Cognition and Histamine:

H1 Receptor Modulation of Prefrontal Cortex

From Fungi in the Amazon to

Working Memory Function in the Rat Brain

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GLOSSARY:

Below contains a list of important terminology and abbreviations used in this thesis, as well as their respective definitions.

Assay
--A test to determine the characteristics of a compound

Cannula
--(plural cannulae) A tube inserted into an organism for delivering or removing fluid

dlPFC
--Dorsolateral Prefrontal Cortex

Endophyte
--A fungus that lives symbiotically within a plant

Ethnobotany
--The study of the relationships between people and plants

H1R
--Histamine 1 Receptor. Histamine is a neurotransmitter arising from neurons in the hypothalamus at the base of the brain.

Histaminergic
--Interacting with the histamine system

NMDA Receptor
--An N-methyl-D-aspartate, glutamate-activated protein ion channel

Receptor Agonist/Antagonist
--A chemical that binds to a receptor to activate/inactivate it

Spines
--A small protrusion, from the dendrite of a neuron, capable of forming a synapse

Stereotaxic
--Using a three dimensional coordinate system to find locations in organs, e.g. brain

Vehicle
--The solution carrying the drug of interest, e.g. saline vehicle is a control

2-Pyridylethylamine Dihydrochloride
--PDEA, an H1 receptor agonist

ABSTRACT:

Background

This thesis investigated the relationship between spatial working memory and histamine actions at the H1R histamine receptor in the prefrontal cortex (PFC) of rats. Neuropsychiatric disorders, such as schizophrenia, are associated with detrimental cognitive impairments involving dysfunction of the PFC. Many medications administered to patients, such as antipsychotics to patients with schizophrenia, block H1R. Yet it is not known whether blocking H1R would help or further hinder cognitive operations dependent upon the PFC. The current study examined how administering H1R agonists into the PFC can influence spatial working memory performance in a rodent model.

Methods

Spatial working memory function within the PFC was assessed in eleven rats trained on a spatial delayed alternation T-maze test. After baseline performance stabilized, they were administered a dose of the H1 receptor agonist 2-Pyridylethylamine (PDEA) dihydrochloride (0.001, 0.01, 0.1, 1.0 μg/0.5μl) or saline vehicle by cannula (placed by stereotaxic surgery) into each side of the medial PFC. The experimenter testing the rat was blind to the drug treatment condition. The percentage of correctly executed maze trials following drug treatment was compared to those following saline control.

Results

Preliminary results to date, from 9 rats, indicate that the 0.01 μg dose of PDEA significantly improved performance compared to saline control: average performance following saline infusion: 73.1 $\pm$ 2.6 % correct; average performance following 0.01 μg PDEA infusion: 85.0 $\pm$ 3.1 % correct, $p<0.05$. All rats were improved by either the 0.01 μg or 0.1 μg doses of PDEA, compared to their performance on saline control ($p<0.05$).

Conclusions

The results provide preliminary support for the ability of H1R agonists to improve spatial working memory. Further testing in larger samples and of additional doses are warranted in order to confirm effects and establish dose-response relationships. Confirmation of effects would provide novel neurobiological information about the role of the histaminergic system in cognitive function and suggest that blockade of these receptors by medications, such as antipsychotic treatments, may worsen cognitive abilities in patients. It also suggests that histamine agonists, potentially ones derived from natural sources such as plants of the rain forest, may enhance cognitive health.

INTRODUCTION:

The Histaminergic and Central Nervous Systems

The histaminergic system is commonly known through the widespread use of antihistamines to treat allergic reactions. However, the greatest potential of treatments that affect histamine might not be in modulating the immune system, but rather the central nervous system, specifically the brain. A regular and often inseparable side effect of taking antihistaminic allergy medications is drowsiness, which served as the first indication that the histaminergic system can modulate the activity of the central nervous system (Tsujii, Yamamoto, Ohira, Takahashi, & Watanabe, 2010; Yanai et al., 2011). It was this drowsiness that stimulated research into how changes in the histaminergic system could modulate brain functioning (Yanai et al., 2011). In 1951, chlorpromazine was synthesized and although it was initially utilized for its antihistaminic anesthetic effects, it became the first antipsychotic medication used commonly to treat the major psychotics disorders such as schizophrenia (Ban, 2007).

Antipsychotic Medications and Histamine

Chlorpromazine, a first-generation antipsychotic medication, was a landmark drug in the treatment of psychotic disorders. This compound led to the development of the first-generation of antipsychotic medications, which all blocked dopamine D2 receptors with high affinity. D2 receptor blockade has been related to antipsychotic efficacy, but also to serious motor side effects, sedation, and cognitive impairment. Second-generation antipsychotics (SGAs), also known as atypical antipsychotics, were developed to treat psychosis, as well as problems in mood and cognition, while minimizing unwanted side

effects (Gaebel & Zielasek, 2015; Wu et al., 2005). These agents all block dopamine D2 receptors with lower affinity than the first-generation compounds, thus lessening their motor side effects. In contrast to their predecessor medications, SGAs target different brain neurotransmitter systems simultaneously, and this is thought to underlie their more benign influences on cognition (Roth, Sheffler, & Kroeze, 2004).

The breakthrough SGA, clozapine, and the commonly used SGA, risperidone, both have diverse receptor affinities, but in particular they share a high affinity for H1 histamine receptors (Shahid, Walker, Zorn, & Wong, 2009). Another current frequently-prescribed SGA is aripiprazole, which can treat psychosis but has mixed effects on cognition. To better elicit patient benefits, aripiprazole has been tailored to effect dopamine and serotonergic receptors. However, aripiprazole also has affinity for the histamine H1 receptor (Girgis et al., 2011; Sumiyoshi, Higuchi, & Uehara, 2013). The balance of H1 receptor effects could be an important, although largely overlooked, component for optimizing treatments. However, it is not known whether these actions should be amplified or diminished to help patients, as there is little known about the role of H1R in the brain circuits that mediate higher cognition. Blockade of the H1R is thought to underlie the rapid weight gain caused by these agents (He, Deng, & Huang, 2013), but clinicians must consider whether diminishing H1R actions would be helpful to patients such as in its effects on cognition.

The study of complex medications to treat complex psychiatric disorders, such as schizophrenia, may limit understanding of other applications of treatments and the potential impact they could have on the population (Wu et al., 2005). Although mental illnesses are complex, this complexity does not completely explain the slow progress of

psychopharmacology; rather, it reveals a foundational lack of understanding for the biological intricacies of the brain, without which, the potential for treating brain dysfunction remains farther out of reach. Until the healthy brain's neurobiology is better understood, medical psychiatry will continue to be relatively behind its true treatment potential in comparison to other medical fields. Research focus needs to continue to broaden to elucidate the impact of understudied systems, such as the histaminergic system. The brain is complicated and in order for research to focus upon those aspects that will yield the greatest medical potential, neurobiologists must become detectives.

Chlropromazine's Backstory

It is the consistent reappearance of something first ignored that often constitutes the grounds of a genuine clue. In the case of trying to treat psychosis, the relevance of the histaminergic system has been relatively overlooked. First, medications that target the histaminergic system were medically pursued as a means to control immune responses. Then, noting the sedative properties of these medications, attention was shifted towards the histaminergic system's potential to aid in anesthesia by affecting the nervous system. Finally, these anesthetic effects pointed investigation into the central nervous system and eventually, when these medications were turned towards psychiatric disorders, the result was the first antipsychotic drug, chlorpromazine. The issue at that moment of psychopharmacology, was that there seemed to be only a coincidental connection between chlorpromazine's histaminergic modulation and the treatment of psychosis.

When attempts were made to explain the therapeutic effects of chlorpromazine, our scientific understanding of the relationship between the histaminergic system and

psychosis was based primarily on conjecture (Miyamoto, Miyake, Jarskog, Fleischhacker, & Lieberman, 2012). While the discovery of first-and second-generation antipsychotics will forever be a turning point in the treatment of psychiatric illness, it should not be the conclusion. Neuroreceptor interactions are clues to important new questions: How does modulating the brain's histaminergic system affect the way we think, our cognition, and in its dysfunction, mental illness?

Cognitive Deficits from Schizophrenia

The symptoms of schizophrenia can be divided into to three types: positive, negative and cognitive. All three are detrimental to the patient, but it is the severity of cognitive symptoms that place a patient with schizophrenia at a more fundamental disadvantage in everyday life (Strassnig et al., 2015). Psychosis is thought to be related to excessive dopamine D2 receptor stimulation in the caudate nucleus of the basal ganglia, as well as to possible abnormalities in the PFC. Given the complexities and relatively poor understanding of the neurobiology of psychosis, this thesis will focus on the cognitive deficits of schizophrenia, which are so impairing. In particular, it is the impairment of working memory that can make functioning in everyday life more difficult (Lesh, Niendam, Minzenberg, & Carter, 2011).

