxyphenylglycine (CPG) reduces glutamate measured by microdialysis, but not glutamate measured by the MEA (Hascup et al., 2010). Therefore, due to increased glial activation by the microdialysis probe, microdialysis techniques may measure non-neuronal pools of glutamate, whereas the MEA measures from neuronal pools with no detectable glutamate measurements from cysteine-glutamate transporters.

Using an MEA that measures neuronally-derived glutamate, the results from this study demonstrate for the first time the ability of α7nAChR PAMs to potentiate glutamate release *in vivo* and additionally, in awake, freely-moving rats. Previous studies investigating PAMs have been limited to *in* vitro measurements or *in vivo* recordings of dopamine in anesthetized animals. Many *in vitro* study findings may have limited implications as it has recently been demonstrated that the type II PAMs PNU-120596 and SB-206553 are considerably less potent at physiological temperatures than at the room temperature, which is the temperature that the majority of *in vitro* experiments occurred (Sitzia et al., 2011). This study demonstrates that *in vivo,* i.e. physiological temperatures, both AVL-3288 and PNU-120596 were capable of modulating neurotransmitter release. The effect of increased temperature on α7nAChR activity is more pronounced at higher agonist concentration, as the percent decrease in current amplitude as a result of increased temperature is more significant at maximal concentrations than at EC_{50} (Jindrichova et al., 2012). Additionally, maximal concentration of agonist caused a more rapid desensitization at physiological temperatures than at room temperature, whereas temperature had no effect on desensitization from agonist at EC_{50} (Jindrichova et al., 2012). Two distinct desensitized states have been proposed for the α7nAChR, one that is destabilized by PNU-120596 and one that is not destabilized by PNU-120596 (Williams et al., 2011a). This ability to destabilize a desensitized receptor configuration may underlie the capability for type II PAMs to block desensitization. However, the second desensitized state proposed by Williams et al. was insensitive to PNU-120596. Greater free energy at physiological temperatures may favor the PNU-120596-insensitive desensitized state over the PNU-120596-sensitive desensitized state and contribute to reduced efficacy of type II PAMs at physiological temperatures. The existence of multiple desensitized states may also contribute to differences demonstrated *in vivo* between the type I PAM, AVL-3288, and the type II PAM, PNU-120596, at the higher dose combinations in this study.

Glutamate signal was attenuated with high levels of NMDA-stimulation in the presence of the high-dose of AVL-3288. This may reflect rapid receptor desensitization as a result of interaction between a high concentration of acetylcholine and a high concentration of AVL-3288. These results are not surprising as AVL-3288 is a type I PAM and, therefore, should have no effect on receptor desensitization. Notably, AVL-3288 was originally developed by screening $GABA_A$ PAMs to find an initial template for an α7nAChR PAM and was then modified to increase selectivity to the α7nAChR and enhance bioavailability (Ng et al., 2007). While AVL-3288 does not have activity at other nicotinic receptors, e.g. α4β2 and α3β4, the PAM still has a low affinity as a $GABA_A$ PAM (Ng et al., 2007). Activity at this receptor may contribute to glutamate attenuation, however, is likely not the sole cause of the response. If $GABA_A$ activity alone was sufficient to significantly attenuate the glutamate response, then a significant attenuation should have been observed when the low-dose of NMDA-stimulation was given in the presence of the high dose of AVL-3288. Because the high-dose of AVL-3288 was not sufficient to attenuate glutamate, the high-dose must interact with the higher NMDA-stimulation to reduce the recorded signal. While α7nAChR desensitization is a plausible and likely explanation of the phenomenon, increased GABA activity cannot be excluded as GABA is primarily an inhibitory neurotransmitter and can reduce glutamate transmission. Although it has not been reported, if mesolimbic stimulation with NMDA dose-dependently increases GABA as well as choline and glutamate, then positive allosteric modulation of $GABA_A$ could lead to increased inhibitory effects at higher levels of NMDA-stimulation. Although, if GABA activity does not vary between levels of NMDA-stimulation, then the increased attenuation observed at higher concentrations of NMDA-stimulation is more likely desensitization or some other interaction between the dose of AVL-3288 and the increased stimulation. When tested on oocytes, i.e. in the absence of GABA, AVL-3288 exhibited an attenuated response in the presence of continued stimulation, indicating that desensitization of the α7nAChR in the presence of AVL-3288 occurs independent of its GABA activity. While the extent of the interaction of GABA and AVL-3288 *in vivo* is not yet known, a clear interaction occurs between the high-dose combination of AVL-3288 and NMDA stimulation that results in an attenuation of evoked glutamate release and this attenuation likely involves α7nAChR desensitization.

In contrast to AVL-3288, PNU-120596 did not cause a significant attenuation in glutamate release at any dose-combinations tested. Although, at higher doses of NMDA, the ability of PNU-120596 to potentiate glutamate was diminished; however, the signal was not less than after vehicle pretreatment. This result is consistent with a PNU-120596-insensitive desensitized state (Williams et al., 2011a). An alternate explanation to the decreased effectiveness of PNU-120596 is open channel block by large cations. While the α7nAChR gates cations, large cations, such as choline, are too large to flow through the channel pore and consequently, can block the influx of smaller cations, such as Ca^{2+} or Na^{+} (Kalappa & Uteshev, 2013). By preventing PNU-120596-sensitive desensitization, the kinetics of the α7nAChR is altered and the channel stays open for longer durations (Hurst et al., 2005; Szabo et al., 2014). The increased duration of channel opening increases the probability that the channel will be blocked by large cations, resulting in open-channel inhibition. Additionally, PAMs generally have a saturability of their effects due to a limited degree of cooperativity between orthosteric and allosteric binding sites, producing diminishing returns with increased orthosteric-allosteric saturation (Christopolous, 2002; Uteshev, 2014a). Together, these mechanisms may contribute to the diminished ability of PNU-120596 to potentiate glutamate release, despite the ability of PNU-120596 to prevent rapid desensitization.

Behaviorally, the lack of potentiation at high levels of stimulation with PNU-120596 may not be relevant. PAMs may be most advantageous when enhancing signal that has low salience. If the signal is strong, i.e. high salience, the signal likely will not need to be further enhanced and positive modulation may not be necessary; therefore, the differential ability to potentiate high levels of stimulation between types of PAMs may not cause changes in cognitive performance. More importantly, both types of PAMs demonstrate the ability to potentiate low-dose stimulation *in vivo*. While enhancing low levels of α7nAChR activity may be essential in enhancing glutamate release in order to ameliorate cognitive deficits, PAMs may also increase the risk of inappropriately activating the receptor in the presence of a subthreshold stimulus. This false alarm would be the result of potentiating a signal that would have not otherwise generated a response. Indeed, PAMs can enable receptor activity in the presence of concentrations of acetylcholine that did not previously induce activity (Hurst et al., 2005). This fine line between enhancing cholinergic function in a state of hypofunction and avoiding false alarms may limit the therapeutic advantage of using PAMs over orthosteric agonists. This is not to say that PAMs will not have advantages over orthosteric agonists, but rather PAMs will inherently cause some inappropriate receptor activation, although likely to a lesser extent than orthosteric agonists. This effect is because PAMs require a minimal amount of orthosteric activity in order to produce a response, even if the afferent activity is subthreshold in the absence of PAM, whereas orthosteric agonists will indiscriminately activate the receptor, even in the absence of any afferent activity. Thus, the ability to potentiate highly salient stimuli, or lack thereof, may not influence PAM efficacy in behavioral tasks; although, PAMs can potentiate stimuli with low salience, but may be limited in their advantage over orthosteric agonists due to their ability to potentiate subthreshold stimuli.

