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Role of Cholinergic Projections to the Orbitofrontal Cortex in Probability Discounting in a Rat Model

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Abstract

Decision-making, especially when rewards are probabilistic, influences our lives on a daily basis and can be affected in some psychological disorders. Thus, understanding the neural basis of decision-making is important to understand how this processing occurs under "normal" conditions and to develop potential targets for treating conditions in which decision-making is affected. Projections from the basal forebrain release acetylcholine in all cortical areas. These projections are damaged in Alzheimer's disease and there has also been interest in this pathway with respect to attention deficit/hyperactivity disorder (ADHD) and schizophrenia. Rats were trained in a probability-discounting task in which the animal choses between a small reward available 100% of the time or a larger reward available 100%, 33%, or 17% of the time. The subjects then received cholinergic lesions to the orbitofrontal cortex using 192IgG-saporin; sham-surgery, or no surgery. Cholinergic lesions to the OFC lead to more risky behavior in the subjects, causing the lesion animals to choose the large reward at higher rates than the control animals especially when the large reward was at 17% availability. Differences in lesion and sham-lesion groups did not reach significance, while lesion and nonsurgical groups showed significant differences, indicating that surgery may have been a large factor in changes seen in task performance.

Introduction

Decision-making is a large part of every-day life and is altered in drug addiction and psychological disorders. There is much evidence to show that decision-making is strongly correlated to other processes such as impulsivity, attention, and memory. Many brain regions modulate these processes, but the prefrontal cortex has been shown to be essential in these higher-order processes. The orbitofrontal cortex, also known as the ventromedial prefrontal cortex, gets its name from its location just above the orbits of the eye. These cognitive processes are disrupted or altered in many psychological conditions such as Alzheimer's disease, attention deficit hyperactivity disorder (ADHD), and schizophrenia (APA, 2000). In addition to cognition related problems, the cholinergic system has been shown to be altered in these conditions as well. Previous research has investigated the role of acetylcholine in relation to learning and memory, but little research has focused on the role of the cholinergic system in the orbitofrontal cortex in relation to impulsivity. The goal of this study was to assess the role of cholinergic projections to the orbitofrontal cortex in a probability discounting task measuring impulsivity.

Impulsivity and Risky Decision-Making

Impulsivity has been implicated in many psychiatric disorders, neurodegenerative diseases, and drug addiction, but there is no clear definition of impulsivity. Impulsivity is linked to cognition, action inhibition and decision making, making a single definition of impulsivity difficult (APA, 2000). Impulsive behavior is often defined as behavior that has a certain abnormally high associated level of risk, or possibility of a negative result (Cardinal, 2006). According to Cardinal (2006), there are many facets to impulsivity, and the idea can be subdivided into preparation impulsivity, execution impulsivity, and outcome impulsivity.

Preparation impulsivity describes when a subject does not properly take all of the given information into account when making a choice. Execution impulsivity occurs when the subject stops an action before finishing a task, and outcome impulsivity occurs when the subject chooses a small, instantly gratifying reward rather than waiting for a larger, more optimal reward (Cardinal 2006). According to Urcelay and Dalley (2012), impulsive choice and propensity to choose the immediate reward may be related to the dysfunction of several neurotransmitter systems in conditions such as ADHD.

Many studies test impulsivity by looking at the subjects' ability to stop a task once it has been started. A 2009 study looked at the effect of several neurotransmitter systems on a rat's stop-task performance (Bari et al., 2009). The rationale behind this type of stop-task performance is that it is commonly inhibited in psychiatric conditions in which the individual is unable to suppress inappropriate behavior. Deficits in this task indicate impulsivity because once subjects start a task, they must exhibit control and action inhibition in order to properly end the task. Another study by Bari et al. (2008) showed the difference in neuropharmacological processes that modulate stop-signal and go/no go tasks. This study indicated that the serotonin network is implicated in go/no-go tasks while the stop-signal reaction seems to be modulated more by noradrenaline. This study shows that while there is significant overlap in the pathways used for these two different impulsivity tests, there are slight differences in how these tasks are processed. Within the realm of action inhibition, there are complicated and distinct neural circuits that modulate very similar tasks. This study speaks to the complexity of the cognitive processing that is responsible for just one aspect of impulsivity and decision-making.

One of the types of impulsivity that Cardinal (2006) referenced, outcome impulsivity, can be assessed by testing the subject's performance in discounting tasks. Discounting refers to a

decision-making task in which the subject must choose between two unequally valued rewards. The availability of the rewards is changed throughout the task; either the time to receive the reward or the probability of receiving the reward is altered throughout the test (Soman et al., 2005). These tasks are referred to as delay discounting and probability discounting, respectively. These discounting tasks model situations in which outcomes of a choice are uncertain either in whether or not the desired outcome will occur or when the desired outcome will occur (Cardinal, 2006). The decisions that the subjects make during the tasks are comparable to decisions that humans make every day. For example, people often must make the decision of whether to purchase something small or save money to buy something bigger later (Green & Myerson, 2004). Both animal and human studies demonstrate similar trends in behavior when confronted with these tasks (Rachlin, Raineri, & Cross, 1991). In delay discounting, when the delay to receive the reward is small, the subject generally chooses the large reward over the smaller reward, but as the delay to receive the large reward is increased, there comes a point at which the subject starts to choose the smaller reward more consistently. Similarly, in probability discounting, when the large reward is available at high probabilities, the subject often chooses the large reward, but when the large reward is often not delivered and the smaller reward is more reliable, subject begin to choose the smaller reward. The point at which the subject's preference reverses indicates that the subject's perception of the value of the large reward has decreased below the smaller reward (Green & Myerson, 2004).

