Control of Intertemporal Choice by Dorsal Raphe Serotonergic Neurons

by

Sangyu Xu

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Signature redacted

Author .....
Department of Brain and Cognitive Sciences
August 12, 2016

Signature redacted

Certified by....
Susumu Tonegawa
Picower Professor of Biology and Neuroscience
Thesis Supervisor

Signature redacted

Accepted by .....
Matthew A. Wilson
Sherman Fairchild Professor of Neuroscience and Picower Scholar
Director of Graduate Education for Brain and Cognitive Sciences

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Abstract

While animals tend to prefer immediate rewards to delayed ones [1], delayed gratification is often advantageous [2]. Appropriate choice about future rewards is critical for survival. The dorsal raphe serotonergic neurons have been long implicated in the control of temporal discounting of reward [3] [4], but it is not clear whether their activities in fact direct the decision making process. In this thesis, I designed a cued intertemporal choice task for mice that allows the combination of highly specific genetic manipulations with sophisticated behavioral interrogations. The task utilizes odors to communicate upcoming reward contingencies to the mouse subjects. I found that optogenetically augmenting or silencing the activities of dorsal raphe serotonergic neurons precisely at decision epochs resulted in an increase or a reduction in the choice for the delayed and larger reward, respectively. These manipulations do not alter the subjects' choice in trials involving immediate rewards, suggesting that serotonin might only be important for conditions in which difficult trade-offs are required. I also demonstrated that the nucleus accumbens, a major component of the mesolimbic reward pathway, is a possible downstream target of the aforementioned serotonin action. Taken together, these results show that serotonergic neurons regulate inter-temporal choice behavior bidirectionally, possibly through actions in nucleus accumbens.

Thesis Supervisor: Susumu Tonegawa
Title: Picower Professor of Biology and Neuroscience

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The Ant and the Grasshopper

In a field one summer's day a Grasshopper was hopping about, chirping and singing to its heart's content. An Ant passed by, bearing along with great toil an ear of corn he was taking to the nest.

"Why not come and chat with me," said the Grasshopper, "instead of toiling and moiling in that way?"

"I am helping to lay up food for the winter," said the Ant, "and recommend you to do the same."

"Why bother about winter?" said the Grasshopper; "we have got plenty of food at present." But the Ant went on its way and continued its toil.

When the winter came the Grasshopper had no food, and found itself dying of hunger, while it saw the ants distributing every day corn and grain from the stores they had collected in the summer. Then the Grasshopper knew:

"IT IS BEST TO PREPARE FOR THE DAYS OF NECESSITY."

Æsop's Fables. Sixth century B.C.

Contents

List of Figures

List of Tables

Chapter 1

Introduction to Intertemporal Choice and Choice Impulsivity

Animals have to make decisions about time all the time. The ants in the famous Aesop's fable have to choose between eating the food they harvested while it was abundant in the summer, and storing the food for later during the winter when it would be scarce. This is an illustration of an intertemporal choice, choosing between options over time. We choose between reaching for a cigarette and preserving long-term health, and we debate between truancy and getting an education for later employability. To spend or to save, to procrastinate or to work: intertemporal decisions pervade our daily life. On a larger scale, we have to choose collectively as a society. Do we select a political candidate who promises to cut taxes or one who is devoted to long-term civil projects that will benefit us as a group for many years to come? Clearly, these choices that we make about time have implications in diverse arenas such as health, economics and politics.

Given the choice, animals tend to prefer a reward that will arrive sooner than one that will arrive later [1]. In some scenarios, however, the delay to the reward and the size of the reward create a dilemma for the decision maker. For example, while one has no difficulty choosing between receiving 10 dollars today and 10 dollars in a week, one might hesitate to choose between 10 dollars today and 20 dollars in a week. The

latter scenario, where a delay-size trade-off has to be performed by the decision maker, is a more interesting case of intertemporal choice. Many problems life poses involve such decisions, in which we must choose between *wanting more* and *wanting it sooner.*

How we choose between reward options that are differently delayed is governed by a few decision variables. Obviously, these include the size of the rewards available and the delay to the rewards. A third variable, our discount function, governs how we valuate the future reward.