The differential effect on rapid desensitization between the two types of PAMs may also affect the therapeutic advantage of PAMs. By blocking one form of receptor desensitization, PNU-120596 allows for an increased receptor response and more calcium entry into the neuron. As such, the potentiation of glutamate by PNU-120596 was far more robust than the potentiation measured in the presence of AVL-3288. While these results are intriguing, it will be interesting to see which type of PAM produces more advantageous results in behavioral tasks. While increased glutamate release is generally construed as beneficial, too much can cause excitotoxic damage. Thus, desensitization of the α7nAChR may serve to protect the brain from destructive levels of stimulation. At room temperature, PNU-120596 was cytotoxic to cell lines and this damage was dependent on α7nAChR activity (Ng et al., 2007). However, when tested at physiological temperatures, PNU-120596 did not demonstrate excitotoxicity (Hu et al., 2009; Sitzia et al., 2011). Although PNU-120596 likely does not decrease cell viability *in vivo* likely due to increased temperature, AVL-3288 did not cause damage to cells at any temperature measured, including at room temperature where the drugs and receptor have significantly increased activity (Ng et al., 2007). Therefore, type II PAMs may have more potential to exacerbate excitotoxic events than type I PAMs due to differential effects on rapid desensitization. Receptor desensitization is also important for agonist-induced α7nAChR up-regulation and by blocking desensitization, PNU-120596 prevents this up-regulation of the receptor (Thomsen & Mikkelsen, 2012). However, increased α7nAChR expression did not correlate with cognitive improvements in these experiments, although localization of receptors on the surface versus within the cell was not determined (Thomsen et al., 2011). In addition to these differences between type I and type II PAMs based on receptor desensitization, the results presented here demonstrate differential effects of type I and type II PAMs in evoking glutamate release at high-dose combinations. Further behavioral studies are needed to determine if these *in vivo* differences produce different results in behavioral tasks and if the inhibition of desensitization is more or less beneficial to cognitive performance.

If the goal of PAMs is to enhance endogenous cholinergic activity, then type I PAMs better preserve the nature and integrity of cholinergic transmission. While type II PAMs may produce more robust effects, they also alter α7nAChR kinetics and allow the channel to be open for a longer duration. Type I PAMs, conversely, have no effect on receptor kinetics and as such, receptor dynamics with low concentrations of acetylcholine and type I PAM are similar to receptor dynamics with higher concentrations of acetylcholine (Ng et al., 2007). Regardless of the effect of receptor kinetics, both types of PAM have demonstrated pro-cognitive effects in sensory gating, working memory, spatial memory, short-term memory, and cognitive flexibility (Hurst et al. 2005; Ng et al., 2007; Timmerman et al., 2007; Dunlop et al., 2008; Faghih et al., 2009; McLean et al., 2012). Despite the promising effects of α7nAChR PAMs in preclinical studies, the type II PAM, JNJ-39393406, failed to reduce sensory gating deficits in patients with schizophrenia when used as an adjunct to antipsychotic treatment (Winterer et al., 2013). In this phase II trial, the PAM was administered acutely. Several preclinical studies have failed to demonstrate an improvement in cognitive performance when administering a PAM alone and acutely (Thomsen et al., 2011; Callahan et al. 2013). It may be that some of the PAMs, particularly type II, require multiple administrations before pro-cognitive effects can be observed and that immediate facilitation of α7nAChR activity may not always be sufficient to enhance cognitive performance. Although they may not always be effective when administered alone, PAMs can be used in conjunction with other drugs that increase orthosteric activity at the α7nAChR to enhance cognitive performance (Callahan et al., 2013). α7 PAMs increase the potency of both acetylcholine and direct agonists, enabling the effective dose to be lowered when these drugs are used together. Lowering the effective dose increases the therapeutic index (also referred to as therapeutic window), making drugs safer while reducing side effect profiles (Williams et al., 2011b). Additionally, while the orthosteric binding side is highly conserved throughout evolution, the allosteric site is less conserved, and therefore, has greater variability in the binding domain between receptor subtypes (Yang et al., 2012). This variation allows for highly selective PAMs. Orthosteric agonists can be less selective to an individual receptor, however, when used in conjunction with a PAM, can effectively become more selective. PAMs will enhance the orthosteric activity at the target receptor, allowing for a reduced dose of agonist required to be effective. Because PAMs generally do not have affinity to other receptors, orthosteric activity at the non-target receptors is not enhanced and the reduced dose of agonist results in a decreased activity at these non-target receptors (Uteshev, 2014b). In other words, as a result of positive allosteric modulation of the target receptor, a lower dose of an orthosteric agonist can be used, reducing activity at receptors with similar orthosteric binding sites. Thus, PAMs may be used to reduce the side effects of orthosteric agonists and have benefits in treatment whether alone, or as a supplement to more direct therapeutics.

α7nAChR PAMs have been demonstrated preclinically to improve performance in a variety of tasks, either alone or in conjunction with other orthosteric agonists. Two classes of α7 PAMs were demonstrated in this study to significantly potentiate glutamate release during low levels of stimulation. During high levels of mesolimbic stimulation, the two classes had differential effects: the type I PAM produced either a potentiation or an attenuation of glutamate release at the low- and high-dose of PAM, respectively, whereas the type II PAM did not have an effect at either dose of PAM. The potentiation profiles of both types of PAM demonstrate the importance of the interaction between the dose of PAM and the level of stimulation as either PAM has varying effects depending on these interactions. Collectively, these results demonstrate the ability of α7nAChR PAMs to potentiate glutamate release *in vivo* and therefore, support further investigation into the use of α7nAChR PAMs in the treatment of cognitive deficits associated with schizophrenia.

References

Alexander, K.S., Wu, H.Q., Schwarcz, R., & Bruno, J.P. (2012). Acute elevations of brain kynurenic acid impair cognitive flexibility: normalization by the alpha7 positive modulator galantamine. *Psychopharmacology*, 220, 627-637.

Alkondon, M., Pereira, E.F.R, Cortes, W.S., Maelicke, A., & Albuquerque, E.X. (1997). Choline is a selective agonist of α7 nicotinic acetylcholine receptors in the rat brain neurons. *European Journal of Neuroscience*, 9, 2734-2742.

American Psychiatric Association. (2013). Schizophrenia Spectrum and Other Psychotic Disorders. In *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). doi:10.1176/appi.books.9780890425596.dsm02

Ban, T.A. (2007). Fifty years chlorpromazine: a historical perspective, *Neuropsychiatric Disease and Treatment*, 3(4), 495-500.

Barch, D.M., Carter, C.S., & Cohen, J.D. (2004). Factors influencing Stroop performance in schizophrenia. *Neuropsychology*, 18(3), 477-484.

Bauer, D., Gupta, D., Harotunian, V., Meador-Woodruff, J.H., & McCullumsmith, R.E. (2008). Abnormal expression of glutamate transporter and transporter interacting molecules in prefrontal cortex in elderly patients with schizophrenia. *Schizophrenia Research*, 104, 108-120.

Berman, K.F., Torrey, E.F., Daniel, D.G., & Weinberger, D.R. (1992). Regional cerebral blood flow in monozygotic twins discordant and concordant for schizophrenia. *General Archives of Psychiatry*, 49, 927-934.

Blot, K., Bai, J., & Otani, S. (2013). The effect of non-competitive NMDA receptor antagonist MK-801 on neuronal activity in rodent prefrontal cortex: an animal model for cognitive symptoms of schizophrenia. *Journal of Physiology*, 107, 448-451.

Bortz, D.M., Jørgensen, C.V., Mikkelsen, J.D., & Bruno, J.P. (2014). Transient inactivation of the ventral hippocampus in neonatal rats impairs the mesolimbic regulation of prefrontal glutamate release in adulthood. *Neuropharmacology*, 84, 19-30.

Bortz, D.M., Mikkelsen, J.D., & Bruno, J.P. (2013). Localized infusions of the partial alpha 7 nicotinic receptor agonist SSR180711 evoke rapid and transient increases in prefrontal glutamate release. *Neuroscience*, 255, 55-67.

Burmeister, J.J. & Gerhardt, G.A. (2001). Self-referencing ceramic-based multisite microelectrodes for the detection and elimination of interferences from the measurement of L-glutamate and other analytes. *Anal. Chem.*, 73, 1037-1042.

Callahan, P.M., Hutchings, E.J., Kille, N.J., Chapman, J.M., & Terry, A.V. (2013). Positive allosteric modulator of alpha 7 nicotinic acetylcholine receptors, PNU-120596 augments the effects of donepezil on learning and memory in aged rodents and non-human primates. *Neuropharmacology*, 67, 201-212.

Castner, S.A., Smagin, G.N., Piser, T.M., Wang, Y., Smith, J.S., Christian, E.P., Mrzljak, L., & Williams, G.V. (2011). Immediate and sustained improvements in working memory after selective stimulation of α7 nicotinic acetylcholine receptors. *Biological Psychiatry*, 69, 12-18.