The literature is split as to whether probability discounting and delay discounting are modulated by the same neural mechanisms or whether they are separate processes. Mazur (1989) tested pigeons' responses to both probability discounting and delay discounting. The study concluded that both types of discounting use the same underlying neural processes and suggested

that it is possible to think of probabilistic reinforcers as equivalent to delayed reinforcers. Rachlin and Logue (1986) confirmed Mazur's assertion that one single fundamental processes controls individual's responses to these two tasks. It is suggested that probability and delay are inverses of each other and that both discounting tasks can reveal a deficit in self-control (Rachlin & Logue, 1986).

Mazur (1987) was able show that delay discounting functions, using a pigeon model, are hyperbolic. Rachlin, Raineri & Cross (1991) were able to confirm his mathematical model and extend the idea of the hyperbolic function to probabilistic discounting in humans. This study used human subjects completing both delay and probability discounting tasks. Both tasks resulted in qualitatively similar hyperbolic curves, further supporting Mazur's idea that both tasks are mediated by the same processes.

However, there is evidence for the two tasks as being mediated by separate processes as well. One facet of this view is rooted in the idea that probability discounting tests risky decision making, but not impulsivity and delay discounting tests impulsivity (Stopper, Green, & Floresco, 2014). Probability discounting tests the subject's decision to choose something with only a chance of reward where delay discounting tests the subject's ability to wait for gratification without risk of not being rewarded. In addition Green and Myerson (1996) argue that from an ecological standpoint, delayed and probabilistic discounting are relevant in different situations, leading to the idea that they are modulated by separate processes. Delay discounting has an added risk when compared to probability discounting because waiting for a reward in nature comes with the risk that it may be taken away; essentially that longer delays inherently discount the reward in both time and probability (Green & Myerson, 1996; 2004). In addition, Green and Myerson (2004) assert that the idea that the fact that temporal and probability discounting can be

modeled by the same type of function indicates that they are controlled by a single discounting process. However, evidence shows that many variables such as the amount of reward offered have differing effects on temporal and probability discounting, indicating that the tasks must be mediated by different processes (Green & Myerson, 2004).

Role of the Orbitofrontal Cortex in Decision-Making

The orbitofrontal cortex is located in the prefrontal cortex and is involved in many aspects of executive function including reward-related processing, learning, and decision-making. The orbitofrontal cortex has many important connections to other subcortical brain regions such as the basolateral amygdala and nucleus accumbens (Schoenbaum et al., 2006; Howard et al., 2015). The basolateral amygdala is important in emotional and fear responses and motivational learning and the nucleus accumbens is primarily involved in pleasure and reward. The bilateral connections from the basolateral amygdala and nucleus accumbens to the orbitofrontal cortex also show the orbitofrontal cortex's importance in associative learning. The connections to these important brain regions make the orbitofrontal cortex essential in many every day circumstances.

One aspect of the orbitofrontal cortex that is particularly important in decision-making and reward is the ability of this brain region to code outcome expectancies. It is crucial that the brain be able to code the value of potential outcomes in order to make informed decisions about potential actions. The connections that the orbitofrontal cortex has with the limbic system also poises it to be essential in processing the consequences and potential rewards of potential outcomes (Schoenbaum et al., 2006). Functional magnetic resonance imaging (fMRI) studies in

humans have shown that blood flow increases to the orbitofrontal cortex in anticipation of expected outcomes (Gottfried et al., 2002), indicating an increase in neural firing in this region.

By manipulating the value and identity of certain food odors, human fMRI studies are able to show that the orbitofrontal cortex is able to code dissociable representations of specific rewards. Of note, the orbitofrontal cortex seems to code both the identity and the value of the reward (Howard et al., 2015). Electrophysiological recordings of macaque monkeys revealed that in addition to coding rewards, the orbitofrontal cortex neurons were also able to code aversive stimuli (Tremblay & Schultz, 1999) and the discounted value of a delayed reward (Roesch & Olson, 2005). When the reward was delayed, neurons fired less frequently, proportional to the delay of the reward. Similarly in rats, when waiting for uncertain delivery of either sucrose or quinine, as many as 20% of neurons in the orbitofrontal cortex fired during the delay (Schoenbaum et al., 2003).

Damage to the orbitofrontal cortex has been shown to have negative consequences on behavior in humans. Phineas Gage, a famous figure in psychology, miraculously survived an accident in which a pole from a railroad pierced through his skull. The rod entered his skull near his left eye, exiting at the top of the skull. While Gage survived the accident, living for 12 more years, he was left with profound personality and behavioral changes. He was unable to hold the same job because of his temperament. Most of the damage was done to the left frontal cortex and many connections between the frontal cortex and subcortical structures were severed. Notably, the connections between the orbitofrontal cortex and parts of the limbic system such as the basolateral amygdala and the hippocampus were split (Van Horn et al., 2012).

In addition, humans with orbitofrontal cortex damage perform badly in decision making tasks in behavioral studies. The Iowa Gambling Task is one example of a decision making task

administered commonly to human subjects. The task is designed to simulate decisions the subject would have to make commonly in real life (Bechara et al., 1994). In the task, the subject must choose cards from different decks of cards and learn which decks are "better," or provide more rewards than others. Patients with orbitofrontal cortex damage are more likely to choose high risk, high reward choices (Bechara et al., 1999). However, these patients were able to generate skin conductance responses, an indication of somatic state activation. It has been shown that in these studies, choosing advantageously is correlated with the detection of somatic state activation (Bechara et al., 1996). A later study by Bechara et al. (2000) showed that patients with orbitofrontal cortex lesions were insensitive to future consequences in the Iowa Gambling Task; instead, these patients are more guided by immediate rewards. This shortsightedness and disregard for future consequences could help explain similar symptoms of psychiatric disorders such as schizophrenia and ADHD in which the orbitofrontal cortex is involved (Bechara et al., 2000).