Working memory can be thought of as the brain's mental sketchpad, in that it holds newly-acquired information so that it can be readily accessed in problem-solving situations. This type of memory impairment could be exemplified by imagining someone coming to a fork in the road and, after picking a direction, subsequently not being able to remember which direction was picked because the spatial information was not being

effectively held in the brain's working memory (Arnsten, 2013). A study focused on working memory is essentially a means of taking a very complicated medical disorder, schizophrenia, and prioritizing a specific behavioral aspect, that is working memory impairment, so as to focus on improving the patient's well-being (Sumiyoshi et al., 2013).

Amazonian Endophytes

My interest in the role of histamine in working memory was originally inspired by my experience in a remarkable Yale course in ethnobotany that included a trip to the Amazonian rainforest in Ecuador to collect specimens. Our Yale research team, led by Professor Strobel, designed experiments to biochemically assay purified endophyte secretions. Endophytes are fungi that live symbiotically within plant tissues. Before traveling, each of my peers and I had to decide what particular subset of plants to choose out of the staggering diversity of the Amazon. Each of us went in different directions; I decided to use the ethnobotanical remedies historically developed by Amazonian tribes to create a connection between plant species and their potential to affect the central nervous system. The overarching goal was to find botanical medicines that might alleviate brain dysfunction on a psychiatric level.

Psychoactive Drug-Screening Program

However, this idea was not new, and I found the research of Dr. Bryan Roth who had traveled to Peru with a team to collect botanical samples. Subsequently he distilled the samples to extracts that could be run through the Psychoactive Drug Screening Program (PDSP), which he now operates through the National Institute of Mental Health and is a supported program at the University of North Carolina (McKenna, Ruiz, Hoye, Roth, &

Shoemaker, 2011). The PDSP is the means with which Dr. Roth assays the ability of a compound to interact with a myriad of neurotransmitter receptors (Catapano & Manji, 2007). His worked showed me two things: it allowed me to not only study the ethnobotanical rationale behind his plant collection list, but it also helped me to refine my list by discounting species and even whole genus of plants which Dr. Roth already showed to have minimal psychoactive potential. By having a list of plants that were both used for ethnobotanical remedies, and that indeed contained compounds with neuroreceptor activity, I could make the collection trip to the Amazon in Ecuador not to study the plants, but instead to study the psychoactive potential of their endophytes (illustrated in Figure 1).

The benefit of studying the secretions of the plant species-specific symbiotic endophytes is that the fungi's past evolutionary specialization to a certain plant species allowed the two species to coevolve over time. This increases the likelihood that the two species produce similar compounds, or that the compound once thought to be produced by the plant was actually produced by the endophyte within it. Either way, by assaying the secretions from the cultured endophytes, through the PDSP, it was possible to gather data on their neuroreceptor activity.

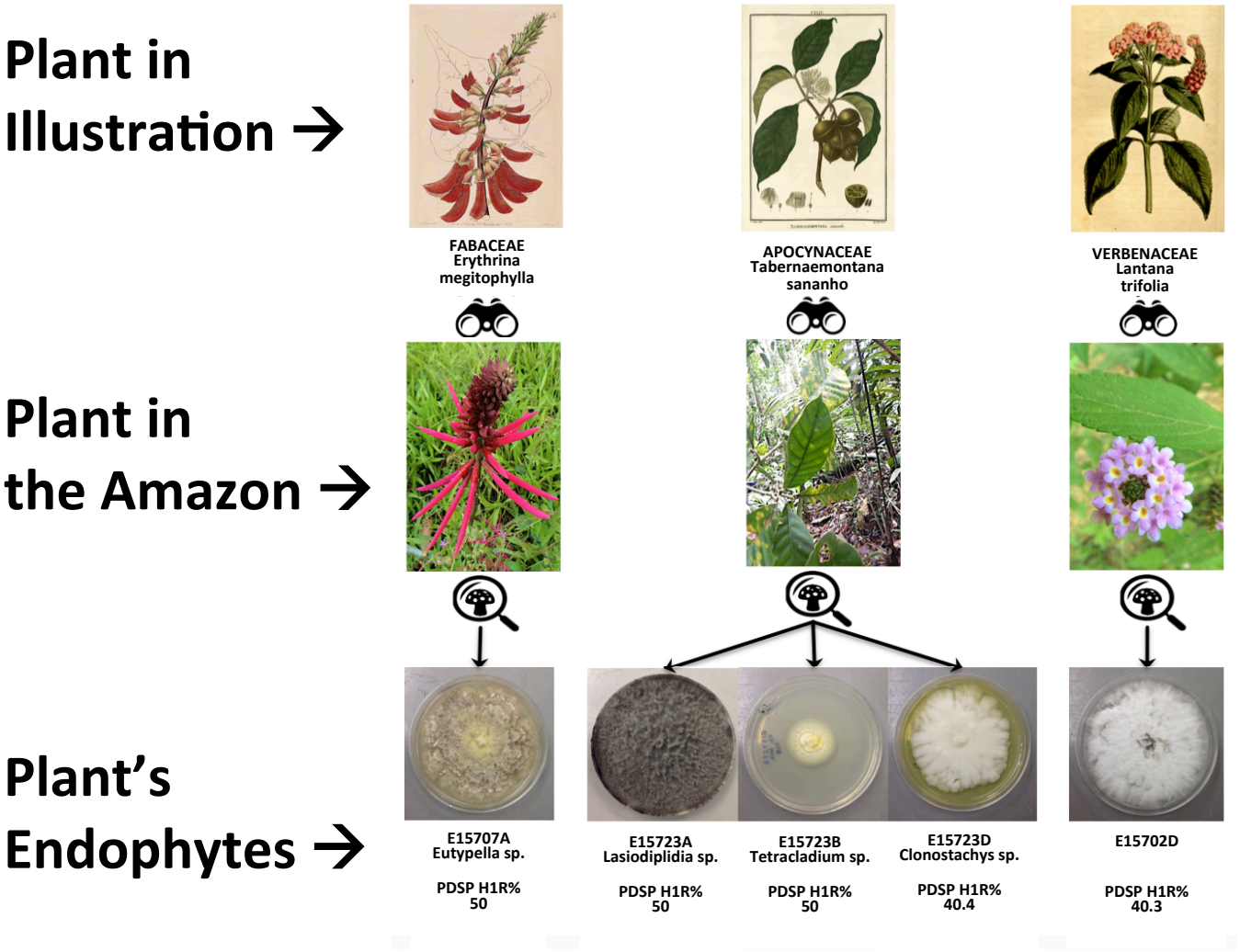

Figure 1. Progression of Research from Target Plant to Found Plant to Cultured Fungi
This figure illustrates the selection of plant species to search for in the Amazon. Plants could then be found by recognizing the correct phenotype. Plant tissue is then collected and used to culture endophytic fungi. Each fungi's extracts are then assayed through the PDSP.

PDSP Results

The PDSP data collected curiously showed that the neuroreceptors most heavily responsive to the endophyte compounds, that I had extracted, were the H1 histaminergic neuroreceptors (illustrated in Figure 2). This, in conjunction with the history of chlorpromazine brought me to Dr. Arnsten, Yale's leading neurobiological researcher in neuroreceptors pertaining to working memory (Arnsten, 2009; Arnsten, Paspalas, Gamo, Yang, & Wang, 2010), who inspired me to focus study on effects of H1R activity on working memory. With Dr. Arnsten's guidance, it was possible to adopt a preexisting experimental model used to reliably evaluate working memory in rats. Using this paradigm in conjunction with drug-based modulation of the H1R histaminergic system in the rat's brain, through direct PFC infusion, allows for the relationship between working memory and the histaminergic system to be explored (Robbins, 2000). The drug being administered is not identical to the compounds found in the endophytes, but the experimental drug does possess the same H1R agonistic capabilities. The rationale behind targeting the PFC is thanks to Yale's Dr. Goldman-Rakic whose historic work determined that it is the dorsolateral PFC (dlPFC) that controls spatial working memory within the primate brain (Goldman-Rakic, 1988, 1996).