1.1 Temporal Discounting of Reward

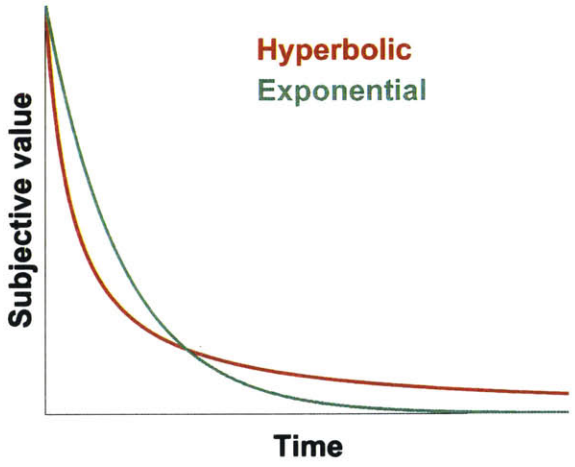

Figure 1-1: Discounting functions.

Temporal discounting of reward is a decrease in the subjective value of a reward as it is delayed into the future. It is also referred to as *delay discounting* in animal experimental psychology. This decrease in value is steep and negatively accelerated, and is often described with a hyperbolic function, exponential function or some combination of both (Fig. 1-1). In the framework of reinforcement learning, temporal discounting can be viewed as the loss of effectiveness for a reinforcer to reinforce an action or a conditioned stimulus.

A constant K, the discount factor, determines how steep the discount function is. Different individuals have different discount factors [5] . An individual's discount factor has been shown to be at least partially hereditary [6], and associated with

age [7], drug use [8] and mental health conditions [9] [10]. As discount factor could be considered an individual trait, it is possible that the ants and the grasshoppers in the fable have different discount factors.

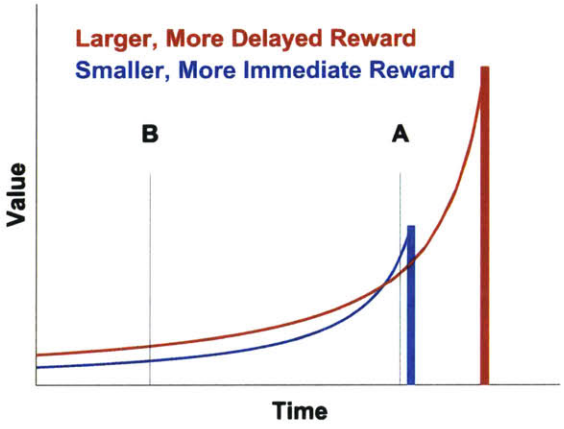

Figure 1-2: Preference reversal: When the choice point is at A, and the two options are offered soon into the future, the subjective value for the small, more immediate reward is higher; however, when the two options are far away into the future, such as when the choice point is set to be at B, the larger reward is more preferable. This is a feature of hyperbolic discount function. Adapted from Ainslie(1981).

Now, back to the example choice between receiving 10 dollars today and 20 dollars some time in the future. If one is asked to pick between 10 dollars today and 20 dollars in a week, 10 dollars today might sound very enticing. However, if the two options are similarly spaced apart but occur much further into the future, say 10 dollars in 50 days versus 20 dollars in 57 days, 20 dollars in 57 days appeals more to most people. This phenomenon is called preference reversal [11] (Fig. 1-2). As Ainslie demonstrated in his analysis, preference reversal can only occur when the subject has a non-exponential discounting function, e.g. it holds for hyperbolic discounting.

1.2 Choice Impulsivity

Impulsivity, acting prematurely without foresight, is a maladaptive behavior. Impulsive behavior can be divided into three categories: *reflection impulsivity* refers to not sampling evidence enough before making a response; *impulsive action* refers to the inability to suppress a motor response; *impulsive choice* refers to choosing a more

immediate reward over a larger reward that is more delayed. The type of impulsivity we deal with in intertemporal choice is *choice impulsivity* i.e. a failure to choose to delay gratification. Choice impulsivity is a feature of many psychiatric conditions, such as ADHD, mania and substance abuse.

1.3 Behavior Paradigms for Intertemporal Choice

Intertemporal choice is a problem that has attracted the attention of economists, psychologists and animal behaviorists alike. As a result of a history of convergent effort, the methods of investigating intertemporal choice are as diverse as the people interested in it. I review below behavior paradigms that have been used over the years to investigate how animals, including people, make choices about rewards in time.

1.3.1 Pigeons and Rodents

Non-primate intertemporal choice tasks can be split into two main categories: *systematic* tasks [12] and *adjusting* tasks [13] [14] [15] [16].