Christopoulos, A. (2002). Allosteric binding sites on cell-surface receptors: novel target for drug discovery. *Nature Reviews: Drug Discovery*, 1, 198-210.

Dajas-Bailador, F. & Wonnacott, S. (2004). Nicotinic acetylcholine receptors and the regulation of neuronal signalling. *Trends in Pharmacological Sciences*, 25(6), 317-324.

Day, B.K., Pomerleau, F., Burmeister, J.J., Huettl, P., & Gerhardt, G.A. (2006). Microelectrode array studies of basal and potassium-evoked release of L-glutamate in the anesthetized rat brain. *Journal of Neurochemistry*, 96, 1626-1635.

Demakis, G.J. (2004). Frontal lobe damage and tests of executive processing: a meta-analysis of the category test, Stroop test, and trail-making test. *Journal of Clinical and Experimental Neuropsychology*, 26(3), 441-450.

Dunlop, J., Lock, T., Jow, B., Sitzia, F., Grauer, S., Jow, F., Kramer, A., Bowlby, M.R., Randall, A., Kowal, D., Gilbert, A., Comery, T.A., LaRocque, J., Soloveva, V., Brown, J., & Roncarati, R. (2009). Old and new pharmacology: Positive allosteric modulation of the α7 nicotinic acetylcholine receptor by the 5-hydroxytryptamine$_{2B/C}$ receptor antagonist SB-206553 (3,5-dihydro-5-methyl-*N*-3-pyridinylbenzo[1,2-*b*:4,5-*b*']dipyrrole-1-(2*H*)-carboxamide). *Journal of Pharmacology and Experimental Therapeutics*, 328, 766-776.

Eastwood, S.L. & Harrison, P.J. (2005). Decreased expression of vesicular glutamate transporter 1 and complexin II mRNAs in schizophrenia: further evidence for a synaptic pathology affecting glutamate neurons. *Schizophrenia Research*, 73, 159-172.

Faghih, R., Gopalakrishnan, S.M., Gronlien, J.H., Malysz, J., Briggs, C.A., Wetterstrand, C., Ween, H., Curtis, M.P., Sarris, K.A., Gfesser, G.A., El-Kouhen, R., Robb, H.M., Radek, R.J., Marsh, K.C., Bunnelle, W.H., & Gopalakrishnan, M. (2009). Discovery of 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1*H*-pyrrol-1-yl)benzenesulfonamide (A-867744) as a novel positive allosteric modulator of the α7 nicotinic acetylcholine receptor. *Journal of Medicinal Chemistry*, 52(10), 3377-3384.

Faraone, S. V., Chen, W. J., Goldstein, J. M., & Tsuang, M. T. (1994). Gender differences in age at onset of schizophrenia. *British Journal of Psychiatry*, 164, 625-629.

Green, M.F., Kern, R.S., Braff, D.L., & Mintz, J. (2000). Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"?. *Schizophrenia Bulletin*, 26(1), 119-136.

Grønlien, J.H., Håkerud, M., Ween, H., Thorin-Hagene, K., Briggs, C.A., Gopalakrishnan, M., & Malysz, J. (2007). Distinct profiles of α7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. *Molecular Pharmacology*, 72(3), 715-724.

Guan, Z.Z., Zhang, X., Blennow, K., & Nordberg, A. (1999). Decreased protein level of nicotinic receptor α7 subunit in the fronal cortex from schizophrenic brain. *NeuroReport*, 10, 1779-1782.

Gubbins, E.J., Gopalakrishnan, M., & Li, J. (2010). α7 nAChR-mediated activation of MAP kinase pathways in PC12 cells. *Brain Research*, 1328, 1-11.

Gupta, D.S., McCullumsmith, R.E., Beneyto, M., Haroutunian, V., Davis, K.L. & Meador-Woodruff, J.H. (2005). Metabotropic glutamate receptor protein expression in the prefrontal cortex and striatum in schizophrenia. *Synapse*, 57, 123-131.

Harrison, P.J. & Weinberger, D.R. (2005). Schizophrenia genes, gene expression, and neuropathology: on matter of their convergence. *Molecular Psychiatry*, 10, 40-68.

Hascup, E.R., af Bjerkén, S., Hascup, K.N., Pomerleau, F., Huettl, P., Strömberg, I., & Gerhardt, G.A. (2009). Histological studies of the effects of chronic implantation of ceramic-based microelectrode arrays and microdialysis probes in rat prefrontal cortex. *Brain Research*, 1291, 12-20.

Hascup, E.R., Hascup, K.N., Stephens, M., Pomerleau, F., Huettl, P., Gratton, A. & Gerhardt, G.A. (2010). Rapid microelectrode measurements and the origin and regulation of extracellular glutamate in rat prefrontal cortex. *Journal of Neurochemistry*, 115, 1608-1620.

Hauser, T.A., Kucinski, A., Jordan, K.G., Gatto, G.J., Wersinger, S.R., Hesse, R.A., Stachowiak, E.K., Stachowiak, M.K., Papke, R.L., Lippiello, P.M., & Bencherif, M. (2009). TC-5619: An alpha7 neuronal nicotinic receptor-selective agonist that demonstrates efficacy in animal models of the positive and negative symptoms and cognitive dysfunction of schizophrenia. *Biochemical Pharmacology*, 78, 803-812.

Haut, M.W., Cahill, J., Cutlip, W.D., Stevenson, J.M., Makela, E.H., & Bloomfield, S.M. (1996). On the nature of Wisconsin Card Sorting Test performance in schizophrenia. *Psychiatry Research*, 65, 15-22.

Hilmas, C., Pereira, E.F.R., Alkondon, M., Rassoulpour, A., Schwarcz, R., & Albuquerque, E.X. (2001). The brain metabolite kynurenic acid inhibits α7 nicotinic receptor activity and increases non-α7 nicotinic receptor expression: physiopathological implications. *The Journal of Neuroscience*, 21(19), 7463-7473.

Hirayasu, Y., Tanaka, S., Shenton, M.E., Salisbury, D.F., DeSantis, M.A., Levitt, J.J., Wible, C., Yurgelun-Todd, D., Kikinis, R., Jolesz, F.A., & McCarley, R.W. (2001). Prefrontal gray matter volume reduction in first episode schizophrenia. *Cerebral Cortex*, 11 (4), 374-381.

Hu, M., Gopalakrishnan, M., & Li, J. (2009). Positive allosteric modulation of α7 neuronal nicotinic acetylcholine receptors: lack of cytotoxicity in PC12 cells and rat primary cortical neurons. *British Journal of Pharmacology*, 158, 1857-1864.

Hurst, R.S., Hajós, M., Raggenbass, M., Wall, T.M., Higdon, N.R., Lawson, J.A., Rutherford-Root, K.L., Berkenpas, M.B., Hoffmann, W.E., Piotrowski, D.W., Groppi, V.E., Allaman, G., Ogier, R., Bertrand, S., Bertrand, D., & Arneric, S.P. (2005). A novel positive allosteric modulator of the α7 neuronal nicotinic acetylcholine receptor: *in vitro* and *in vivo* characterization. *The Journal of Neuroscience*, 25(17), 4396-4405.

Jindrichova, M., Lansdell, S.J., & Millar, N.S. (2012). Changes in temperature have opposing effects on current amplitude in α7 and α4β2 nicotinic acetylcholine receptors. *PLos One*, 7(2), e32073, 1-8.

Kalappa, B.I. & Uteshev, V.V. (2013). The dual effect of PNU-120596 on α_7 nicotinic acetylcholine receptor channels. *European Journal of Pharmacology,* 718, 226-234.

Kapur, S. & Mamo, D. (2003). Half a century of antipsychotics and still a central role for dopamine D2 receptors. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 27, 1081-1090.

Karagianis, J., Novick, D., Pecenak, J., Haro, J.M., Dossenback, M., Treuer, T., Montgomery, W., Walton, R., & Lowry, A.J. (2009). Worldwide-schizophrenia outpatient health outcomes (W-SOHO): baseline characteristics of pan-regional observational data from more than 17,000 patients. *The International Journal of Clinical Practice*, 63 (11), 1578-1588.

Keefe, R.S.E, Bilder, R.M., Davis, S.M., Harvey, P.D., Palmer, B.W., Gold, J.M., Meltzer, H.Y., Green, M.F., Capuano, G., Stroup, T.S., McEvoy, J.P., Swartz, M.S., Rosenheck, R.A., Perkins, D.O., Davis, C.E., Hsiao, J.K., & Lieberman, J.A. (2007a). Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. *Archives of General Psychiatry*, 64, 633-647.