PDSP Data: Endophyte Compound Binding Properties for a Myriad of Neuroreceptors

Data represent mean % binding inhibition (N = 4 determinations) for compound tested at receptor subtypes.
Significant inhibition is considered > 50%. In cases where negative inhibition (-) is seen, this represents a stimulation of binding

Color Key: >10 >20 >30 >40 >50 % Inhibition

CMPD / ID	5-HT1B	5-HT2C	5-ht5a	Alpha1A	Alpha1B	Alpha2C	D1	D5	DAT	GABAA	H1	M4	M5	Sigma 1	Sigma 2
33976 / **E15723A**	11.3	-6.7	-11.1	-5	-6.3	4.2	-7.7	13.3	-3.4	40	**50**	-1.8	-0.5	-12.7	5
33977 / **E15723B**	3.2	11	-12.8	0.3	14.9	-4.1	-10.2	28	-4.1	-10.7	**50**	-8.4	-11.2	-6.3	-3
33978 / **E15702D**	16.5	0.5	-0.8	6.9	2.9	0.6	24	-4	5.6	5.7	**40.3**	-12	39.9	-0.6	14.1
33979 / **E15723D**	3.6	-1.7	-2.4	1.3	-18.3	-1.3	-10.8	25	10.5	15.6	**40.4**	-16	25.9	14.7	12.2
33980	8	3.3	-10.4	18.2	-15.3	-5.8	-6.9	15.8	4.2	-1.2	34.3	1.4	17.8	10.3	3.2
33981	8.1	5.2	-2.1	-11.6	-14.3	-0.8	-1.8	8.1	0.5	9.7	12.5	-27.5	20	8.8	3.6
33982	12.4	10.1	20.4	-10.6	-23.1	12.5	5.3	9.1	16.7	15.7	-25.6	-16.2	22.9	7.3	10
33983	10	-1.9	-0.7	-10.2	-19.7	14.2	6	13.1	7.6	8.9		8.4	8.3	12.4	5.6
33984 / **E15707A**	7.8	13	-4.2	-14.4	-23.6	2.3	14.4	15.7	-13	24.1	**50**	21.7	1.6	16.2	15.1
33985	6.9	15.6	-2.7	-9.5	-17.7	2.7	10.1	19.4	-6.4	28	-7.2	20.2	1	12.4	-0.3

Figure 2. The Capability of Different Fungal Extracts to Bind to Neuroreceptors
This figure illustrates the data received from the PDSP. The PDSP's assay records the ability of each sample to displace a signal compound occupying different neuroreceptor types. This displacement is recorded as a percentage. The larger the percentage, the more capable a sample is at binding to that specific neuroreceptor. Percentages at or below 50% are not considered significant by the PDSP, but they are still valid data. This data table specifically shows the H1R binding capability for five extracts.

Species Differences in PFC and Working Memory

Nonhuman primates have highly developed brains, including an extensive dlPFC that confers powerful working memory abilities. However, infusion experiments in nonhuman primates are extremely expensive, and thus are rarely the appropriate starting place for neuropsychopharmacological research. Rodents have a much smaller PFC, and do not have the newly evolved dlPFC, but they do have a medial PFC region that has been shown to be necessary for performance of spatial working memory tasks (Kolb & Robbins, 2003). As rodents are nocturnal, their sensory systems are not well-equipped to process visual features. In particular, albino rats have exceptionally weak vision and do not even possess a ventral stream for visual feature analysis; only primates have that. Instead, the nocturnal rodent brain is proficient at processing visual spatial information. Due to this species-specific specialization, the ideal method of observing working memory in rodents is to use a paradigm that relies on their spatial working memory specifically, especially as the neurobiology of spatial working memory has been extensively studied in primates, thereby allowing for cross-species research comparisons.

Spatial Working Memory

On the cellular level, pyramidal neurons within layer III of the monkey dlPFC are crucial for spatial working memory. When an animal attempts to remember the recently learned location of something in order to solve a puzzle, layer III pyramidal neurons excite each other through NMDA receptor synapses on dendritic spines to keep the spatial position “in mind”. These pyramidal cell microcircuits have a preferred orientation, i.e. they preferentially fire to the memory of an event in a particular area of visual space, but not to

other areas. These neurons are called “Delay cells” because they are able to maintain firing across the delay period in a spatial working memory task.

For example, one cluster of Delay cells maintains firing for the memory of stimuli at 90 degrees, while a different cluster of pyramidal neurons fires for the memory of stimuli at 45 degrees. It is this persistent neural activity that allows for the spatial information to be available for immediate recall as a working memory (Goldman-Rakic, 1995). To assess the spatial working memory of an animal is to assess how their pyramidal neurons, in the PFC, are behaving. Parallel physiological studies in the rat PFC have shown that there are also neurons that are able to fire during the delay period (Yang, Shi, Wang, Peng, & Li, 2014). However, rodent PFC neurons cannot maintain firing for as long a time as those in monkeys, and thus must create a chain of responsive neurons to hold memories as long as required. The PFC neurons also interconnect with the hippocampus to allow correct performance at longer delays.

H1 Receptors and the PFC

The brain’s histamine pathway originates from the tuberomammillary nucleus (TMN) of the posterior hypothalamus and from this location it makes connections to many other brain regions including the PFC (Blandina, Munari, Provensi, & Passani, 2012; Brown, Stevens, & Haas, 2001; Haas & Panula, 2003). In reaction to the histamine signal, there are four known histamine neuroreceptor subtypes, H1, H2, H3 and H4, which are distributed throughout the brain (Funke et al., 2013; Martinez-Mir et al., 1990).

Interestingly, the PFC does have a population of H1 receptor-containing neurons and further intriguing is evidence that individuals with schizophrenia have a reduced H1

neuroreceptor binding profile within their PFC (Iwabuchi et al., 2005). This suggests that, should someone ingest a compound that has H1R agonist activity, perhaps from an ethnobotanical remedy, that this compound could affect the brain's H1 histaminergic system, including the H1 neuroreceptors within the PFC (Panula & Nuutinen, 2013). If that person has schizophrenia, perhaps it could treat the H1 activity reductions in the PFC. Within a rat model, it has been shown that upon the administration of SGAs, the rat medial PFC shows a greater histamine efflux.

Specifically, when systemic administration of the SGA clozapine is compared to that of the H1 antagonist pyrilamine in rat models, both induce similar histamine efflux in the PFC. This implicates antipsychotics such as clozapine as influential on the H1 neuroreceptor system in its relevance to memory (Fell et al., 2012; Roegge, Perraut, Hao, & Levin, 2007). In addition, it has been shown that the SGA olanzapine has a significant occupancy effect on the subpopulation of H1 neuroreceptors in humans (Sato et al., 2015). Thus, if compounds that modulate the H1 neuroreceptor's activity are administered directly to the PFC, then effects of directly modulating PFC H1 receptors can be assessed (Schwartz, Arrang, Garbarg, Pollard, & Ruat, 1991).

There has been one intriguing study demonstrating that mice with a knockout for the H1 receptor showed impaired spatial working memory performance (Zlomuzica, Ruocco, Sadile, Huston, & Dere, 2009). However, there have been no studies of effects of histamine agonists on the working memory functions of the PFC, despite the clinical relevance of this issue. The current study provides the first examination of histamine H1R actions on the working memory functions of the rodent PFC. By infusing an H1 receptor

agonist directly into the rat PFC, in conjunction with the animal trying to solve a spatial working memory puzzle, it is possible to observe the potential effect of modulating the H1 histaminergic neurons of the PFC on spatial working memory functioning.

T Maze Paradigm

The experiment performed utilizes H1 neuroreceptor agonists being infused into the rat PFC, such that the animal's subsequent ability to solve a T maze spatial working memory puzzle can be observed. The fundamental principle of a T maze is that it positions the animal at the base of a T-shaped maze and then, as the animal travels the vertical length of the T, it approaches the opportunity to turn right or left at the top branch point. After it has made a decision, the rat is given a reward and placed at the base of the T again, thus completing trial 0 of 10. When the rat reaches the juncture point for trials 1-10, it is only rewarded if it chooses the path opposite the path previously rewarded in prior trial. Through training, the rat learns to alternate with proficiency which arm of the maze it chooses, to the point at which a stable model of spatial working memory is created. Then, by infusing the H1 histamine receptor agonist, PDEA into the rat PFC, it is possible to observe subsequent changes in working memory dependent maze solving behavior.

METHODS:

Animal Model

Male Sprague Dawley rats (n=11), weighing 300-550g, were individually housed in filter frame cages. These rats were kept on a 12 hr light/dark cycle, and the experiment was conducted during the light phase. The animals were fed a diet of auto-claved Purina rat chow (14-16g/rat per day) immediately after behavioral testing. Water was available *ad libitum*. Rats were weighed weekly, and maintained at ~ 300-400g for young rats (age 4-12 months) and 400-550g for aged rats (age 24-36 months). Food rewards during cognitive testing were highly palatable miniature chocolate chips; this minimized the need for dietary regulation to account for caloric intake through food rewards. Rats were assigned a single experimenter who handled them extensively before behavioral testing. The experimenter testing the animal was blind to the drug treatment conditions. All animal procedures were performed in accordance with the protocol approved by the Yale Institutional Animal Care and Use Committee.