In *systematic* tasks, the experimenter decides on a set of delay and size contingencies, and tests the subjects systematically on them, only changing variables between sessions. In most cases, the tasks are not cued. Sometime the free choices are preceded with a block of forced choices, so that the subject can be sure to sample the contingencies on both sides. These tasks have a range of shortcomings. First, since the subjects are not informed trial-by-trial about upcoming contingencies, they have to remember what previous trials entailed, and then make their choice. This leads to a memory confound in any effect found in the subjects' preferences. Second, since often the delay contingencies are varied by session, or by blocks of trials, there is no efficient way of randomizing the contingencies. Third, when subjects respond to blocks of the same reward contingencies, they may not make every decision in real-time, since it is good enough to rely on the last decision. This amplifies the influence of the previous trial on the current choice and may result in trials where the decision epoch is absent.

In *adjusting* tasks, the reward contingencies are adjusted according to the be-

havioral responses of the subjects. For example, a rule could be set to increase the delay to a particular reward option if the subject chooses it at high frequencies, and decrease the delay to that option if the subject fails to choose it. Adjusting tasks aim to find indifference points by titrating one of the independent variables. Pigeons and rats have been found to discount hyperbolically in a series of adjusting procedures [17] (See his reasoning and derivation for the indifference function in Appendix A).

Adjusting tasks share some of the pitfalls of the systematic tasks, such as a lack of guaranteed real-time decision epochs. In addition, adjusting tasks do not stabilize consistently, even over long periods of training, and the subjects do not respond to any rapid changes in reward contingencies [18]. The biggest problem with adjusting reward contingencies across testing is the introduction of other confounding variables, probably learning-related, into the testing of decision-making, leading to erroneous conclusions. For example, the involvement of orbitofrontal cortex (OFC) in intertemporal choice has been a point of contention. OFC was found to promote impulsive choice [15], suppress impulsive choice [16] or both depending on context [19]. The effect seemed to depend on whether OFC was lesioned before or after training. Considering especially that OFC is critically involved in the acquisition of reward value representations, the conflict in the OFC delay discounting results seems to be due to a learning effect. This mystery was partially clarified when rats were lesioned in OFC after training in a 6-arm maze, which cued the rats about the delay contingencies associated with the arms. No effect was found [20]. OFC appears not to be involved in delay discounting *per se*.

The 6-arm maze version of the delay discounting task was a marked improvement on previous tasks. The crucial component that the maze task provided was cue. When rodents could be cued, trials could be randomized rapidly, decorrelating the preference from many other variables. While it was probably very labor intensive, it was very much like a primate task.

1.3.2 Humans and Non-Human Primates

In humans, a delay discounting task in a systematic and cued form is very simply administered. Most human already speak a language the experimenter is able to communicate in, or they can be taught quickly to look at a computer screen where the size or color of certain cues represent reward contingencies [21] [22]. Monkeys typically can be taught the latter [23]. As mentioned above, cueing the subjects allows the randomization of trials, which in turn allows the isolation of decision epochs. These epochs are essential for monitoring real-time neural activities relevant to the decisions, and for dynamic transient manipulations.

1.3.3 Motivations for a Cued Intertemporal Choice Task and Olfaction as a Mode of Communication

Sections 1.3.1 and 1.3.2 make it abundantly clear that there is a need to design a rodent intertemporal task with cues. Rodents are much lower maintenance and faster in growth and reproduction than monkeys. Transgenic mice have revolutionized the study of many aspects of biology, including neuroscience. Optogenetics, still very much a difficult technology in monkeys, is extremely accessible in mice.

One of the hurdles with using mice is communication. The final task we want to achieve contains complex rules and requires mice to respond to large arrays of stimuli. Hence, we need a mode of communication that is quick to learn and is quantitative. Mice, as it turns out, see terribly, but hear much better and smell extremely well. It was demonstrated that olfactory cues were efficient conditioned stimuli that worked on very fast timescales [24], and at a high level of discrimination between concentration levels [25]. These are the threads that inspired the design of an odor-guided intertemporal choice task. The task is done with an apparatus consisting of an operant chamber and an olfactometer. The setup is described in the next chapter.

Chapter 2

The Odor-Guided Intertemporal Choice (OGIC) Task for Mice

In this chapter, I describe a novel cued intertemporal choice task I have designed for mice, and briefly describe sample behavior data from the task.

2.1 Design Goals

2.1.1 Automated Behavioral Testing

The equipment that the behavior task relies on is completely automated, including the operant chamber, the olfactometer that delivers the odor-carrying air and the delivery of any laser pulses. Automation allows high throughput collection of data with minimal human interaction.