There is evidence to suggest that in addition to the orbitofrontal cortex, the anterior cingulate cortex also plays a role in cost-benefit decisions. Direct and indirect pathways link the two areas, however their roles in decision-making have been found to be dissociable. The anterior cingulate, located medially in the cerebral cortex, is involved in effort-based decision-making (Rudebeck et al., 2006). Lesions to the orbitofrontal cortex and the anterior cingulate cortex caused dissociable effects to decision making in maze tasks testing impulsivity and effort-based decision making. Orbitofrontal cortex lesions caused impulsivity in delay tasks, while anterior cingulate lesions did not affect behavior in these tasks. In effort-based decision tasks, rats choosing high reward options had to climb a 30cm high barrier to receive reward. Animals with anterior cingulate lesions showed a significant decrease in frequency of high reward choices

after lesion surgery whereas orbitofrontal cortex lesion animals did not change their behavior on this task (Rudebeck et al., 2006). Both of these neural circuits understood together may be important in explaining apathy that often accompanies risky decision making in many psychiatric conditions.

A similar study tested the effects of activation of the cannabinoid receptor system in both the anterior cingulate cortex and the orbitofrontal cortex (Khani et al., 2014). Rats received microinjections of either a vehicle or ACEA, a cannabinoid type-1 receptor agonist into either of the brain regions. As in the previously mentioned study, animals were trained in a delay-based or effort-based maze tasks. ACEA in the anterior cingulate influenced rats to make choices to lessen physical exertion to get rewards whereas ACEA in the orbitofrontal cortex caused rats to prefer smaller immediate rewards to the larger delayed rewards, consistent with the idea of orbitofrontal cortex dysfunction causing impulsivity (Khani et al., 2014).

While many studies point to orbitofrontal cortex dysfunction as a cause for impulsivity and risky decision making, others indicate that the correlation is not that simple. A study by Orsini et al. (2015) found that lesions to the orbitofrontal cortex made rats more risk averse under risk of punishment. In this task, the rat had the option of choosing one lever with a small reward or another lever that always delivered a large reward but came with the possibility of also receiving a foot shock. Neurotoxic lesions were induced by bilateral infusion of NMDA into the orbitofrontal cortex and the basolateral amygdala. Subjects with bilateral basolateral amygdala lesions were more likely to choose the more risky reward, while subjects with orbitofrontal cortex lesions became more risk averse (Orsini et al., 2015). These results suggest that reciprocal connections between the orbitofrontal cortex and basolateral amygdala may serve to modulate the two brain regions' differing responses to decision-making scenarios. The data suggest that

the lesions did not affect other aspects of the task such as anxiety-like behavior, food reward motivation, or reward discrimination, which could confound the results. The result of this study is interesting because it runs contrary to the common belief that lesions to the orbitofrontal cortex result in more risky and impulsive behavior. This study suggests that reward and punishment may be coded differently in the prefrontal cortex. In addition, lesions to the orbitofrontal cortex disrupt modeling of potential outcomes, leading to less adaptive decision making when probability of foot shock is increased (Pickens et al., 2005).

A study by Winstanley et al. (2004), shows a similar contradiction of the vast majority of the literature that shows that orbitofrontal cortex lesions cause an increase in impulsivity. This study shows that rats with excitotoxic lesions of the orbitofrontal cortex showed more preference for the larger, delayed reward in probability discounting paradigms. This result is rationalized by the idea that the orbitofrontal cortex is necessary for adapting to changing outcomes of tasks. With a dysfunctional orbitofrontal cortex, the lesioned rats were not able to properly encode the devaluation of the large reward with the increasing delays (Winstanley et al., 2004).

The Role of the Cholinergic System

The cholinergic system is one of the main neurotransmitter systems in the central nervous system. Acetylcholine plays many roles in the brain including modulation of learning, memory, attention, decision-making, and states of arousal (Celesia & Jasper, 1966; Sarter et al., 2001; Sarter & Bruno, 1997). The major cholinergic output of the brain is the basal forebrain including the nucleus basalis and the substantia innominata. These regions produce acetylcholine which is then distributed around the brain to subcortical areas as well as the cortex (Mesulam et al., 1983; Sarter et al., 2001). There are two main types of acetylcholine receptors in the brain that are

generally targeted, the nicotinic acetylcholine receptor including the $\alpha_4\beta_2$ and α_7 subtypes, and metabotropic muscarinic receptors.

In experimental studies, the cholinergic system is targeted and manipulated by the use of receptor agonists or antagonists or by using neurotoxins to lesion certain regions. Over the past few decades, many advances have been made in drugs that can target the cholinergic system or specific acetylcholine receptors. Prior to the synthesis of the immunotoxin 192 IgG-saporin, researchers generally used amino acid excitotoxins which produced more non-specific effects (McGaughy et al., 1996). The development of 192 IgG-saporin, a low affinity nerve growth factor receptor coupled to a ribosome inactivating protein, has allowed for much more targeted approaches to understanding the cholinergic system. 192 IgG-saporin works by binding to p75 nerve growth factor expressed selectively on acetylcholine releasing neurons. The immunotoxin is then endocytosed and the ribosome-inactivating component, saporin, leads to inhibition of protein synthesis and subsequent cell death (Everitt & Robbins, 1997). The immunotoxin can be infused into cortical areas to destroy cholinergic neurons in precise areas, making it a powerful tool to use when assessing the role of specific cholinergic inputs.

The cholinergic system has been shown many times to be essential in attentional processing. In an attention task in which rats were trained to discriminate visual signal trials from non-signal trials, rats that received systemic injections of the muscarinic receptor antagonist scopolamine were shown to have less ability to detect 500ms trials (McQuail & Burk, 2006). In addition, as the dose of scopolamine was increased, the number of omissions in the trials increased as well, but administration of scopolamine did not affect the animal's number of correct rejections of non-signal trials. Mecamylamine, a nicotinic receptor antagonist, did not have a significant effect on the animals' performance other than to increase the number of

omissions (McQuail & Burk, 2006), indicating that different receptor types play differing roles in modulation of attention.