Keefe, R.S.E., Sweeney, J.A., Gu, H., Hamer, R.M., Perkins, D.O., McEvoy, J.P., & Lieberman, J.A. (2007b). Effects of olanzapine, quetiapine, and resperidone on neurocognitive function in early psychosis: a randomized, double-blind 52-week comparison. *American Journal of Psychiatry*, 164, 1061-1071.

Kerns, J.G. Nuechterlein, K.H., Braver, T.S., & Barch, D.M. (2008). Executive functioning component mechanisms and schizophrenia. *Biological Psychiatry*, 64, 26-33.

Konradsson-Geuken, Å., Wu, H.Q., Gash, C.R., Alexander, K.S., Campbell, A., Sozeri, Y., Pellicciari, R., Schwarcz, R., & Bruno, J.P. (2010). Cortical kynurenic acid bi-directionally modulates prefrontal glutamate levels as assessed by microdialysis and rapid electrochemistry. *Neuroscience*, 169, 1848-1859.

Kristiansen, L.V., Beneyto, M., Haroutunian, V., & Meador-Woodruff, J.H. (2006). Changes in NMDA receptor subunits and interacting PSD proteins in dorsolateral prefrontal and anterior cingulate cortex indicate abnormal regional expression in schizophrenia. *Molecular Psychiatry*, 11, 737-747.

Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers, M.B., & Charney, D.S. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in human. *Archives of General Psychiatry*, 51, 199-214.

Lahti, A.C., Koffel, B., LaPorte, D., & Tamminga, C.A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology,* 13, 9-19.

Leonard, S. Adler, L.E., Benhammou, K., Berger, R., Breese, C.R., Drebing, C., Gault, J., Lee, M.J., Logel, J. Olincy, A., Ross, R.G., Stevens, K., Sullivan, B., Vianzon, R., Virnich, D.E., Waldo, M., Walton, K., & Freedman, R. (2001). Smoking and mental illness. *Pharmacology, Biochemistry and Behavior*, 70, 561-570.

Leonard, S., Gault, J., Hopkins, J., Logel, J., Vianzon, R., Short, M., Drebing, C., Berger, R., Venn, D., Sirota, P., Zerbe, G., Olincy, A., Ross, R.G., Adler, L.E., & Freedman, R. (2002). Association of promoter variants in the α7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia. *Archives of General Psychiatry*, 59, 1085-1096.

Leonard, S., Mexal, S., & Freedman, R. (2007). Smoking, genetics and schizophrenia: evidence for self medication. *Journal of Dual Diagnosis*, 3, 43-59.

Lieberman, J.A. & Stroup, T.S. (2011). The NIMH-CATIE schizophrenia study: What did we learn?. *American Journal of Psychiatry,* 168(8), 770-775.

Linderholm, K.R., Skogh, E., Olsson, S.K., Dahl, M.L., Holtze, M., Engberg, G., Samuelsson, M., & Erhardt, S. (2012). Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophrenia Bulletin*, 38(3), 426-432.

Livingstone, P.D., Dickinson, J.A., Srinivasan, J., Kew, J.N.C., & Wonnacott, S. (2010). Glutamate-dopamine crosstalk in the rat prefrontal cortex is modulated by alpha7 nicotinic receptors and potentiated by PNU-120596. *Journal of Molecular Neuroscience*, 40, 172-176.

Lowry, J.P. & O'Neill, R.D. (1992). Homogenous mechanism of ascorbic acid interference in hydrogen peroxide detection at enzyme-modified electrodes. *Analytical Chemistry*, 64(4), 453-456.

Matthew, S.V., Law, A.J., Lipska, B.K., Dávila-García, M.I., Zamora, E.D., Mitkus, S.N., Vakkalanka, R., Straub, R.E., Weinberger, D.R., Kleinman, J.E., & Hyde, T.M. (2007). α7 nicotinic acetylcholine receptor mRNA expression and binding in postmortem human brain are associated with genetic variation in neuregulin 1. *Human Molecular Genetics*, 16(23), 2921-2932.

McLean, S., Idris, N.F., Grayson, B., Gendle, D.F., Mackie, C., Lesage, A.S., Pemberton, D.J., & Neill, J.C. (2012). PNU-120596, a positive allosteric modulator of α7 nicotinic acetylcholine receptors, reverses a sub-chronic phencyclidine-induced cognitive deficit in the attentional set-shifting task in female rats. *Journal of Psychopharmacology*, 26(9), 1265-1270.

Miller, E.K. (2000). The prefrontal cortex and cognitive control. *Nature Reviews Neuroscience*, 1, 59-65.

Molina, C., Sanz, J., Sarramea, F., Luque, R., Benito, C., & Palomo, T. (2006). Dorsolateral prefrontal and superior temporal volume deficits in first-episode psychoses that evolve into schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience*, 256, 106-111.

Neigh, G. N., Arnold, H. M., Rabenstein, R. L., Sarter, M., & Bruno, J. P. (2004). Neuronal activity in the nucleus accumbens is necessary for performance-related increases in cortical acetylcholine release. *Neuroscience*, 123, 635-645.

Ng, H.J., Whittemore, E.R., Tran, M.B., Hogenkamp, D.J., Broide, R.S., Johnstone, T.B., Zheng, L., Stevens, K.E., & Gee, K.W. (2007). Nootropic α7 nicotinic receptor allosteric modulator derived from GABA_A receptor modulators. *PNAS*, 104(19), 8059-8064.

Olincy, A., Harris, J.G., Johnson, L.L., Pender, V., Kongs, S., Allensworth, D., Ellis, J., Zerbe, G.O., Leonard, S., Stevens, K.E., Stevens, J.O., Martin, L., Adler, L.E., Soti, F., Kem, W.R., & Freedman, R. (2006). Proof-of-concept trial of an α7 nicotinic agonist in schizophrenia. *Archives of General Psychiatry*, 63, 630-638.

Papke, R.L., Kem, W.R., Soti, F., López-Hernández, G.Y., & Horenstein, N.A. (2009). Activation and desensitization of nicotinic α7-type acetylcholine receptors by benzylidene anabaseines and nicotine. *Journal of Pharmacology and Experimental Therapeutics*, 329(2), 791-807.

Paxinos, G. & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th Ed.). San Diego, CA: Academic Press.

Perala, J., Suvisaari, J., Saarni, S, I., Kuoppasalmi, K., Isometsä, E., Pirkola, S., Partonen, T., Tuulio-Hendriksson, A., Hintikka, J., Kieseppä, T., Härkänen, Y., Koskinen, S., & Lönnqvist, K. (2007) Lifetime prevalence of psychotic and bipolar I disorders in a general population. *Archives of General Psychiatry*, 64, 19-28.

Pomerleau, F., Day, B.K., Huettl, P., Burmeister, J.J., & Gerhardt, G.A. (2003). Real time *in vivo* measures of L-glutamate in the rat central nervous system using ceramic-based multisite microelectrode arrays. *Annals New York Academy of Sciences*, 1003, 454-457.

Redrobe, J.P., Nielsen, E.Ø., Christensen, J.K., Peters, D., Timmermann, D.B., & Olsen, G.M. (2009). α7 nicotinic acetylcholine receptor activation ameliorates scopolamine-induced behavioral changes in a modified continuous Y-maze task in mice. *European Journal of Pharmacology*, 602, 58-65.

Robinson, D.G., Woerner, M.G., Alvir, J.M.J., Bilder, R.M., Hinrichsen, G.A., & Lieberman, J.A. (2002). Predictors of medication discontinuation by patients with first-episode schizophrenia and schizoaffective disorder. *Schizophrenia Research*, 57, 209-219.

Rosenheck, R., Leslie, D., Keefe, R., McEvoy, J., Swartz, M., Perkins, D., Stroup, S., Hsiao, J.K., & Lieberman, J. (2006). Barriers to employment for people with schizophrenia. *American Journal of Psychiatry*, 163, 411-417.

Rutherford, E. C., Pomerleau, F., Huettl, P., Strömberg, I., & Gerhardt, G. A. (2007). Chronic second-by-second measures of L-glutamate in the central nervous system of freely moving rats. *Journal of Neurochemistry*, 102, 712-722.