2.1.2 Isolating the Decision Epoch

To achieve this goal of isolating the decision epoch, I used an olfactory cue to signal to the mouse subjects the delay contingencies of the upcoming reward options. When a subject intiated the task, a mixture of two odors was delivered. The concentration of odor A (caproic acid) signaled the delay of the left reward, while the concentration of odor B (hexanol) signaled the delay of the right reward. The reward contingencies

were randomized trial-by-trial, so that decisions were independent of history. Compared to block-wise task designs, this procedure ensured real-time decision making in every trial. Compared to the various adjusting-delay tasks where preferences often fail to converge [18], this task prevented strategy-forming in the subject, and was relatively stationary across days. After training to associate odor concentration with reward delay, mice reliably chose the less delayed reward option. When offered a choice between a large and a small reward, the subjects' preferences readily shifted to the large reward. Overall, this procedure isolated the decision epoch for manipulation and monitoring.

2.1.3 Testing the Choice to Wait

Many mouse waiting tasks that utilize infrared beams for registering nose pokes require the mice to remain in the port [26] [27] [28] [29]. For a naturally hyperactive rodent species, this could introduce unnecessary effort confound. In my task, once the subjects made a commitment poke into the chosen reward port, they did not have to stay there to receive the reward (Fig. 2-1). Once the delay elapsed, a water reward was delivered with an audible click of the water valve. Since the subjects were well-trained and motivated, they were able to go back to the reward port to collect the water droplet. During the waiting period, the subjects were free to wait anywhere in the chamber. The subjects collected more than 95% of the rewards. The subjects then had to wait for the remaining portion of the trial duration to lapse before they could initiate a new trial. The trial duration is kept the same regardless of the side chosen. These considerations ensured that only the time component of the waiting is tested.