Delayed –Alternation Task

The delayed-alternation task was selected as the paradigm to assess PFC function, as it is widely-used in testing of PFC function in rats and is considered to be a valid task for sensitively assessing alterations in PFC function. Use of this task provided the opportunity for comparison with previous studies in Dr. Arnsten's laboratory of (1) PFC dopamine depletion and (2) stress, for which this paradigm was also used. Optimal performance of the delayed-alternation task requires processes subserved by the PFC that include spatial working memory (Goldman-Rakic, 1987), egocentric spatial processing (Kesner,

Farnsworth, & DiMattia, 1989) and inhibition of proactive interference and inappropriate motor responses (Mishkin, 1964). Cognitive testing methods were similar to those developed previously in this laboratory by Murphy and Arnsten to examine the effects of stress on spatial working memory in rats (Murphey & DavisGw, 1994).

Rats were initially habituated to a T-maze (dimensions, 90 by 65 cm) (illustrated in Figure 3) for 5 days until they were readily eating chocolate chips placed in the food wells at the end of each maze arm. After habituation, rats were trained to perform the delayed-alternation task. On the first trial, animals were rewarded for entering either arm. In subsequent trials, for a total of 10 trials per session, rats were rewarded only if they entered the maze arm that was not chosen for reward in the previous trial. Between trials the maze was wiped with alcohol to remove any olfactory clues.

The delay between trials was "0" sec during the initial training. After approximately 2-4 weeks of training sessions, animals underwent surgery to implant indwelling guide cannulae directed to the PFC location. Testing on the delayed-alternation task was reinstated only after the implant had healed completely, ~1 week after surgery. For each animal, delays were adjusted to produce performance levels stabilized at between 60-80% correct. Delays averaged anywhere from 0-20sec for aged rats, to 60+ seconds for young rats. This baseline level of performance allowed for the detection of either maze solving improvement or impairment with drug administration.

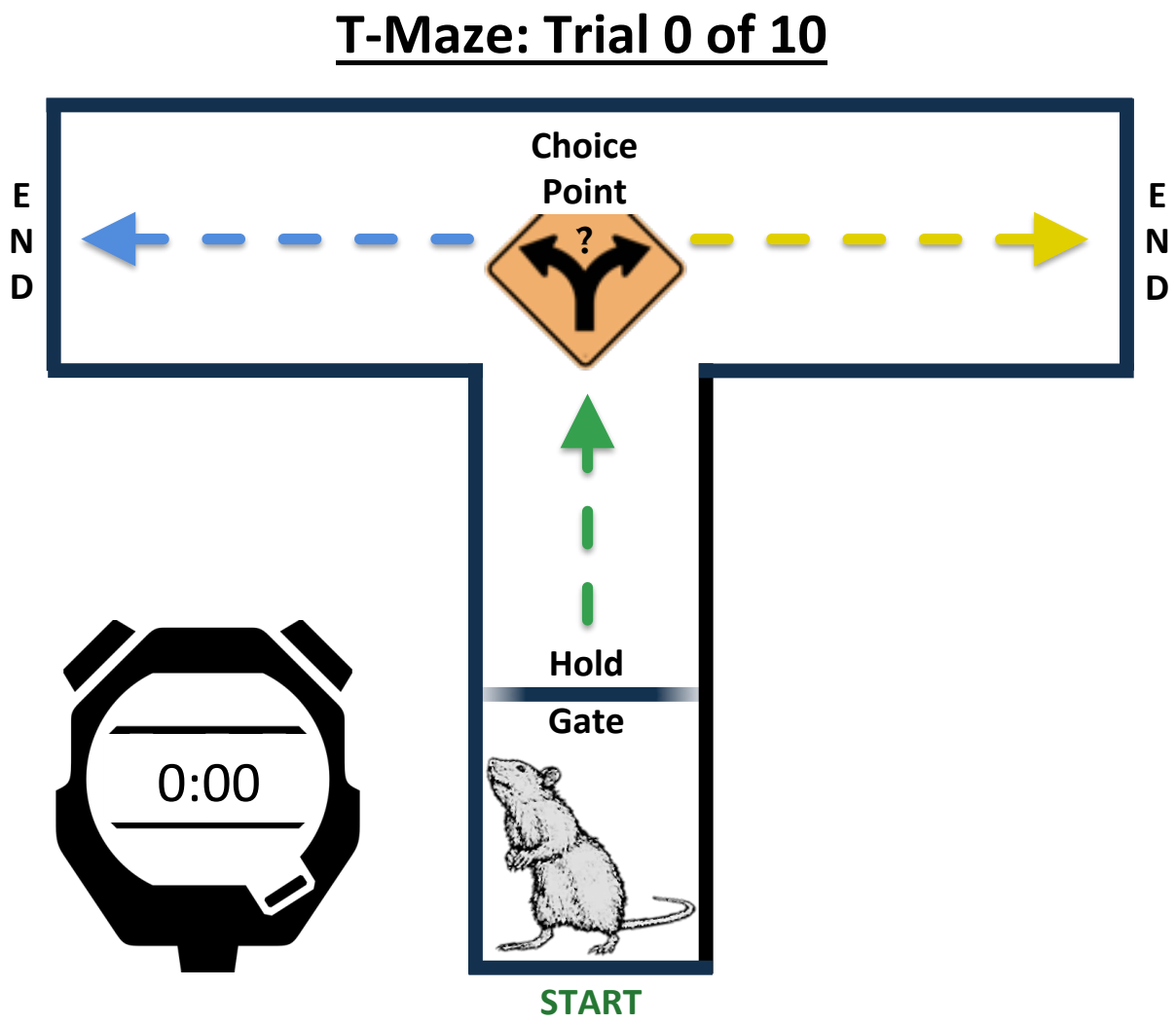

Figure 3. T Maze for Delayed-Alternation Task for Prefrontal Cortex Testing
The figure illustrates the T-maze for testing rats on a spatial delayed alternation task. To perform a trial correctly, rats must recall the arm of the maze it went to on the previously rewarded trial so the rat can then go to the other side for a chocolate chip reward. Baseline performance is stabilized at ~80% trials correct before each drug or saline control. The intertrial delay is 5-30 seconds.

Cannula Implantation

After training on the delayed-alternation task, rats underwent stereotaxic implantation of chronic guide cannulae. Surgery was performed under injectable (IP)ketamine + xylazine, with inhalation of isoflurane if needed as a supplement anesthesia, using aseptic methods. Guide cannulae consisted of 9.0mm of 23 ga stainless steel directed immediately dorsal to the medial PFC [prelimbic (PL) PFC; stereotaxic coordinates: anteroposterior, +3.2mm; mediolateral, +/- 0.75mm; dorsoventral, -4.2mm] (illustrated in Figure 4). Cannulae (Plastics Products) were affixed to the skull using dental cement secured with sterile stainless steel screws. A sterile stylette was screwed into place in each guide cannulae to prevent occlusion. Stylettes were changed on a regular basis to maintain patency. Great care was taken to minimize pain and infection after the operation to decrease stress to the animal. Rats were monitored on a daily basis for signs of distress or infection, and were initially treated with subcutaneous injectable metacam (1.0mg/kg) to decrease pain. Rats were housed singly during the period after the operation.

Infusion Procedure

Animals were initially adapted with a mock infusion protocol to minimize any stress associated with the procedure. Rats were gently restrained while stylettes were removed and replaced with 30 ga sterile infusion needles that extended 1mm below the guide cannulae. The rats received bilateral infusions of 2-Pyridylethylamine dihydrochloride at concentrations of either 0 (saline vehicle), 0.01, 0.1 or 1 µg/0.5µl The doses were chosen to provide a range for pilot testing. Infusions were driven by a Harvard Apparatus syringe pump set at flow rate of .225 µl/min using 25 µl Hamilton syringes for an infusion time of 2

min. Needles remained in place for 2 min after the completion of the infusion. Stylettes were inserted back into the cannulae and behavioral testing began immediately after the infusion procedure. It is also worth noting that the administered drug is likely metabolized out of the rat's system by the next day, but to be sure an additional week is given between infusions, during which it is verified that the performance returned to normalized baseline. The experimenter testing the rat was blind to drug treatment conditions.