Saha, S., Chant, D., Welham, J., & McGrath, J. (2005). A systematic review of the prevalence of schizophrenia. *PLoS Medicine*, 2(5), 413-433.

Saha, S., Welham, J., Chant, D., & McGrath, J. (2006). Incidence of schizophrenia does not vary with economic status of the country. *Social Psychiatry and Psychiatric Epidemiology*, 41, 338-340.

Schwarcz, R., Rassoulpour, A., Wu, H.Q., Medoff, D., Tamminga, C.A., & Roberts, R.C. (2001). Increased cortical kynurenate content in schizophrenia. *Biological Psychiatry*, 50, 521-530.

Seeman, P. (2002). Atypical antipsychotics: mechanism of action. *Canadian Journal of Psychiatry*, 47(1), 27-38.

Sitzia, F., Brown, J.T., Randall, A.D., & Dunlop, J. (2011). Voltage- and temperature-dependent allosteric modulation of α7 nicotinic receptors by PNU120596. *Frontiers in Pharmacology,* 2(81), 1-9.

St. Peters, M., Demeter, E., Lustig, C., Bruno, J. P., & Sarter, M. (2011). Enhanced control of attention by stimulating mesolimbic-corticopetal cholinergic circuitry. *The Journal of Neuroscience,* 31(26), 9760-9771.

Swartz, M.S., Perkins, D.O., Stroup, T.S., Davis, S.M., Capuano, G., Rosenheck, R.A., Reimherr, F., McGee, M.F., Keefe, R.S.E., McEvoy, J.P., Hsiao, J.K., & Lieberman, J.A. (2007). Effects of antipsychotic medications of psychosocial functioning in patients with chronic schizophrenia: findings from the NIMH CATIE study. *American Journal of Psychiatry,* 164, 428-436.

Szabo, A.K., Pesti, K., Mike, A., & Vizi, E.S. (2014). Mode of action of the positive modulator PNU-120596 on α7 nicotinic acetylcholine receptors. *Neuropharmacology*, 81, 42-54.

Thomsen, M.S. & Mikkelsen, J.D. (2012). Type I and II positive allosteric modulators differentially modulate agonist-induced up-regulation of α7 nicotinic acetylcholine receptors. *Journal of Neurochemistry*, 123, 73-83.

Thomsen, M.S., Christensen, D.Z., Hansen, H.H., Redrobe, J.P., & Mikkelsen, J.D. (2009). A α7 nicotinic acetylcholine receptor activation prevents behavioral and molecular changes induced by repeated phencyclidine treatment. *Neuropharmacology,* 56, 1001-1009.

Thomsen, M.S., El-Sayed, M., & Mikkelsen, J.D. (2011). Differential immediate and sustained memory enhancing effects of alpha7 nicotinic receptor agonists and allosteric modulators in rats. *PLoS ONE*, 6(11), e27014, 1-9.

Timmerman, D.B., Grønlien, J.H., Kohlhaas, K.L., Nielsen, E.Ø., Dam, E., Jørgensen, T.D., Ahring, P.K., Peters, D., Holst, D., Christensen, J.K., Malysz, J., Briggs, C.A., Gopalakrishnan, M., & Olsen, G.M. (2007). An allosteric modulator of the α7 nicotinic acetylcholine receptor possessing cognition-enhancing properties in vivo, *Journal of Pharmacology and Experimental Therapeutics*, 323(1), 294-307.

Tuppurainen H., Kuikka J.T., Viinamäki H., Husso M., & Tiihonen J. (2010) Extrapyramidal side-effects and dopamine D2/3 receptor binding in substantia nigra. *Nordic Journal of Psychiatry,* 64(4), 233–238.

Umbrecht, D., Schmid, L., Koller, R., Vollenweider, F.X., Hell, D., & Javitt, D.C. (2000). Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers. *Archives of General Psychiatry*, 57, 1139-1147.

Uteshev, V.V. (2014a). Allosteric modulation as a therapeutic alternative to orthosteric activation in nicotinic acetylcholine receptors. *Calcium Signaling*, 1(1), 24-35.

Uteshev, V.V. (2014b). The therapeutic promise of positive allosteric modulation of nicotinic receptors. *European Journal of Pharmacology,* 727, 181-185.

van der Zeyden, M., Oldenziel, W.H., Rea, K., Cremers, T.I., & Westerink, B.H. (2008). Microdialysis of GABA and glutamate: analysis, interpretation and comparison with microsensor. *Pharmacology, Biochemistry and Behavior*, 90, 135-147.

Vasey, M.W. & Thayer, J.F. (1987). The continuing problem of false positives in repeated measures ANOVA in psychophysiology: a multivariate solution. *Psychophysiology*, 24(4), 479-486.

Wahono, N., Qin, S., Oomen, P., Cremers, T.I.F., de Vries, M.G., & Westerink, B.H.C. (2012). Evaluation of permselective membranes for optimization of intracerebral amperometric glutamate biosensors. *Biosensors and Bioelectronics*, 33, 260-266.

Werkheiser, J.L., Sydserff, S., Hubbs, S.J., Ding, M., Eisman, M.S., Perry, D., Williams, A.J., Smith, J.S., Mrzljak, L., & Maier, D.L. (2011). Ultra-low exposure to alpha-7 nicotinic acetylcholine receptor partial agonists elicits an improvement in cognition that corresponds with an increase in alpha-7 receptor expression in rodents: implications for low dose clinical efficacy. *Neuroscience*, 186, 76-87.

White, T., Anjum, A., & Schulz, S.C. (2006). The Schizophrenia Prodrome. *American Journal of Psychiatry*, 163 (3), 376-380.

Williams, D.K., Wang, J., & Papke, R.L. (2011a). Investigation of the molecular mechanism of the α7 nicotinic acetylcholine receptor positive allosteric modulator PNU-120596 provides evidence for two distinct desensitized states. *Molecular Pharmacology*, 80(6), 1013-1032.

Williams, D.K., Wang, J., & Papke, R.L. (2011b). Positive allosteric modulators as an approach to nicotinic acetylcholine repcetor-targeted therapeutics: advantages and limitations. *Biochemical Pharmacology,* 82, 915-930.

Winterer, G., Gallinat, J., Brinkmeyer, J., Musso, F., Kornhuber, J., Thuerauf, N., Rujescu, D., Favis, R., Sun, Y., Franc, M.A., Ouwerkerk-Mahadevan, S., Janssens, L., Timmers, M.,

& Streffer, J.R. (2013). Allosteric alpha-7 nicotinic receptor modulation and P50 sensory gating in schizophrenia: A proof-of-mechanism study. *Neuropharmacology*, 64, 197-204.

Wu, E.Q., Birnbaum, H.G., Shi, L., Ball, D.E., Kessler, R.C., Moulis, M., & Aggarwal, J. (2005). The economic burden of schizophrenia in the United States in 2002. *Journal of Clinical Psychiatry*, 66(9), 1122-1129.

Wu, H.Q., Pereira, E.F.R, Bruno, J.P., Pellicciari, R., Albuquerque, E.X., & Schwarcz, R. (2010). The astrocyte-derived α7 nicotinic receptor agonist kynurenic acid controls extracellular glutamate levels in the prefrontal cortex. *Journal of Molecular Neuroscience*, 40, 204-210.

Yang, J.S., Seo, S.W., Jang, S., Jung, G.Y., & Kim, S. (2012). Rational engineering of enzyme allosteric regulation through sequence evolution analysis. *PLos Computational Biology*, 8(7), e1002612, 1-10.

Zmarowski, A., Sarter, M., & Bruno, J. P. (2007). Glutamate receptors in nucleus accumbens mediate regionally selective increases in cortical acetylcholine release. *Synapse*, 61, 115-123.

Zwart, R., Filippi, G.D., Broad, L.M., McPhie, G.I., Pearson, K.H., Baldwinson, T., & Sher, E. (2002). 5-hydroxyindole potentiates human α7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release in cerebellar slices. *Neuropharmacology*, 42, 347-384.