2.2 Task Description

2.2.1 Apparatus

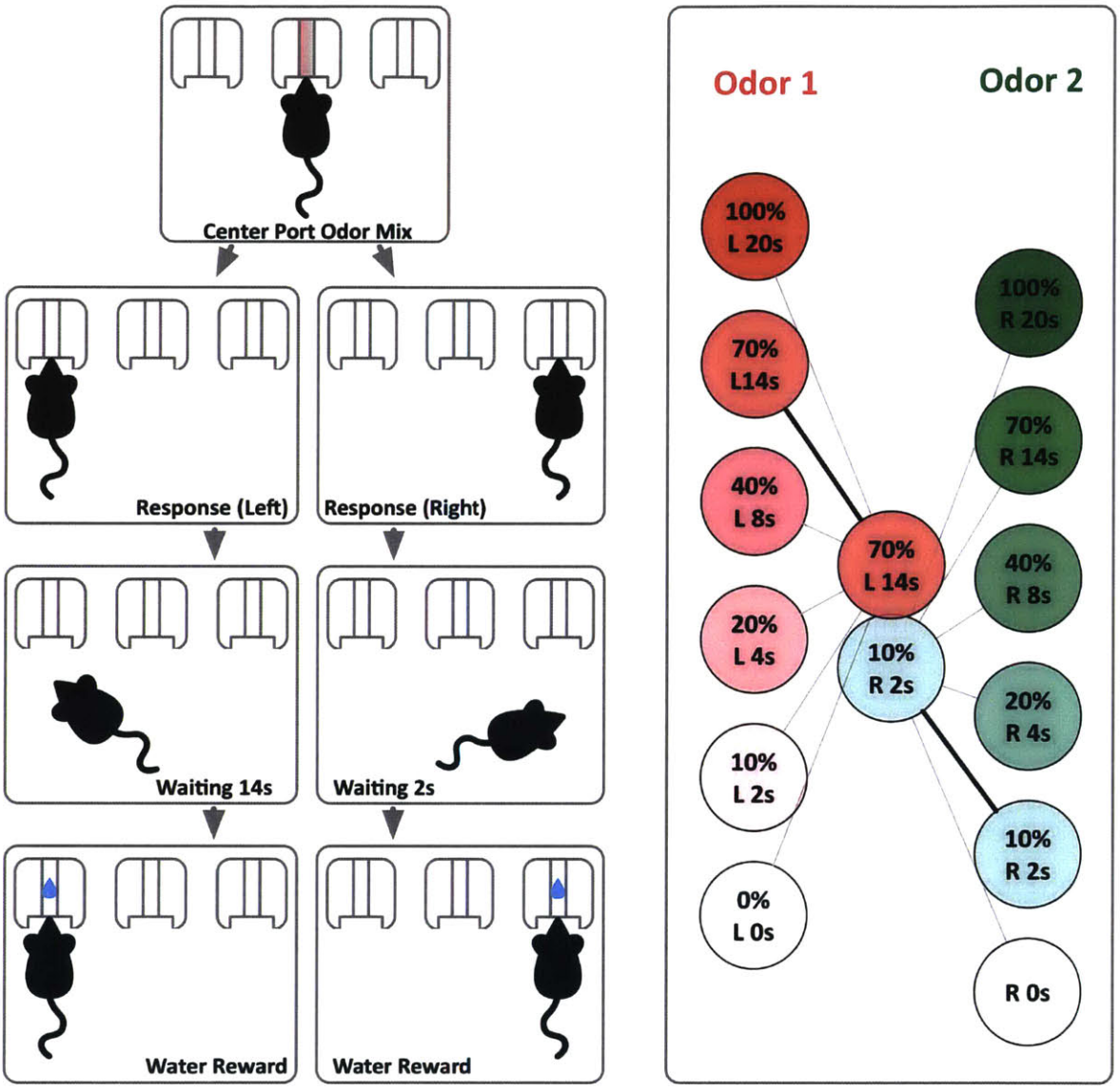

Figure 2-1: Task structure and cue delay correspondence. The left panel shows the sequence of events in a typical trial. Subject initiates the tasks by sampling odors in the center odor port. They then make a choice by poking in the left or right reward port, wait the prescribed amount of time and receive water reward on the chosen side. Right panel shows the linear variation of reward delays in correspondence to their respective odors.

The task took place in a rectangular custom-designed operant chamber [24] (see Fig. 2-2). There were three ports that the subject could nosepoke into. Each port was equipped with an infrared beam for registering the nosepoke and a white LED for signaling purposes. The center port was the odor port and was connected via tubing to the carrier air flow from an olfactometer (Island Motion, Tappan, NY, USA). The carrier air was joined at the odor manifold by streams of odor-carrying air flow. This odor flow was created by passing clean air through a filter loaded with odorants. The odor flows were mixed at the manifold and delivered together to the subject. The side ports were reward ports and were connected to a reservoir of water, via solenoid valves. Water was dispensed when a TTL signal opened the solenoid valves. The amount of water dispensed was controlled by the length of time the valves were open. All electrical components were controlled via RJ45 cables by a state machine. The state machine was in turn controlled by a custom-designed software suite (MATLAB, The MathWorks Inc., Natick, MA, USA). Beam break data was automatically logged by the Matlab software.

2.2.2 Subjects

Subjects were transgenic mice in the C57BL/6J background between 2 and 6 months of age. They were housed and water-restricted in accordance with guidelines from the Committee on Animal Care at the Massachusett Institute of Technology.

2.2.3 Shaping

Subjects were water restricted for a week (administered 1.2 milliliters of water in a single session per day), and then gradually shaped to associate odor concentration with reward delays. On day one, subjects were placed into the chamber with the shaping protocol already in place. Subjects were required to poke in the center port and collect a reward from either side port (2-1). The concentration of each odor was correctly associated with the delay on the same side as in the final task. The carrier flow was increased gradually over the course of the shaping process. The subjects

performed up to 200 trials per session. The set of odor flow values, reward delays and total flow rate were as shown in Table 2.1.

2.2.4 Final Task

Once the subjects reliably preferred the less delayed reward to the more delayed reward, the left reward was made twice as large as the right reward. This was done by triggering two valve openings on the left and one on the right. This was maintained throughout the remainder of testing. The array of left reward delays used were (0 s, 2 s, 4 s, 8 s, 14 s and 20 s). The array of right reward delays used were (0 s, 2 s, 4 s, 8 s, 14 s and 20 s). In all optogenetic experiments, a smaller set of right reward delays were used (0s, 2s and 8s). The left reward will be interchangeably referred to as the large reward, and the right reward as the small reward.