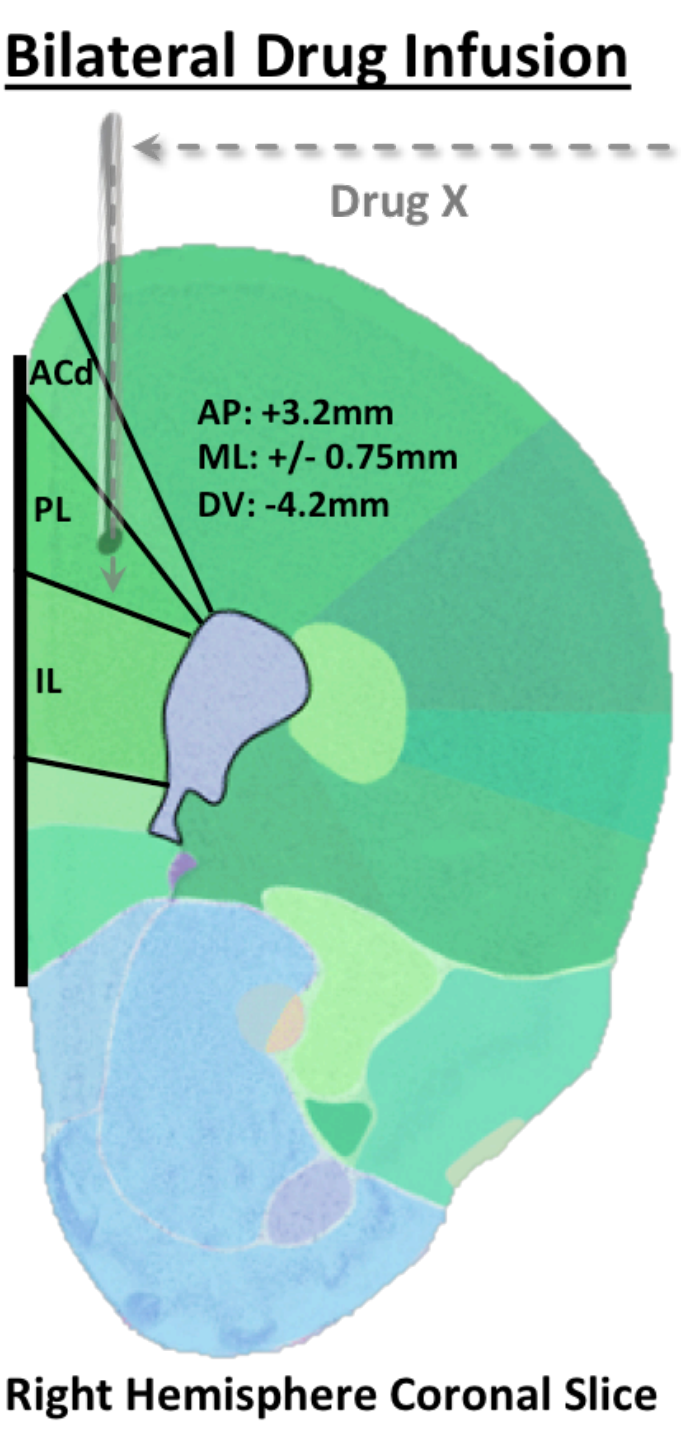

Figure 4. Prelimbic Prefrontal Location For Cannula Infusions

The figure illustrates a coronal slice of rat prefrontal cortex. The cannula is placed at specific coordinates in the prelimbic region by stereotaxic surgery. Infusions are made into the cannulae at varying concentrations of 2-Pyridylethylamine, in the treatment conditions, and saline vehicle, in the control condition.

Abbreviations:

Brain regions:
ACd, dorsal anterior cingulate cortex
PL, prelimbic cortex
IL, infralimbic cortex

Cannula placement Coordinates: AP, anterior-posterior
ML, medial-lateral
DV, dorsal-ventral

Data Analysis

The percent of correct trials was calculated for each rat for each drug concentration tested. Performance for each drug concentration was compared to performance with vehicle, including data from all rats that received that drug concentration, with independent two-tailed t-tests. The peak performance on drug for each rat was also compared to performance on vehicle. Results were considered significant at $p<0.05$. Means and standard errors were calculated for performance at vehicle and each drug dose and graphed to interpret relationships between conditions.

RESULTS:

Statistics

The study is still in progress. To date, 9 of the 11 animals were tested with the saline vehicle to establish control values. Nine animals were tested at 0.01 μg, 8 at 0.1 μg and 6 at 1.0 μg of PDEA. Ten rats were tested at 0.01 μg and/or 0.1 μg. Seven rats were tested at both doses; two rats were tested only at 0.01 μg and one at 1.0 μg only. 50% of rats exhibited peak performance at .01 μg and the remaining 50% exhibited peak performance at .1 μg. As this was a pilot study, peak performance was also calculated for each rat at either the 0.01 μg or 0.1 μg concentration and also compared to vehicle. The graph below (Figure 5) demonstrates the significant improvement of performance in the rats administered drug, in comparison to peak performance to vehicle: [vehicle mean percent trials-correct 73.1±2.6 (standard error), PDEA 0.01-0.1 μg trials-correct 83.5±3.15 (standard error), $p=0.037$] (Figure 5).

Results to date from 9 rats indicate that the 0.01 μg dose of PDEA significantly improved performance compared to saline control: average performance following saline infusion: 73.1 $\pm$ 2.6 % correct; average performance following 0.01 PDEA infusion: 85.0 $\pm$ 3.1 % correct, $p<0.05$. All rats were improved by either the 0.01 μg or 0.1 μg doses of PDEA compared to their performance on saline control ($p<0.05$).