Figures

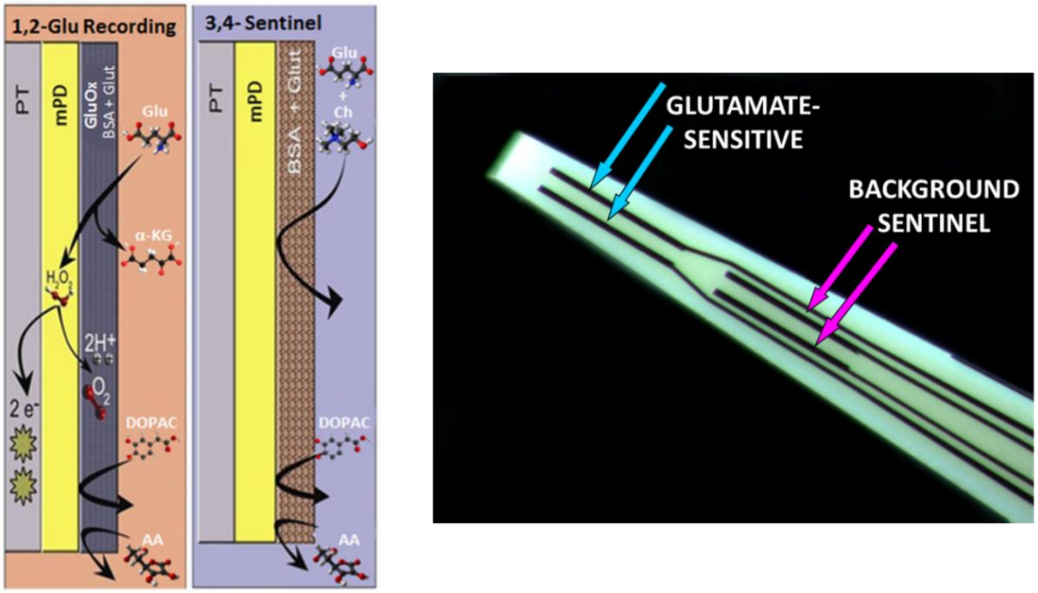
1,2-Glu Recording
3,4- Sentinel
PT
mPD
GluOx
BSA + Glut
Glu
α-KG
H_2O_2
2H+
O_2
2 e-
DOPAC
AA
PT
mPD
BSA + Glut
Glu
+
Ch
DOPAC
AA
GLUTAMATE-
SENSITIVE
BACKGROUND
SENTINEL

Figure 1: enzyme detection scheme and photomicrograph of recording channels on MEA. Glutamate is oxidized by GluOx on glutamate-sensitive channels producing H_2O_2 and α-ketoglutarate. H_2O_2 is then reduced, generating a measurable current. Glutamate is not oxidized on sentinel channels. mPD on all channels prevents dopamine and ascorbic acid from generating a signal. Using this exclusion barrier and self-reference technique, glutamate can be measured selectively with sub-second resolution.

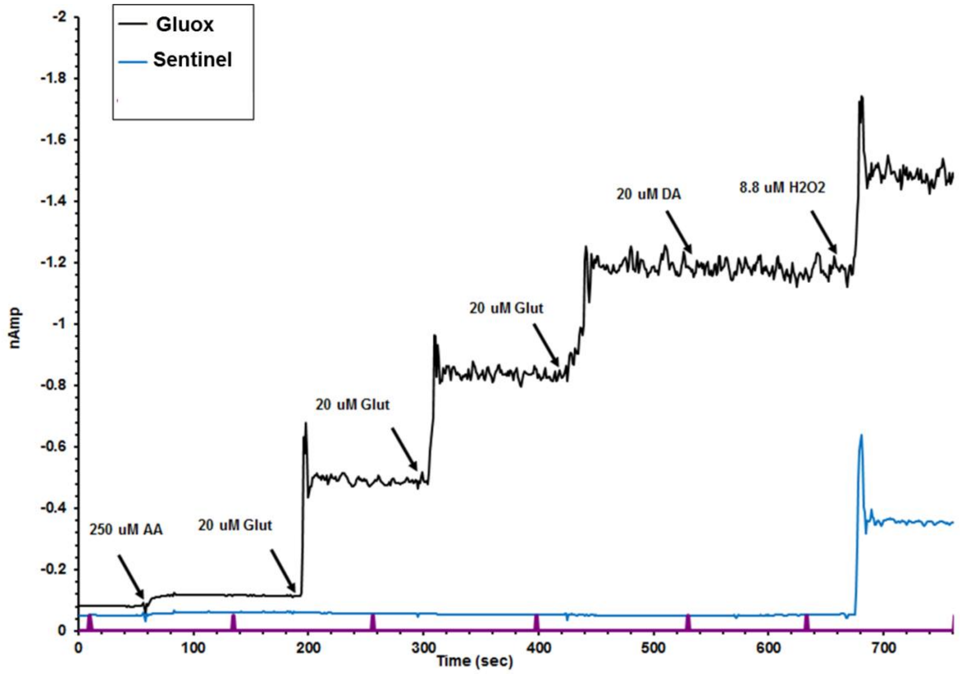

Figure 2: a representative *in vitro* calibration of the MEA immediately prior to implantation. Glutamate-sensitive channels are represented by the black upper tracing whereas sentinel channels are below in blue. Time is represented on the x-axis (in seconds) and current is represented on the y-axis (in nA). Three additions of glutamate produce three equal increases in current. The slope of these additions is used to relate current and concentration of glutamate (nA/µM Glu). No change in current is detected on the sentinel channel after glutamate additions. Additionally, no response is observed after the addition of ascorbic acid or DA on either channel. Lastly, an addition of H_2O_2 is observed equally on all channels indicated equal sensitivity of all channels to the reporting molecule.