It was found that the functioning basal forebrain is necessary for proper performance in attentional demanding tasks such as a five choice serial task. In this task, an animal must correctly choose one of five response locations at which a light is illuminated for a given period of time. Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) can be used to induce excitotoxic lesions in the basal forebrain; these lesions result in significant deficits in the five choice serial task (Muir et al., 1994). While cholinergic processing has been found to be essential for attentional functions, acetylcholine does not seem to be related to mnemonic processing. Excitotoxic lesions in the basal forebrain of monkeys did not significantly affect their performance in spatial discrimination, concurrent discrimination, or delayed response tasks (Voytko, 1996).

Acetylcholine has been specifically implicated in attention tasks when visual stimuli are involved (Dalley et al., 2004; Dalley et al., 2001). Immunotoxic lesions induced by 192 IgG-saporin to the rat basal forebrain impairs performance in visual attention tasks. Animals with these lesions were found to exhibit perseverative responding when under high attentional demand. This type of responding increased the number of anticipatory errors. Systemic administration of scopolamine further disrupted task performance (Dalley et al., 2004). During visual attention tasks, there is an elevation in levels of acetylcholine in certain areas of the brain including the prefrontal cortex. Microdialysis studies showed a strong correlation between cortical acetylcholine efflux in the prefrontal cortex and task performance (Dalley et al., 2001).

Sustained attention, also referred to as behavioral vigilance, describes an organism's ability to detect unpredictable signals over a long period of time. Sustained attention is

modulated by the basal forebrain cholinergic system and requires top-down processing to discriminate signal trials from distractors (Sarter et al., 2001). In addition to the basal forebrain, the pedunculopontine tegmental nucleus (PPTg) cholinergic cells in the brainstem are also thought to be necessary for sustained attention. Rats with PPTg lesions tested in a 5-Choice Serial Reaction Time Task, as described previously, had deficits in performance correlated with the loss of PPTg cholinergic cells, as determined by immunohistochemistry (Cyr et al., 2015).

McGaughy et al., (1996) were able to show that performance worsened in behavioral vigilance tasks after infusion of 192 IgG-saporin into the basal forebrain. Furthermore, they showed that behavioral vigilance task performance correlated with the acetylcholinesterase fiber density found in many cortical areas. Acetylcholinesterase fiber density can help elucidate the degree to which cholinergic projections are killed off by the cholinergic immunotoxin (McGaughy et al., 1996). This study provides strong evidence that the presence of cholinergic projections is directly involved in attentional processing.

It has been known for many years that cholinergic system dysfunction is one of the characteristics of psychiatric disorders and neurodegenerative disorders such as ADHD, Alzheimer's disease, and Schizophrenia. In all of these conditions, decision-making, impulsivity, and attentional processes are affected. Many different neural mechanisms are implicated in the etiologies of these diseases including dysfunction of neurotransmitter systems. Research has shown the key role the cholinergic system plays in the symptoms of these three conditions.

It was first indicated by Deutsch (1971) that the cholinergic system may be key to understanding the progression of Alzheimer's disease. It was noted by Drachman (1977) that anticholinergic drugs were able to induce Alzheimer's-like cognitive impairments in healthy individuals, and cholinergic enhancing drugs were able to improve cognitive performance in

those with dementia. Alzheimer's disease is a progressive neurodegenerative disease characterized by memory deficits, personality changes, and eventual death. In addition to other neural changes, much research has shown degradation of the cholinergic system to be a consistent feature of Alzheimer's disease (Dunnet & Fibiger, 1993; Schliebs & Arendt, 2006).

Cholinergic neurons in the basal forebrain undergo significant degeneration in Alzheimer's disease, leading to lack of cholinergic modulation in important brain regions such as the prefrontal cortex, hippocampus, nucleus accumbens and hypothalamus (Schliebs & Arendt 2006). Much of the memory deficit that is characteristic of Alzheimer's disease can be attributed to cholinergic degeneration in the hippocampus (Gron et al., 2006). General cholinergic hypofunction including reduction in choline acetyltransferase, nicotinic and muscarinic receptor binding, and lower levels of acetylcholine have been correlated with cognitive decline seen in the progression of Alzheimer's disease (Moll et al., 1990). However, decline in cognitive function as determined by Mini Mental State scores, is only seen after about 30% loss of basal forebrain cholinergic neurons (Arendt, 1999).

Administration of cholinergic toxins has been shown to lead to deficits in performance in various cognitive function tasks (Fibiger, 1991). Patients administered scopolamine to induce memory and cognitive impairments had little improvement when subsequently administered amphetamine to increase alertness. This result suggests that cognitive decline in Alzheimer's is not simply due to level of arousal and attentional deficits. However, co-administration of a cholinergic receptor agonist was able to produce marked improvement in memory and cognitive performance (Drachman, 1977). In addition, NMDA agonist induced lesions of the basal forebrain in rats lead to decreased cortical levels of acetylcholine and impairment in learning the Morris Water Maze and radial maze tasks (Dunnet & Fibiger, 1993).

In addition to learning and memory related cognitive deficits, personality changes, psychotic symptoms, aggression, and impulsive behaviors are also characteristic of Alzheimer's disease. It is postulated that these symptoms are due to cholinergic hypofunction in the prefrontal cortex, hippocampus, and limbic system (Bidzan et al., 2012). A study in an elderly residential community in Cache County, Utah found that out of over 329 patients with dementia, 61% exhibited behavioral and mental disturbances in the past month, and 24% of patients showed agitation and aggressive symptoms. Aggression was more common in patients with advanced dementia, and correlated with estimated cholinergic system dysfunction (Lykestos et al., 2000).