FIGURES

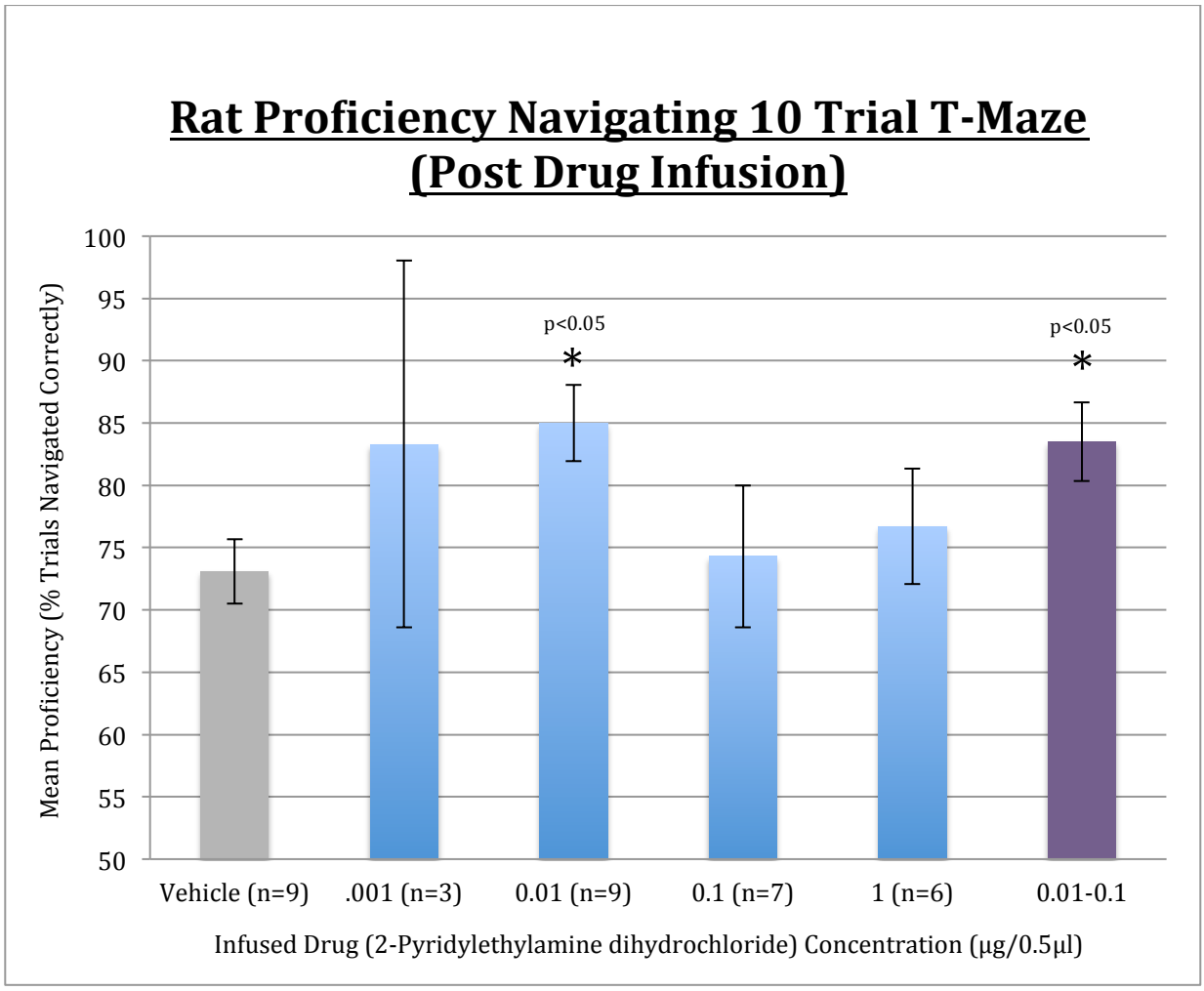

Figure 5. Effects of Histamine H1 Receptor Agonist 2-Pyridylethylamine Dihydrochloride on Delayed-Alternation Task Performance
This graph displays the performance (% correct out of 10 trials) on the spatial working memory task in rats administered 2-Pyridylethylamine Dihydrochloride at doses of 0.001, 0.01, 0.1 and 1.0µg/0.5µl compared to those administered the control vehicle. Means and standard errors are graphed. A cohort of 11 individual rats was tested. The varying N values indicate the number of rats that were tested with the specific dose. The mean for the right-most bar was calculated by including the optimal performance at either 0.01 or 0.1: 16 data points were collected from 10 rats for this range; 7 rats were tested at both doses; 50% of rats had optimal performance at 0.01 and the other 50% had optimal performance at 0.1. *Denotes significance level $p<0.05$ in the drug condition, compared to the control vehicle.

DISCUSSION:

The preliminary findings of this study suggest that stimulation of H1R in the rat PFC with a low dose of PDEA significantly improves spatial working memory performance on the delayed alternation task. This provides some of the first evidence supporting beneficial cognitive effects for H1R agonists in the PFC.

Moving Forward

The foundation of the experiment consists of a cohort of 11 rats, all of which have been successfully trained to navigate the delayed-alternation T-maze working memory assessment paradigm, and have received the proper surgery to allow for drug infusions to be made directly into their PFC. Effectively, the animals are now in a state where they can perform further experimental trials and more data can be consistently gathered. At this point 33 data points have been collected between three drug dosages and a control vehicle. The first drug being tested is PDEA at concentrations of either 0 (vehicle), 0.001, 0.01, 0.1, or 1 μg. This drug is a known H1R agonist but it has never been administered directly to the rat PFC to assess its effects on cognition.

Ideally, future experimentation would prioritize: the acquisition of more data points to perform redundant testing, replication of already-collected data, increase in the size of the animal cohort to collect data across more individuals, and lastly, expansion in the range of dosages administered to better smooth data trends and assess dose-response relationships with more incremental dose variation. Pursuing this increase in data collection will not only serve to strengthen the validity and significance of conclusions, but

will also open the experiment to drawing new conclusions especially as previously unstudied dosages will be tested.

Expanding Dosages

Of the four dosages tested, the two lower dosages, 0.01 and 0.1 ug, together exhibited a statistically significant ability ($p<0.05$, at $p=0.016$, compared to performance with saline control administration) to positively alter maze solving proficiency. For the determination of additional doses to be tested, several options present themselves. Dosages can be added either between, above or below already tested dosages. Of these three options, the trend of the collected data serves to indicate that the option with the most promise is to add dosages below the already tested lower dosages of 0.01 and 0.1. The justification results from the data collected at the 1 μg dosage. This dosage, the highest dosage tested, was unique. It contained no data points where the maze proficiency score was any greater than that of a score received, by the same animal, at a lower dosage, i.e. .001, .01 or .1 μg. By not exceeding any of the results produced from the lower dosages, the findings at the highest dosage of 1 μg becomes a rationale for not pursuing further dosage increases. The trend of the four dosages suggests that the greatest promise for cognitive enhancement is with lower rather than higher dosages.

Inverted U-Shaped Curve

The speculation regarding the potential optimal dose is grounded in the principle of the inverted U-shaped dosage response curve, which remains as a foundation of advisor Dr. Arnsten's research. The inverted U-shaped dosage response curve, for behavioral neurobiology, fundamentally asserts that more is not necessarily better and less is also not

necessarily better, but rather that there is an inherent “sweet spot” at which a dosage can be maximally beneficial. Dr. Arnsten and colleagues performed pioneering research showing that the PFC has a window of neurotransmitter receptor activity within which its performance is optimal. For example, they have shown for receptors, for the neurotransmitters dopamine and norepinephrine, that either too little or too much impairs PFC function (Arnsten, 2009; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007)(illustrated in Figure 6). When it comes to considering cognitive enhancing potential of a medication, this principle helps explain why continually increasing dosages does not increase benefit because after the “sweet spot” is surpassed, the excessive dosage can become not only less beneficial but also potentially detrimental to cognitive performance. If the H1R agonist is indeed a cognitive enhancer, then it is anticipated that upon increasing the breadth of tested dosages, an inverted U-shaped pattern will arise.

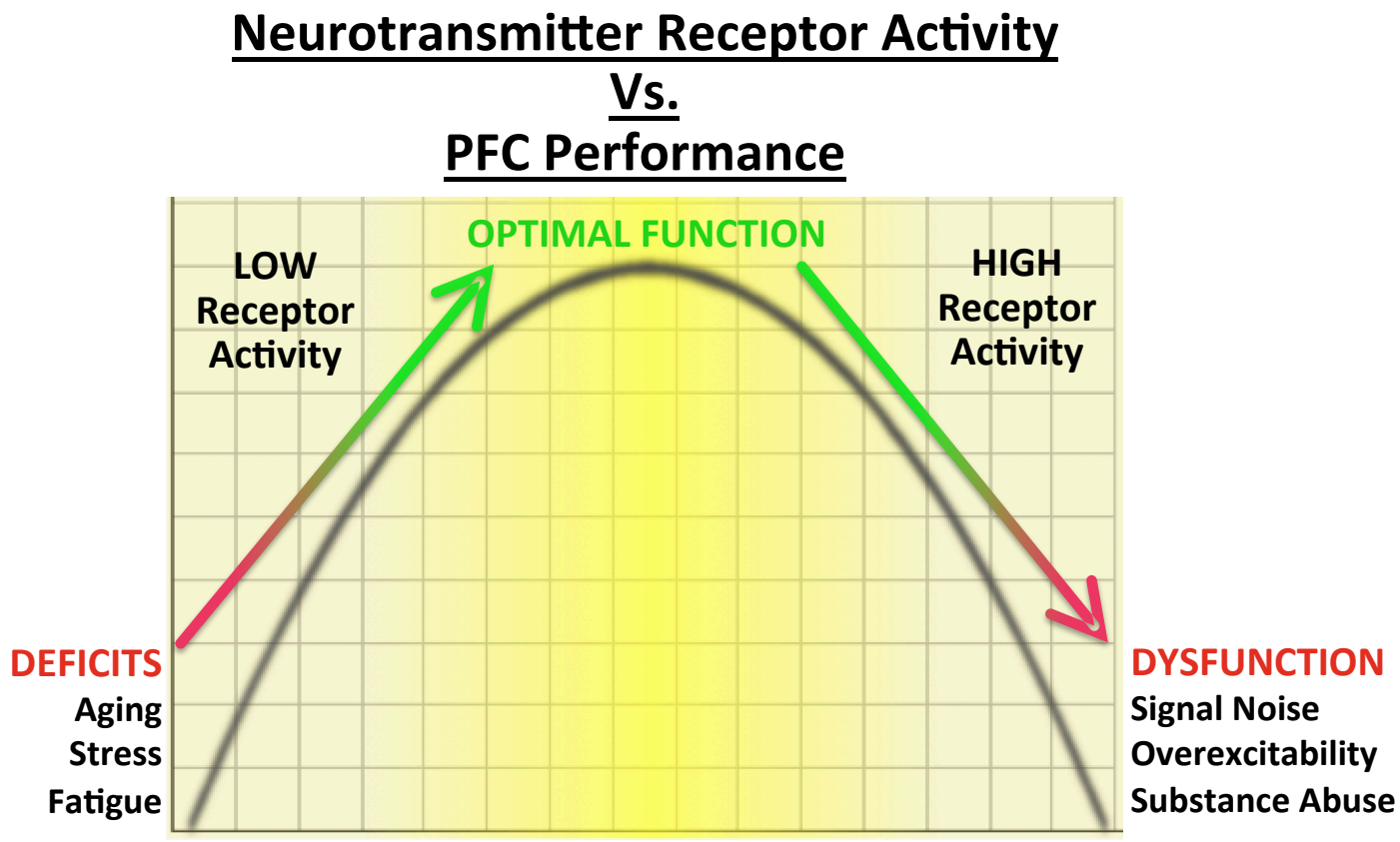

Figure 6. The Inverse U-Shaped Relationship Between Receptor Activity and PFC Performance
The figure illustrates the observed relationship for receptor activity, of neurotransmitter systems such as dopamine and norepinephrine, compared to PFC functioning. When activity is low, there is impaired PFC functioning and deficits in PFC-mediated functions such as working memory. Impairments in PFC are also observed when PFC neurons fire excessively, causing dysfunction. For optimal PFC-mediated cognition, receptor activity needs to be within an optimal range.