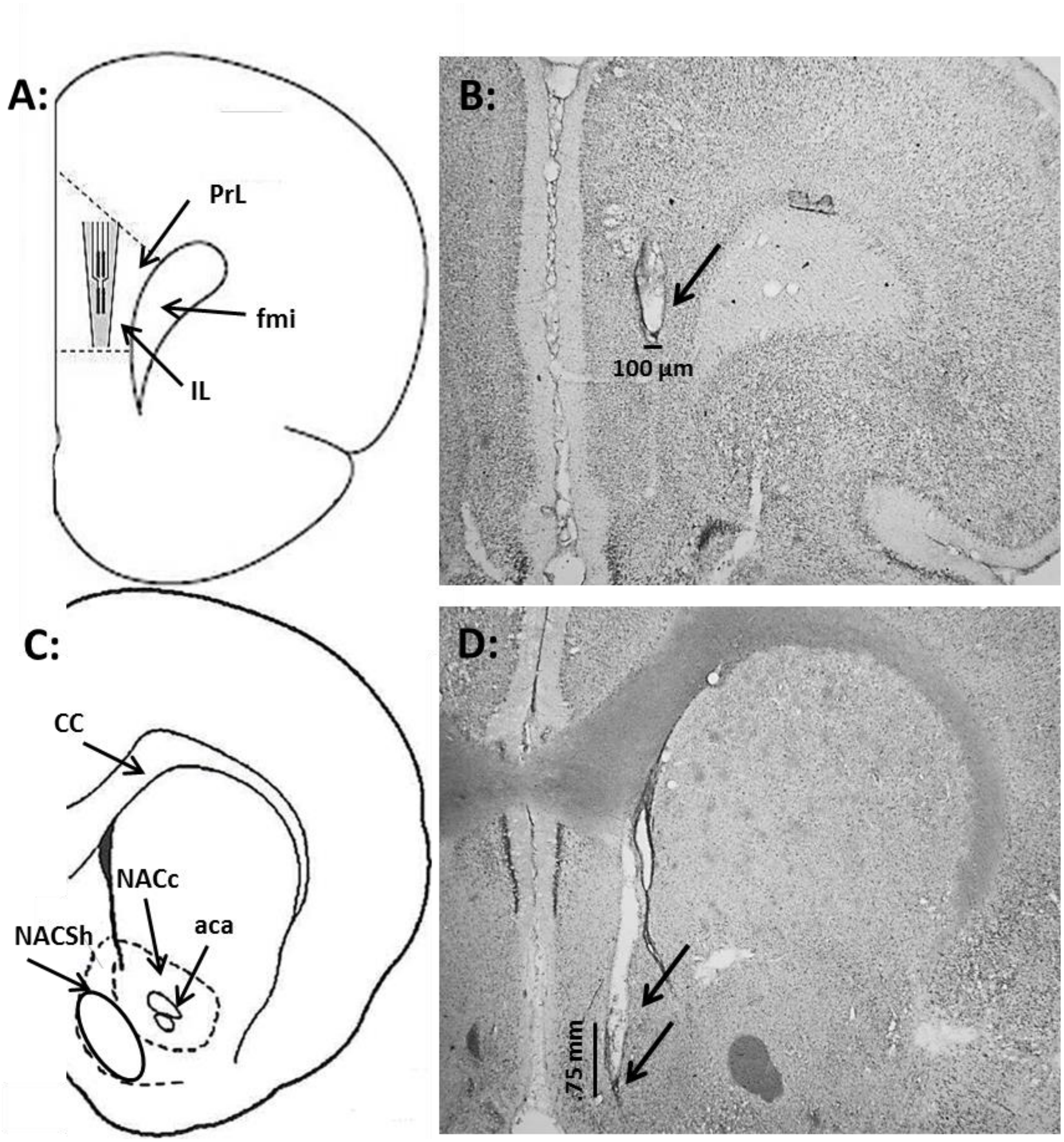

Figure 3: coronal illustrations and sample placement of MEA and cannula. (A) Illustration of MEA between infralimbic and prelimbic corticies within the mPFC. (B) Photomicrograph of MEA placement indicated with arrow. (C) Illustration of cannula placement into the anterior shell of the NAc (NAcSH). (D) Photomicrograph of cannula placement into NAcSH. The upper arrow indicates the end of the steel guide cannula whereas the lower arrow indicates the location of the tip of the infusion cannula.

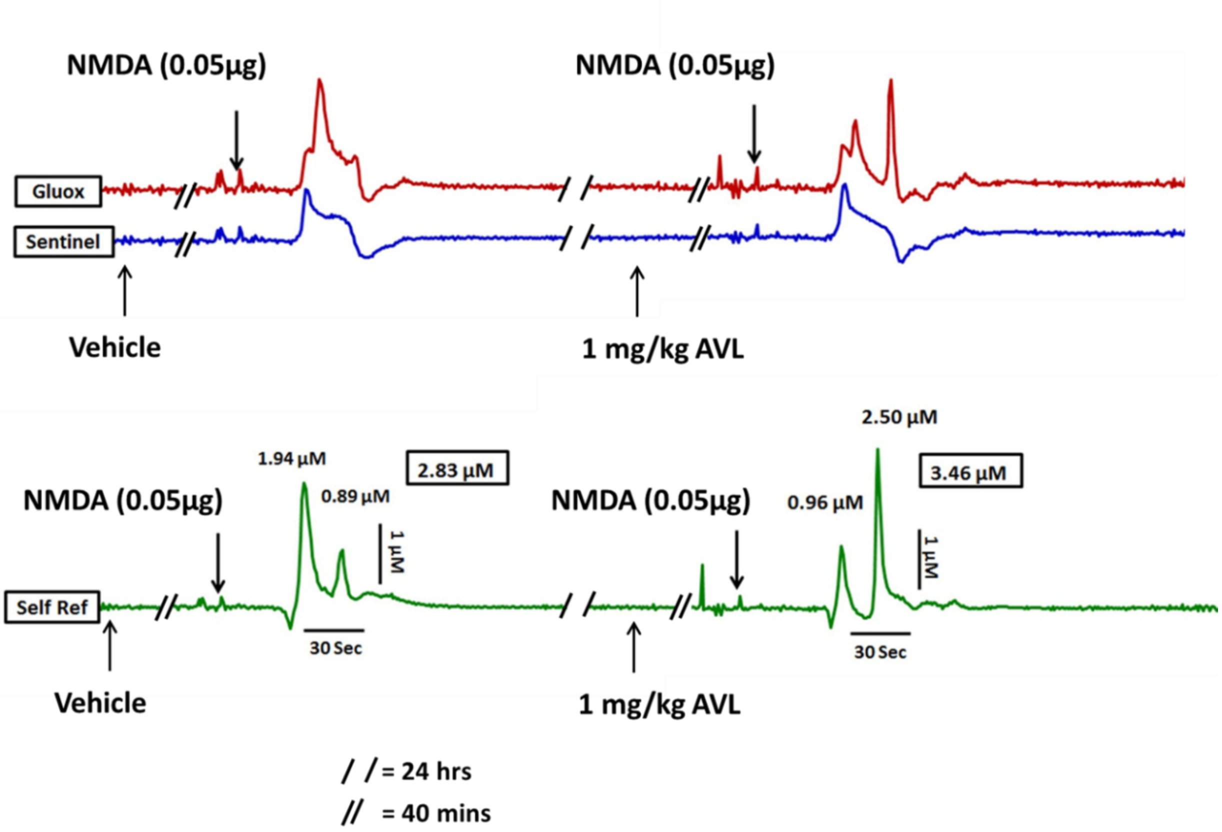

Figure 4: sample tracing from a single animal receiving low-dose of NMDA stimulation after both vehicle and low-dose AVL-3288. The top tracing (red) is from a glutamate-sensitive channel (GluOx) and the middle tracing (blue) is from a sentinel channel. The bottom tracing is the self-referenced tracing obtained by subtracting the sentinel channel from the GluOx channel. In this representative example, the response of 0.05µg NMDA during vehicle treatment was potentiated in the presence of 1 mg/kg AVL-3288 from 2.83µM to 3.46µM glutamate, a percent change of 22%.

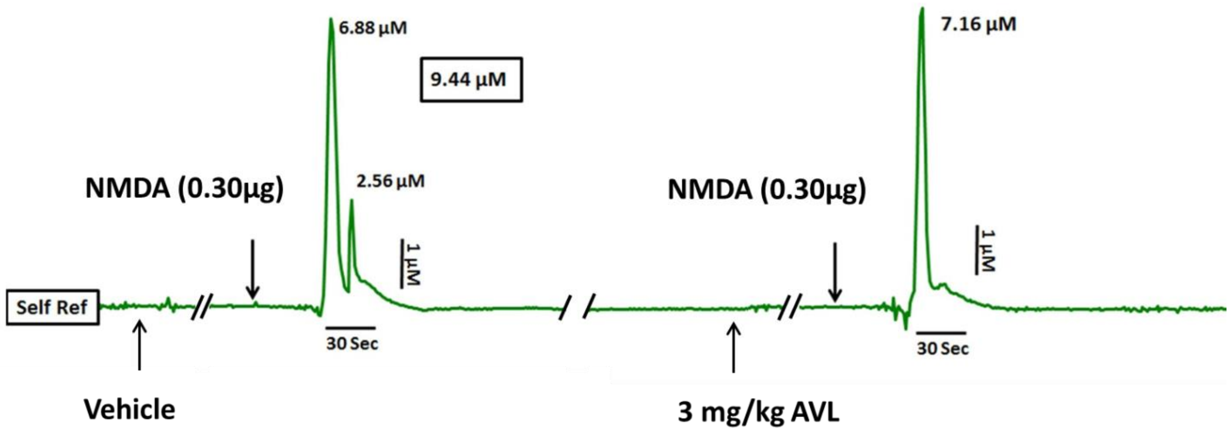

Figure 5: sample self-referenced tracing from a single animal receiving high-dose of NMDA stimulation after both vehicle and high-dose AVL-3288. 3 mg/kg AVL-3288 attenuated glutamate release from 9.44µM to 7.16µM glutamate, a percent change of -24%.