The most common pharmacological treatment for Alzheimer's disease is acetylcholinesterase inhibitors, or drugs that inhibit the breakdown of acetylcholine, leading to higher cortical levels of acetylcholine (Trinh et al., 2003). These drugs have been shown to have varying levels of efficacy when treating different symptoms of the disease. Cholinesterase inhibitors are effective in treating attentional symptoms that cause other cognitive impairments such as executive function and memory (Bracco et al., 2014). Pharmacological enhancement of cholinergic function with acetylcholinesterase inhibition in patients with mild cognitive impairment, a precursor to Alzheimer's, has been shown to improve hippocampal function (Gron et al., 2006). In addition, administration of the cholinesterase inhibitor donezepil to healthy patients, improved both verbal and visual episodic memory when compared to a placebo (Gron et al., 2005).

More recently, studies have been conducted to test the possibility of treating behavioral and psychological symptoms of dementia with acetylcholinesterase inhibitors as well. Rosler (2002) showed that rivastigmine, a dual acetylcholinesterase/butyrylcholinesterase inhibitor, has potential for the treatment of behavioral symptoms including apathy, hallucinations, and

depression. In addition, treatment with rivastigmine decreased the need for additional antipsychotic medications to manage these symptoms (Rosler, 2002). However, Trinh et al. (2003) concluded that cholinesterase inhibitors only cause a modest decrease in psychotic symptoms for patients with moderately progressed Alzheimer's disease. It is suggested that these patients rely on mood stabilizers for symptom management.

Schizophrenia is another psychiatric disorder that has been correlated with cholinergic dysfunction. Common symptoms of schizophrenia include increases in risky decision making, impulsivity, and psychotic episodes (Montes et al., 2015). Schizophrenia has high comorbidity with drug abuse and patients often score highly on the Barratt Impulsivity Scale Evidence (Gut-Fayand et al., 2001). Muscarinic acetylcholine receptors seem to play a key role in antipsychotic benefits of cholinergic enhancers in schizophrenia patients. Administration of the muscarinic receptor agonist arecoline decreased psychotic symptoms in patients with schizophrenia (Pfeiffer & Jenney, 1957). Primate studies show that amphetamine-induced psychotic behavior can be normalized by administration of muscarinic agonist BuTAC (Andersen et al., 2015).

Evidence also exists to support nicotinic acetylcholine receptor modulation of schizophrenic symptoms in addition to muscarinic receptors. There is a high comorbidity of schizophrenia and tobacco abuse (about 80%), and it has been postulated that nicotine helps improve neuropsychological deficits in these patients (Machowick et al., 2014). Both $\alpha 7$ and $\alpha_4\beta_2$ nicotinic acetylcholine receptors have emerged as possible targets to treat cognitive and psychotic symptoms associated with schizophrenia, especially in patients that are non-responsive to commonly used antipsychotics (Freedman, 2014; Eden et al., 2008).

Finally, attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder often diagnosed in children characterized by problems in executive function, inhibitory control,

regulation of attention, all processes mediated by the prefrontal cortex (Wodushek & Neumann, 2003; Barkley & Biederman, 1997), especially the dorsolateral prefrontal cortex and the orbitofrontal cortex (Urcelay & Dalley, 2012). Stimulants are often effective treatments for ADHD as they upregulate the functioning of the prefrontal cortex, especially catecholamine neurotransmission (Halperin & Schulz, 2006).

In addition to prefrontal cortex hypofunction, deficits in cholinergic function may also play a role in ADHD symptoms. It was previously believed that dopamine and norepinephrine were the most essential neurotransmitters involved in the etiology of the condition, but more recent research shows that acetylcholine, especially the functioning of nicotinic acetylcholine receptors, may play a large role in the condition as well. Similarly to what is seen in schizophrenic populations, tobacco abuse is often comorbid with ADHD in adults (Kollins et al., 2005). Treatment of ADHD by nicotinic acetylcholine receptor agonists has showed moderate treatment efficacy (Williams et al., 2012; Potter et al., 2014), especially for enhancement of cognitive impairments (Wilens & Decker, 2007).

The present study sought to investigate the role of cholinergic projections to the orbitofrontal cortex on impulsivity and risky decision-making. Because of the selectivity of the immunotoxin, 192 IgG-saporin was used to destroy cholinergic neurons in the orbitofrontal cortex in rats. To test impulsivity, a probability discounting task was used because of its similarity to real-life situations.

Methods

Subjects

Subjects were 11 Sprague Dawley female rats 2-3 months old at the beginning of the experiment. Subjects were housed in pairs in a room with a 14/10 hour light/dark cycle. Food pellets were available to the animals at all times in their cages, but water was restricted to during testing and 30 minutes per day after the daily test session. Subjects were tested 5-7 days per week for the entire duration of the experiment. Rats received 60 min of water access on days they were not tested. The experimental procedures were approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Apparatus

The rats were trained in one of four chambers, each enclosed in a sound-attenuating box. One side of the chamber was equipped with two water ports with dippers for water administration. Each water port was equipped with photocells to detect the animal entering the port. When a port-entry was detected, the water dipper was raised. In between the two water ports was a retractable lever. One dipper cup in each chamber held 0.01mL of water and the other held 0.06mL of water. The large reward was located at the right port in two of the chambers and in the left port in the other two chambers (Figure 1). A houselight in the back of the chamber provided dim illumination. Training programs were computerized and controlled by MED-PC IV software.

Behavioral Training Procedures

The house light was illuminated for the duration of the testing session. Behavioral procedures started with training to enter the water ports. In this stage, both rewards were

available following each nose-poke into the ports. To move onto the next stage of training, subjects needed to enter water ports 80 times in the test session.