For the dose range of 0.01-0.1 μg there is a p value of less than .05, at p=0.016, which supports that this dose range's ability to improve maze-solving proficiency is not an artifact of experimental error. While the data is only preliminary, it does suggest that an H1R agonist has the capability to improve a rat's PFC functioning and ability to solve a working memory dependent maze task. As this was observed in healthy rats, it suggests that the nature of this behavioral modification is indicative of cognitive enhancement. That being said, it is not possible to know where the "sweet spot" dosage is. It is possible that it exists within the most beneficially tested dosage range of 0.01-0.1 μg, but it is more likely that it exists somewhere below the 0.01 μg dosage. Regardless of where the "sweet spot" is, it will not be discernable unless more dosages are tested below the current best minimum dose of 0.01ug. If those dosages yield compelling data, then the next area to focus on would be dosages within the range of 0.01-0.1 μg. Ultimately the data does indicate that the least promising dosages to test further would be those exceeding 1.0 μg.

Nic-α7R and H1R Agonists

The plan to study lower dosages is also supported by the similarity between this preliminary data and a past study conducted by Dr. Arnsten as she investigated how administering a nicotinic nic-α7R agonist into the primate dlPFC altered Delay cell firing as the monkey performed a spatial working memory task (Yang et al., 2014). These data showed that it was the lower dosages of nic-α7R agonist that improved Delay cell firing for the neurons' preferred spatial direction, while the higher dosages produced nonspecific increases in excitability that impaired the neural representation of visual space.

These conclusions are from the primate *in vivo* electrophysiological recording of dlPFC Delay neurons specific to different orientations of visual stimuli. At the "sweet spot" low dosage it was possible to increase the persistent firing of only the Delay neurons associated with the correct stimuli orientation, but at the higher dosages it generated large amounts of neuronal noise. This noise was generated at higher dosage by persistent firing of Delay neurons specific to other visual stimuli orientations, other than the tested one. The result is a muddy or noisy working memory representation of the orientation of the visual stimulus, thus leading the primate to become increasingly inaccurate with their attempts to exercise their spatial working memory in the experimental task. This prior experiment provided further highly compelling support for the inverted U-shaped dosage response curve for cognitive enhancers and the idea that lower dosages may, counterintuitively, be the most beneficial.

It is possible that the H1R also influences neuronal excitability with an inverted U-shaped dose-response pattern. The H1R is coupled to Gq proteins, which increase internal calcium release and activate protein kinase C. Future studies could examine these, and other potential specific mechanisms, through which H1R stimulation alters Delay cell firing, and whether the representation of visual space is enhanced by low but not higher doses.

Implications of Research

In the event that future research can significantly confirm that H1R agonists are indeed cognitive enhancers for spatial working memory in the rat PFC, then the immediate implications for the research community will be a novel contribution. It will serve to identify a new mechanism that modulates PFC functioning. Due to the history of our limited

understanding of the histaminergic system as it pertains to cognition, the histaminergic system has generally not readily been associated with cognitive function. If conclusive future data does bring the spotlight onto the histaminergic system for cognition, then it will encourage further exploration into the relationship between cognition and the H1 receptor system, and potentially other neurotransmitter systems that were overlooked/deprioritized in the past. Further discoveries will help the research community decipher the biological complexities underlying cognition.

Nature and Neurobiology

Findings of H1R agonist effects on the PFC could also bring attention to using nature as a means to find clues pertaining to the cognitive capabilities we have evolved. It is a general but significant shift in mindset for the research community to increase the attention it pays to the ways plants and fungi affect our bodies, which will hopefully distill down to discovering new molecular mechanisms. This could have broad-ranging implications contributing to support for the preservation of natural ecosystems as yet another scientific community, neurobiologists, become more attentive to studying naturally occurring compounds and how they can interact with our brains.

Medical Applications

New information about the histaminergic system could lead to novel histaminergic medication strategies and improved tailoring of the receptor affinity profiles of currently existing medication classes. An example of a result of this could be the tweaking of atypical antipsychotics to reduce their H1R blocking actions, which appear to have harmful effects on weight gain, (He et al., 2013) and from this study, it appears may also have harmful

effects on cognition. This alteration in receptor binding may help to alleviate these life-impairing side effects. It would be exciting if this research could result in a new type of psychiatric medication derived from naturally-occurring substances, but even if this research only serves to help to better tailor the activity profiles of existing drugs, then that will be a great step forward for alleviating the suffering of patients with a psychotic disorder and potentially other causes of cognitive deficits.

Cognitive Enhancement

Lastly, the broader implications of this research pertain to the future of cognitive enhancement. Initially, research of this nature will serve to improve the degree to which neurobiologists understand the mechanisms that underlie cognition, down to the molecular level. This knowledge could then improve the existing capabilities to medically treat psychiatric disorders, and then, perhaps might even be considered to apply to developing methods to improve the cognitive functioning of healthy individuals.

That being said, there is a very big difference between compensating for a psychiatric disorder and raising the threshold above what our brains are capable of functioning at a healthy baseline. Reaching what some consider to be the holy grail of a true "smart pill" will likely be preceded by smaller steps and will require careful consideration of ethical issues. The first step forward would be increasing the ability to treat those individuals who are not psychiatrically healthy. After being able to fix things when they go biologically wrong, the next step will be to create the ideal neurological conditions for us to achieve our natural potential. Rather than exceed our potential, an important aim could be to achieve our potential, i.e. instead of a "smart pill" to design a "cognitive health pill."

A related example is the role caffeine has played in our culture. Arguably an individual who is ideally well rested shouldn't need caffeine to function at peak mental capacity; in fact, it may impair them by causing overshooting side effects, like jitteriness for example. Caffeine has thus become a means with which we can adjust for our lives not providing the ideal conditions needed for cognitive function. It might be impossible for an average college student to get the amount of sleep required for optimal cognitive performance, but luckily caffeine can step in to help accommodate for the handicaps that our way of life places on our health, particularly allowing us to function on less sleep than we may need. This principle could be applied to next steps towards a "cognitive health pill." It suggests that before exceeding our biological capabilities under ideal conditions, first it is necessary to accounting for the lack of ideal conditions around us. By this logic, the predecessor for the "smart pill" may not be a cognitive enhancer so much as a medication to address more subtle cognitive impairments to bring them into optimal homeostatic range for an individual, a "cognitive health pill."

It is hard to rule out the future possibility of a "smart pill" hypothetically being able to push human intelligence and cognitive function beyond where they have ever been before. However, more realistically the interim step will be leveling the playing field between our neurobiology and our way of life such that our cognitive function is as healthy as it can be even if our lifestyle directly interferes with that capacity. Who knows, maybe a naturally-derived compound that modulates the H1R activity in someone's PFC to optimal levels could bring cognitive science one step closer to a "cognitive health pill".

REFERENCES:

Arnsten, A. F. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci, 10*(6), 410-422. doi: 10.1038/nrn2648

Arnsten, A. F. (2013). The neurobiology of thought: the groundbreaking discoveries of Patricia Goldman-Rakic 1937-2003. *Cereb Cortex, 23*(10), 2269-2281. doi: 10.1093/cercor/bht195

Arnsten, A. F., Paspalas, C. D., Gamo, N. J., Yang, Y., & Wang, M. (2010). Dynamic Network Connectivity: A new form of neuroplasticity. *Trends Cogn Sci, 14*(8), 365-375. doi: 10.1016/j.tics.2010.05.003

Ban, T. A. (2007). Fifty years chlorpromazine: a historical perspective. *Neuropsychiatr Dis Treat, 3*(4), 495-500.

Blandina, P., Munari, L., Provensi, G., & Passani, M. B. (2012). Histamine neurons in the tuberomamillary nucleus: a whole center or distinct subpopulations? *Front Syst Neurosci, 6*, 33. doi: 10.3389/fnsys.2012.00033

Brown, R. E., Stevens, D. R., & Haas, H. L. (2001). The physiology of brain histamine. *Prog Neurobiol, 63*(6), 637-672.