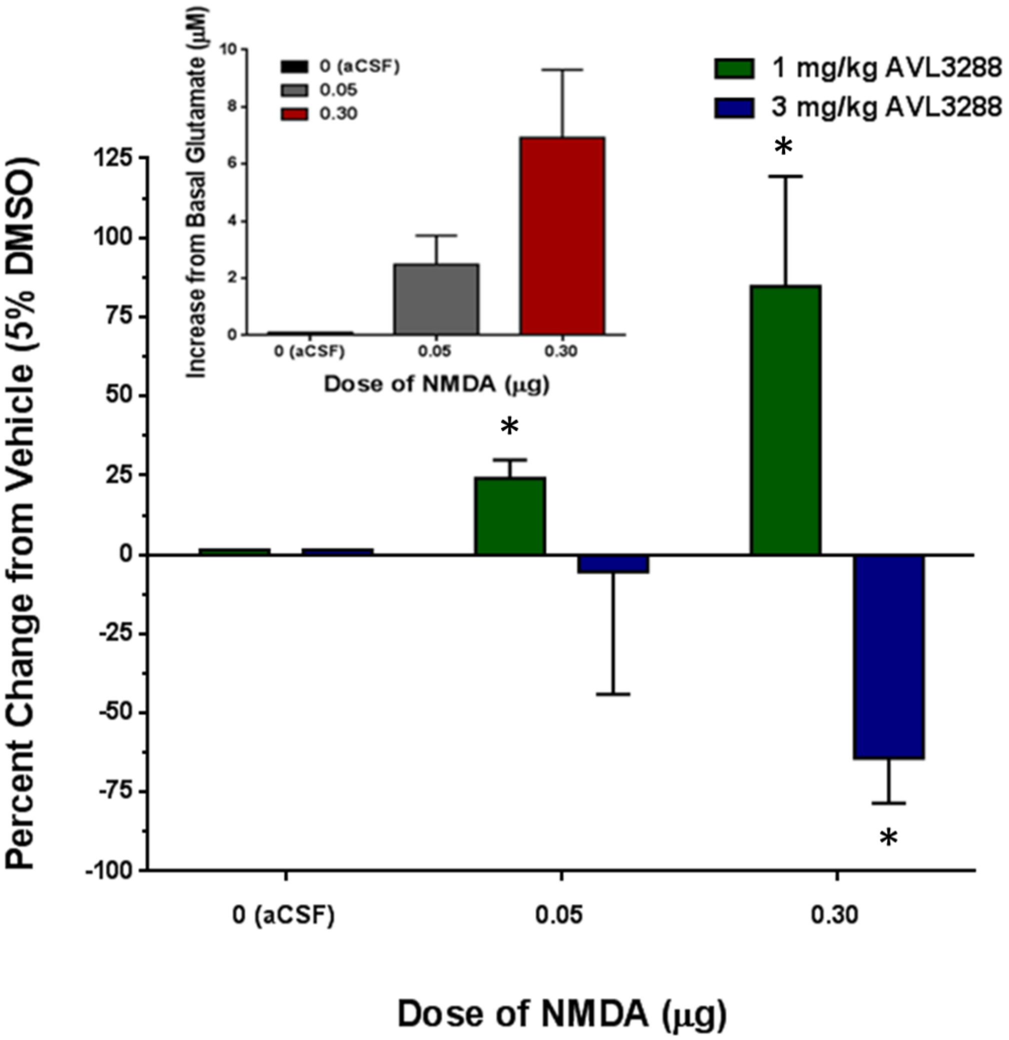

Figure 6: AVL-3288 interacts with mesolimbic stimulation in evoked glutamate release. AVL-3288 data is presented as the percent change from NMDA-evoked glutamate after vehicle treatment. Absolute increases of glutamate during NMDA stimulation is reported in the inset graph. 1 mg/kg AVL-3288 significantly potentiated 0.05µg NMDA-evoked glutamate release. When pretreated with 3 mg/kg AVL-3288, 0.05µg NMDA-evoked glutamate release was potentiated whereas 0.30µg NMDA-evoked glutamate release was attenuated.

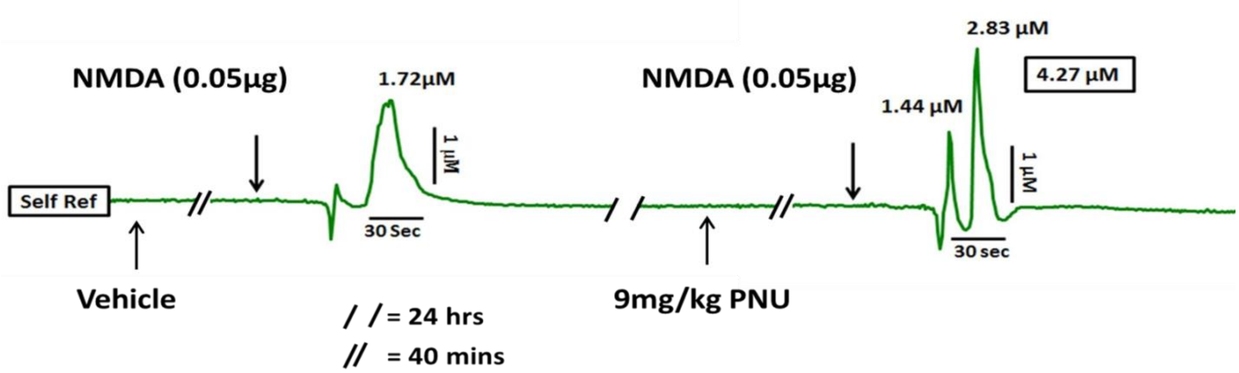

Figure 7: sample self-referenced tracing from a single animal receiving low-dose of NMDA stimulation after both vehicle and high-dose PNU-120596. 9 mg/kg AVL-3288 potentiated glutamate release from 1.72µM to 4.27µM glutamate, a percent change of 148%.

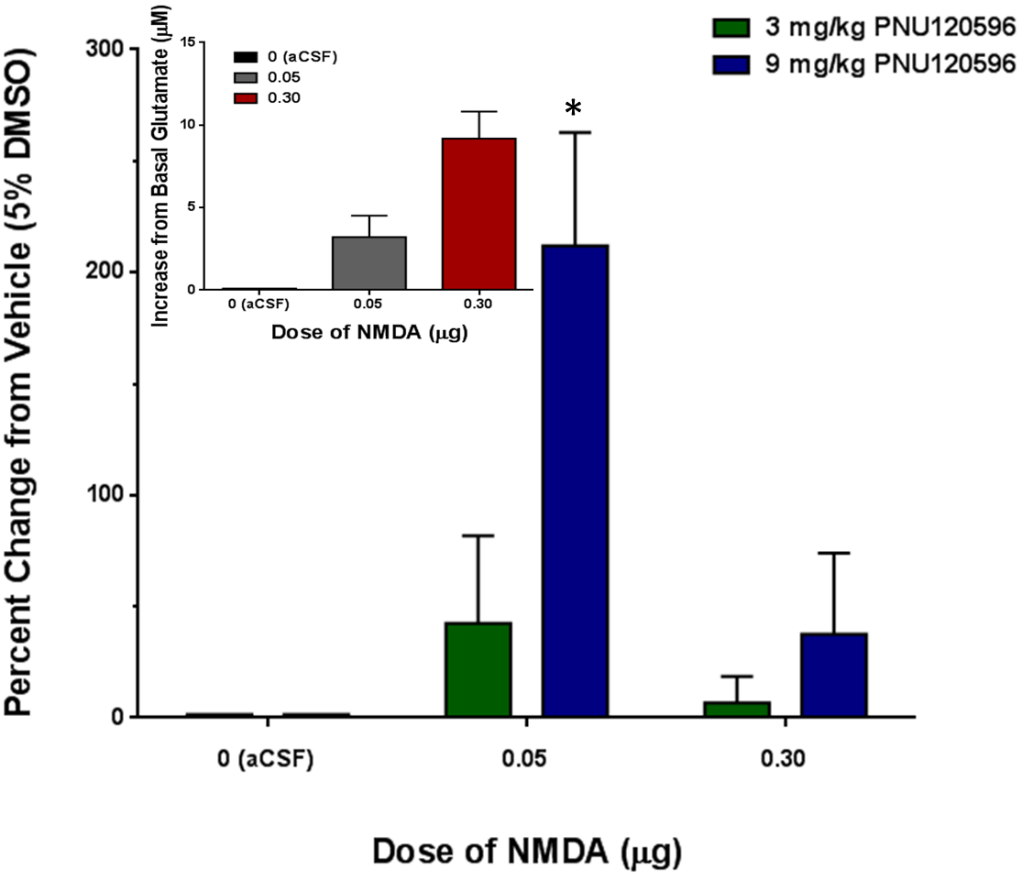

Figure 8: PNU-120596 interacts with mesolimbic stimulation in evoked glutamate release. PNU-120596 data is presented as the percent change from NMDA-evoked glutamate after vehicle treatment. Absolute increases of glutamate during NMDA stimulation is reported in the inset graph. 9 mg/kg AVL-3288 significantly potentiated 0.05µg NMDA-evoked glutamate release. Glutamate release was not significantly potentiated or attenuated after 0.30µg NMDA stimulation at either dose of PNU-120596.