The next stage trained entry of the correct water port, as directed by the illumination of one of the panel lights above the water ports. In this stage, each trial was initiated by extension of the lever in between the ports. A lever press initiated the illumination of one of the panel lights above one of the water ports. A nose poke under the illuminated port allowed the dipper to be raised for 3 seconds.

The final training phase was a probability-discounting task. These training sessions included a lever press to initiate a trial. The training sessions were divided into three sets of 24 trials. Each set of 24 trials started with 12 forced trials and ended with 12 free choice trials. In all of the sets, the probability of receiving the small reward by entering the port was 100% during the first set of trials. The probability of receiving a large reward by entering the port decreased throughout the test session with the first set at 100%, second set at 33%, and the third set at 17%.

In the forced trial section of each set, only one panel light was illuminated at a time indicating the animal would only receive a reward by entering that specific water port. Six of the 12 trials in each forced trial section were for the large reward, and six were for the small reward. The order of the forced trials for large and small rewards was random. The small reward was always available, and the large reward was available at the 100%, 33%, or 17%, proportional to the probability being used in the upcoming set.

The next section was the 12 free choice trials. In this section, the panel lights above both of the rewards were illuminated, allowing the animal to choose which side to go to. If the animal failed to enter a water port during the 10 seconds the lights were illuminated, the trial was ended

and recorded as an omission. After omissions, both panel lights turned off for a 60 second inter-trial interval. The test session was ended after all three blocks were completed by the subject.

Procedure for 192 IgG-saporin administration/Surgery procedure

Rats were divided into lesion (n=4), vehicle (n=4), and non-surgery (n=3) groups. Rats receiving surgery were anesthetized with 90.0 mg/kg ketamine and 9.0 mg/kg xylazine. During surgery, lesion animals received three injections bilaterally for a total of six injections of 0.3μL of 0.4μg/μL of the cholinergic immunotoxin 192 IgG-saporin (Advanced Targeting Systems, San Diego, CA) into the orbitofrontal cortex. The coordinates of the infusions relative to the bregma were anterior/posterior (AP) 4.0mm, mediolateral (ML) +-0.8, and dorsoventral (DV) -3.4, for the first site of injections. For the second injections, the coordinates were AP 3.7, ML +/-2.0, DV -3.6, and for the last injection coordinates were AP 3.2, ML +/-2.6, DV -4.4 relative to the bregma. Sham-lesioned animals were infused with saline. Following surgery, animals were allowed free access to water and restarted re-testing in the probability-discounting task approximately 4-5 days later when water restriction was reinstated.

Behavioral measures and Statistical Analyses

Performance in the task was determined by the number of times the animal chose the large reward out of the 12 total trials in each of the three blocks of free-choice trials. Data was counted for 15 test days post-surgery for the animals. The 15 days post-surgery were broken into 5 blocks of 3 days and averaged (B1-B5). Statistical analyses were carried out using SPSS. Analyses included ANOVAs on the effects of the lesion, surgery, and blocks over time. Statistical significance was given at a level of $\alpha = 0.05$.

Results

Presurgical performance

There were no statistically significant differences between lesion, sham-lesion, and non-surgery animals in average number of entries to the large reward port during the three days prior to surgery.

Effect of 192 IgG-Saporin on Task Performance

Probability of the animals choosing the large reward did tend to vary over the five time blocks for the three sets in the task for all groups of animals (Figure 2). To quantify this observation, a set (100%, 33%, 17%) x block (B1-5) x group (lesion, sham, no surgery) ANOVA was conducted. The analysis indicated that the interaction between these variables approached a level of significance ($F(16,64) = 1.572$, $p = .103$). Given the small sample size, we tested the basis for this trend.

Subsequent ANOVA's revealed a trend for a significant effect of Group during the last block of testing trials when the large reward was available following 17% of port entries, $F(2, 10) = 4.321$, $p = .053$ (Figure 3). Follow-up independent samples t tests indicated, that, during the final set of trials when the probability of receiving the large reward was 17%, the lesioned animals' number of port entries was significantly different from the nonsurgical animals, $t(5) = 2.600$, $p < .05$. There was a trend for the sham-lesioned animals also to differ from the

nonsurgical animals, $t(5) = 2.150$, $p = .084$. Lesioned and sham-lesioned animals did not differ from each other, $t(6) = 1.383$, $p > .20$.

An ANOVA conducted to determine significance related to omissions, when the animal did not respond during a trial, showed that there were no Group differences in omissions rates. Subsequent independent samples t tests indicated there was a main effect of omissions as the probability of receiving the large reward was varied (Figure 4). Animals had fewer omissions when the probability of receiving a large reward was 100% compared to higher rates at 33% and 17%. Animals omit more at 33% than 100% ($t(10) = -2.698$, $p = 0.022$), and even more at 17% than at 33% ($t(10) = -1.713$, $p = .117$).

Discussion

The present experiment sought to investigate the effects of infusions of 192 IgG-saporin into the orbitofrontal cortex on a probability discounting task in rats. The differences seen in the animals' rates of choosing the large reward port shows differences in the groups' risky decision-making, or impulsivity. While there were no significant differences between the lesion, sham-lesion, and non-surgery groups prior to surgery, there was a significant difference between lesion and non-surgery animals after surgery. The difference in response between these groups was observed at the 17% large reward availability (Figure 2). While the lesioned animals did choose the large reward at a higher rate than the sham lesioned animals, the difference was not statistically significant. A comparison between sham lesioned and nonsurgical animals approached significance. Given the small sample size, it is possible that the difference between the sham-lesioned and nonsurgical animals would have been significant with more animals, however, that was not a key research question in this experiment.