Catapano, L. A., & Manji, H. K. (2007). G protein-coupled receptors in major psychiatric disorders. *Biochim Biophys Acta, 1768*(4), 976-993. doi: 10.1016/j.bbamem.2006.09.025

Fell, M. J., Katner, J. S., Rasmussen, K., Nikolayev, A., Kuo, M. S., Nelson, D. L., . . . Svensson, K. A. (2012). Typical and atypical antipsychotic drugs increase extracellular histamine levels in the rat medial prefrontal cortex: contribution of histamine h(1) receptor blockade. *Front Psychiatry, 3*, 49. doi: 10.3389/fpsyt.2012.00049

Funke, U., Vugts, D. J., Janssen, B., Spaans, A., Kruijer, P. S., Lammertsma, A. A., . . . Windhorst, A. D. (2013). (11) C-labeled and (18) F-labeled PET ligands for subtype-specific imaging of histamine receptors in the brain. *J Labelled Comp Radiopharm, 56*(3-4), 120-129. doi: 10.1002/jlcr.3038

Gaebel, W., & Zielasek, J. (2015). Schizophrenia in 2020: Trends in diagnosis and therapy. *Psychiatry Clin Neurosci, 69*(11), 661-673. doi: 10.1111/pcn.12322

Girgis, R. R., Merrill, D. B., Vorel, S. R., Edward, K., Kimberly, P., You, M., . . . Lieberman, J. A. (2011). Aripiprazole versus haloperidol treatment in early-stage schizophrenia. *J Psychiatr Res, 45*(6), 756-762. doi: 10.1016/j.jpsychires.2010.09.003

Goldman-Rakic, P. S. (1987). Development of cortical circuitry and cognitive function. *Child Dev, 58*(3), 601-622.

Goldman-Rakic, P. S. (1988). Topography of cognition: parallel distributed networks in primate association cortex. *Annu Rev Neurosci, 11*, 137-156. doi: 10.1146/annurev.ne.11.030188.001033

Goldman-Rakic, P. S. (1995). Cellular basis of working memory. *Neuron, 14*(3), 477-485.

Goldman-Rakic, P. S. (1996). The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. *Philos Trans R Soc Lond B Biol Sci, 351*(1346), 1445-1453. doi: 10.1098/rstb.1996.0129

Haas, H., & Panula, P. (2003). The role of histamine and the tuberomamillary nucleus in the nervous system. *Nat Rev Neurosci, 4*(2), 121-130. doi: 10.1038/nrn1034

He, M., Deng, C., & Huang, X. F. (2013). The role of hypothalamic H1 receptor antagonism in antipsychotic-induced weight gain. *CNS Drugs, 27*(6), 423-434. doi: 10.1007/s40263-013-0062-1

Iwabuchi, K., Ito, C., Tashiro, M., Kato, M., Kano, M., Itoh, M., . . . Yanai, K. (2005). Histamine H1 receptors in schizophrenic patients measured by positron emission tomography. *Eur Neuropsychopharmacol, 15*(2), 185-191. doi: 10.1016/j.euroneuro.2004.10.001

Kesner, R. P., Farnsworth, G., & DiMattia, B. V. (1989). Double dissociation of egocentric and allocentric space following medial prefrontal and parietal cortex lesions in the rat. *Behav Neurosci, 103*(5), 956-961.

Kolb, B., & Robbins, T. (2003). The rodent prefrontal cortex. *Behav Brain Res, 146*(1-2), 1-2.

Lesh, T. A., Niendam, T. A., Minzenberg, M. J., & Carter, C. S. (2011). Cognitive control deficits in schizophrenia: mechanisms and meaning. *Neuropsychopharmacology, 36*(1), 316-338. doi: 10.1038/npp.2010.156

Martinez-Mir, M. I., Pollard, H., Moreau, J., Arrang, J. M., Ruat, M., Traiffort, E., . . . Palacios, J. M. (1990). Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates. *Brain Res, 526*(2), 322-327.

McKenna, D. J., Ruiz, J. M., Hoye, T. R., Roth, B. L., & Shoemaker, A. T. (2011). Receptor screening technologies in the evaluation of Amazonian ethnomedicines with potential applications to cognitive deficits. *J Ethnopharmacol, 134*(2), 475-492. doi: 10.1016/j.jep.2010.12.037

Mishkin, S. (1964). The Interdependence of Clinical Neurology and Neurophysiology. An Historical Review of the Vestibulo-Ocular Reflex. *McGill Med J, 33*, 80-97.

Miyamoto, S., Miyake, N., Jarskog, L. F., Fleischhacker, W. W., & Lieberman, J. A. (2012). Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Mol Psychiatry, 17*(12), 1206-1227. doi: 10.1038/mp.2012.47

Murphey, R. K., & DavisGw. (1994). Retrograde signalling at the synapse. *J Neurobiol, 25*(6), 595-598. doi: 10.1002/neu.480250602

Panula, P., & Nuutinen, S. (2013). The histaminergic network in the brain: basic organization and role in disease. *Nat Rev Neurosci, 14*(7), 472-487. doi: 10.1038/nrn3526

Robbins, T. W. (2000). From arousal to cognition: the integrative position of the prefrontal cortex. *Prog Brain Res, 126*, 469-483. doi: 10.1016/S0079-6123(00)26030-5

Roegge, C. S., Perraut, C., Hao, X., & Levin, E. D. (2007). Histamine H1 receptor involvement in prepulse inhibition and memory function: relevance for the antipsychotic actions of clozapine. *Pharmacol Biochem Behav, 86*(4), 686-692. doi: 10.1016/j.pbb.2007.02.014

Roth, B. L., Sheffler, D. J., & Kroeze, W. K. (2004). Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov, 3*(4), 353-359. doi: 10.1038/nrd1346

Sato, H., Ito, C., Hiraoka, K., Tashiro, M., Shibuya, K., Funaki, Y., . . . Yanai, K. (2015). Histamine H1 receptor occupancy by the new-generation antipsychotics olanzapine and quetiapine: a positron emission tomography study in healthy volunteers. *Psychopharmacology (Berl), 232*(19), 3497-3505. doi: 10.1007/s00213-015-4002-2

Schwartz, J. C., Arrang, J. M., Garbarg, M., Pollard, H., & Ruat, M. (1991). Histaminergic transmission in the mammalian brain. *Physiol Rev, 71*(1), 1-51.

Shahid, M., Walker, G. B., Zorn, S. H., & Wong, E. H. (2009). Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. *J Psychopharmacol, 23*(1), 65-73. doi: 10.1177/0269881107082944

Strassnig, M. T., Raykov, T., O'Gorman, C., Bowie, C. R., Sabbag, S., Durand, D., . . . Harvey, P. D. (2015). Determinants of different aspects of everyday outcome in schizophrenia: The roles of negative symptoms, cognition, and functional capacity. *Schizophr Res, 165*(1), 76-82. doi: 10.1016/j.schres.2015.03.033

Sumiyoshi, T., Higuchi, Y., & Uehara, T. (2013). Neural basis for the ability of atypical antipsychotic drugs to improve cognition in schizophrenia. *Front Behav Neurosci, 7*, 140. doi: 10.3389/fnbeh.2013.00140

Tsujii, T., Yamamoto, E., Ohira, T., Takahashi, T., & Watanabe, S. (2010). Antihistamine effects on prefrontal cortex activity during working memory process in preschool children: a near-infrared spectroscopy (NIRS) study. *Neurosci Res, 67*(1), 80-85. doi: 10.1016/j.neures.2010.01.010

Vijayraghavan, S., Wang, M., Birnbaum, S. G., Williams, G. V., & Arnsten, A. F. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci, 10*(3), 376-384. doi: 10.1038/nn1846

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. *J Clin Psychiatry, 66*(9), 1122-1129.

Yanai, K., Zhang, D., Tashiro, M., Yoshikawa, T., Naganuma, F., Harada, R., . . . Okamura, N. (2011). Positron emission tomography evaluation of sedative properties of antihistamines. *Expert Opin Drug Saf, 10*(4), 613-622. doi: 10.1517/14740338.2011.562889

Yang, S. T., Shi, Y., Wang, Q., Peng, J. Y., & Li, B. M. (2014). Neuronal representation of working memory in the medial prefrontal cortex of rats. *Mol Brain, 7*, 61. doi: 10.1186/s13041-014-0061-2

Zlomuzica, A., Ruocco, L. A., Sadile, A. G., Huston, J. P., & Dere, E. (2009). Histamine H1 receptor knockout mice exhibit impaired spatial memory in the eight-arm radial maze. *Br J Pharmacol, 157*(1), 86-91. doi: 10.1111/j.1476-5381.2009.00225.x

Image Component References:

http://plantgenera.org/illustration.php?id_illustration=98791&mobile=broad&code_category_taxon=0

http://plantillustrations.org/illustration.php?id_illustration=8458

http://tropical.theferns.info/image.php?id=Tabernaemontana+sananho