It is important to note that while differences were seen at 17% availability of the large reward, rats in all of the groups chose the large reward at 100% availability at relatively the same rate (Figure 2). In this first set of free trials, all of the rats chose the large reward almost all of the time. This suggests that in all experimental groups, animals were able to discriminate between the small and the large reward. In addition, there was no significant difference between the rates of omissions between different experimental groups for any of the three sets of free trials (100%, 33%, 17%). The lack of lesion or surgery-induced effects on omissions suggests that neither the immunotoxin nor the surgical procedures affected the rats' motivation to receive the reward. Difference in omission rates reached significance in independent t tests comparing the three sets of free-choice trials in a test session. As the probability of receiving the large reward declined throughout the test session, the animal's rates of omissions increased. This may be due to fatigue as the session progressed, or disengagement from the task when the large reward was rarely available. However the omission rate remained low for all animals.

The comparison between the lesioned and the nonsurgical animals suggests that cholinergic projections to the orbitofrontal cortex are important for task performance. However, this interpretation must be considered with the lack of significant difference between the lesion and the sham-lesioned animals. The lack of significance between the lesion and sham lesion groups runs contrary to much of the literature indicating that orbitofrontal lesions increase risky decision making. Thus, we cannot rule out that aspects of the surgery including anesthesia, needle penetration, or damage from infusions, are sufficient to impair task performance. It is known that the orbitofrontal cortex is involved in coding both the identity and value of a reward (Howard et al., 2015) including the discounted value of a delayed reward (Roesch & Olson, 2005). After surgery, 192 IgG-saporin lesioned animals still exhibited the same trend as sham-

lesioned animals in decreased affinity for the large reward as the large reward was discounted. The lack of significance between the lesion and sham-lesioned animals for 17% availability of large rewards suggest that other neurotransmitter systems within the orbitofrontal cortex, affected by the blunt trauma associated with surgery could play a role in mediating this type of decision-making.

It is notable to compare the results of this study with Winstanley et al. (2004), in which excitotoxic lesions in either the orbitofrontal cortex or basolateral amygdala were induced in rats. They found that, contrary to previous research, lesions to the orbitofrontal cortex actually decreased animals' impulsivity in a delay discounting task. This result was attributed to lesion-induced deficits in integration of consequences. It was postulated that the punishing quality of not receiving a reward when making a risky choice was not properly encoded in rats with orbitofrontal cortex lesions.

Interestingly, in this study, the comparison between sham-lesioned animals and nonsurgical animals approached significance at $p = .084$. This result suggests that the surgery itself had a large effect on the performance of the animal. Over the 5 time blocks, performance did not change significantly for the groups undergoing surgery, indicating that they were given adequate time to recover. Thus, the increase in risky decision-making seen in the sham animals when compared to the nonsurgical animals can be attributed to sensitivity of the orbitofrontal cortex to surgical procedures. Similar surgical procedures had been performed to target other brain regions in studies such as McGaughy et al. (1996) with no notable effect of surgery, suggesting that the orbitofrontal cortex is a very sensitive brain region. Trauma from surgery including effects of anesthesia, needle penetration, or damage from saline infusion may have been sufficient to alter task performance.

Due to the large effect of surgery that was noted in this study, the ethical choice was made to refrain from conducting more surgeries. A clearer understanding of the cause of the surgery induced changes in task performance is necessary before being able to clearly address questions related to the role of cholinergic inputs to the orbitofrontal cortex. It is possible that refinement of the training or surgical procedures will minimize surgery-induced changes in task performance. For example, smaller cholinergic lesions to different sub-regions of the orbitofrontal cortex would decrease the extent of the blunt surgical trauma in sham-lesioned animals.

In summary, as the results of this study do not align with the majority of the related research, it is important to further investigate the role of cholinergic projections to the orbitofrontal cortex. It is clear from previous studies that the orbitofrontal cortex plays a critical role in regulation of goal-directed behavior. These cholinergic neurons have been shown to be important in neuropsychiatric disorders, so greater understanding of how this brain system modulates decision-making is necessary to developing more targeted treatments.

Acknowledgements

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Figures

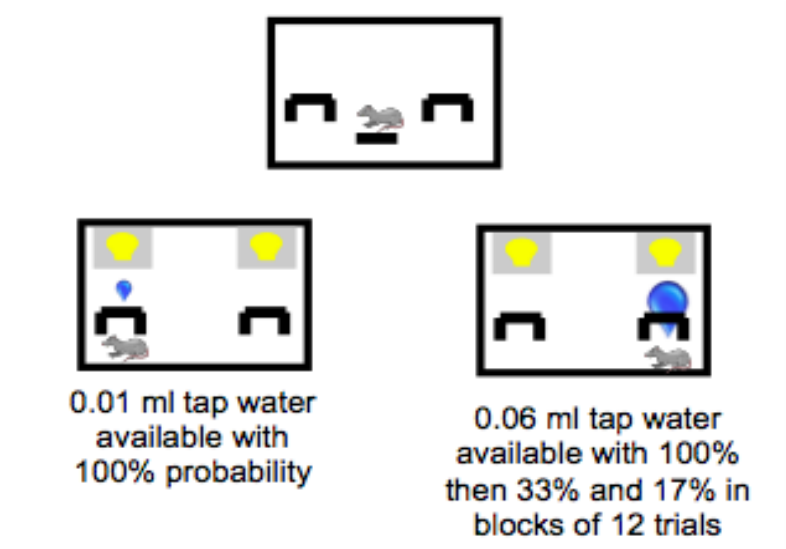

Figure 2. The set-up of the testing chambers. One wall of the test chamber was equipped with two water ports, two panel lights, and one retractable lever in the center. During a free-choice trial, the lever would be extended in the center of the panel wall. A lever-press initiated the illumination of the two panel lights, indicating that the rat could make a choice as to which side port to enter. The large reward port gave 0.06ml of water, and the small reward port gave 0.01ml of water.

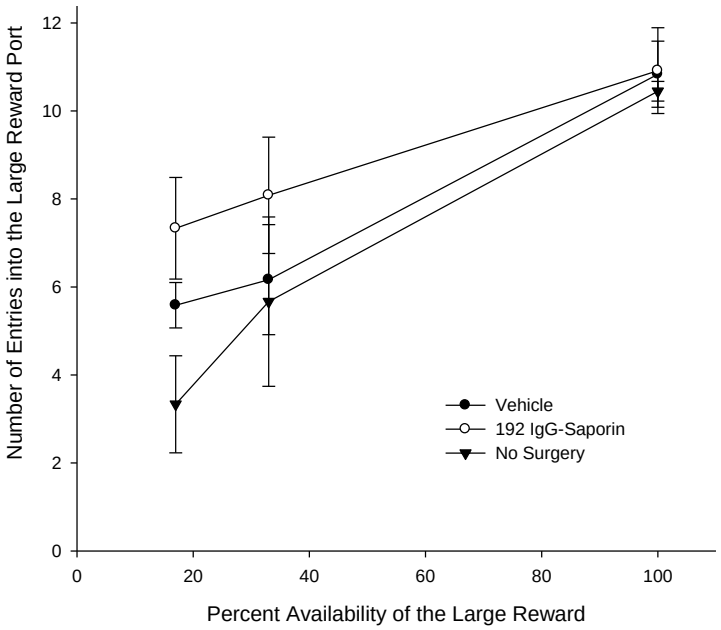

Figure 2. Mean number of entries into the large reward port for animals in the three experimental groups for the three probabilities of receiving the large reward during block 5 (B5) post-surgery. All experimental groups exhibited the trend of choosing the large reward port less frequently as the probability of receiving the large reward decreased within a test session. 192 IgG-saporin lesion animals chose the large reward port more often than the sham-lesioned animals, but the interaction did not reach significance. A comparison between the lesion animals and nonsurgical animals showed a significant difference in their behavior in the task.

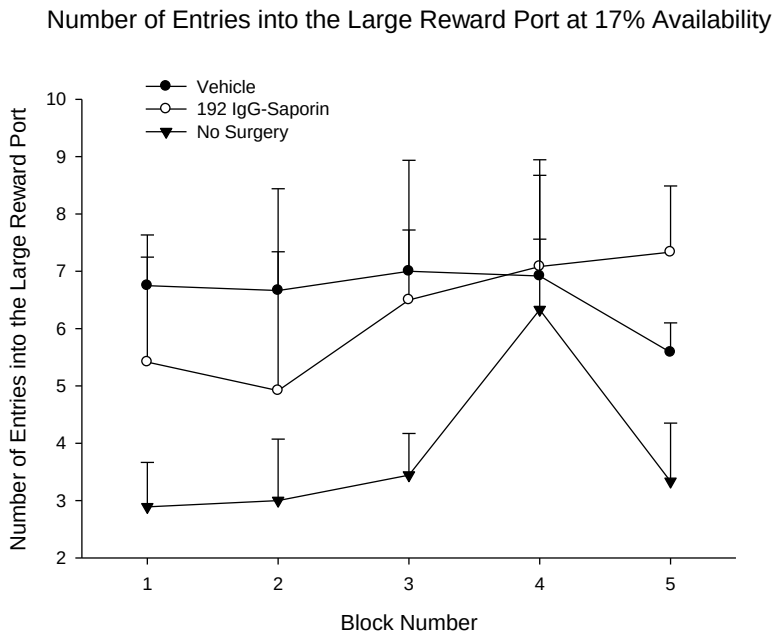

Figure 3. The number of mean entries into the large reward port for the three groups over the five blocks (B1-B5) for the 17% large reward availability trials. Over the five time blocks, 192 IgG-saporin animals slightly increased their affinity for the large reward port, and by block 5, lesion animals chose the large reward port more often than both the sham-lesioned animals and the nonsurgical animals.

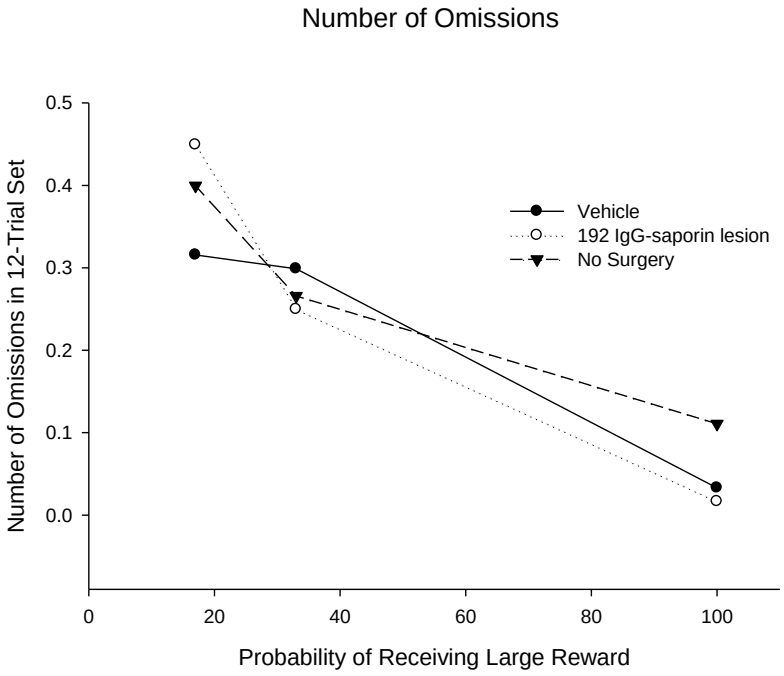

Figure 4. Number of mean omissions made by the three groups from B1-B5. Omissions stayed low for all groups throughout testing. This figure shows the trend in the increase in omissions in all groups as probability of receiving the large reward was decreased throughout a test session. No significant difference in omissions were found between any of the groups.

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