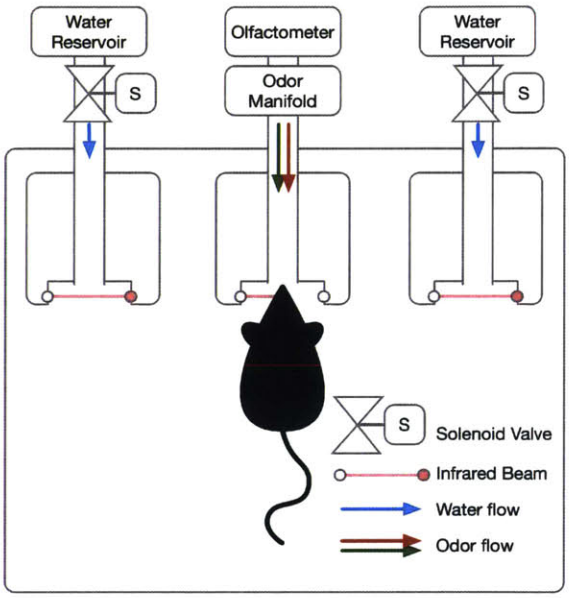

Figure 2-2: Operant chamber connected to olfactometer.

Day	Odor Conc. (mL/min)	Reward Delay (s)	Max Carrier Flow (mL/min)
1	0, 10	0, 2	200
2	0, 10, 20	0, 2, 4	400
3-5	0, 10, 20, 40	0, 2, 4, 8	600
6-8	0, 10, 20, 40, 70	0, 2, 4, 8, 14	800
9 - finish	0, 10, 20, 40, 70, 100	0, 2, 4, 8, 14, 20	1000

Table 2.1: Shaping parameters.

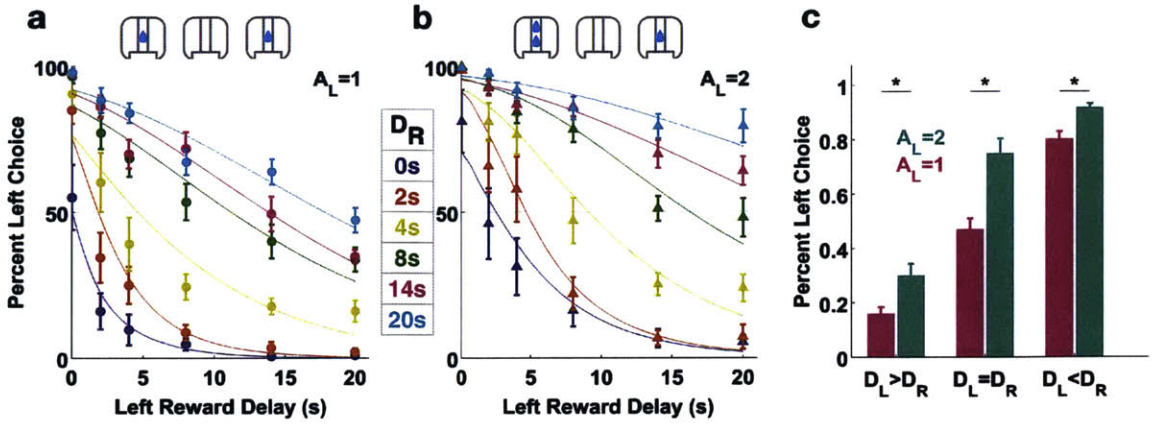

Figure 2-3: Psychometric curves for a group of subjects. ***a.*** *Psychometric curves when the left reward is as large as the right reward.* ***b.*** *Psychometric curves when the left reward is twice as large as the right reward. Inset legend indicates the right reward delays.* ***c.*** *The subjects chose the left option more when the left reward was twice as large as the right reward. A_L: Left reward size. A_R: Right reward size. D_L: Left reward delay. D_R Right reward delay.*

2.2.5 Example Psychometric Curves

Fig. 2-3a and b shows the psychometric curves for a sample group of subjects. Each data point is the percent left choice given the set of two left and right delays, error bars indicating standard errors of population (n=7). Each data series, distinguished by color, is preference data grouped by right reward delays. For each data series, a general linear model with *comploglog* link function was used to fit a preference curve. As the left reward delay increased, the subjects' preference for the left reward decreased, as indicated by the downward trajectory of each preference curve. As the right reward delay increased, the subjects' preference for the left reward increased, as indicated by the upward and rightward fanning of the preference curves. The left panel shows the psychometric curves for equal reward sizes on the left and right, and the right panel shows the curves for when the left reward size was increased to twice as large as the right reward. Given the same delay contingencies, subjects' preference for the left reward was greater when the left reward was twice as large (fig. 2-3c) for all cases of relative delay contingencies (Wilcoxon Signed-rank Test, correcting for multiple comparisons, $p<0.017$). The above results show that mouse subjects were sensitive toward both reward size and delay when making the choice in the task.

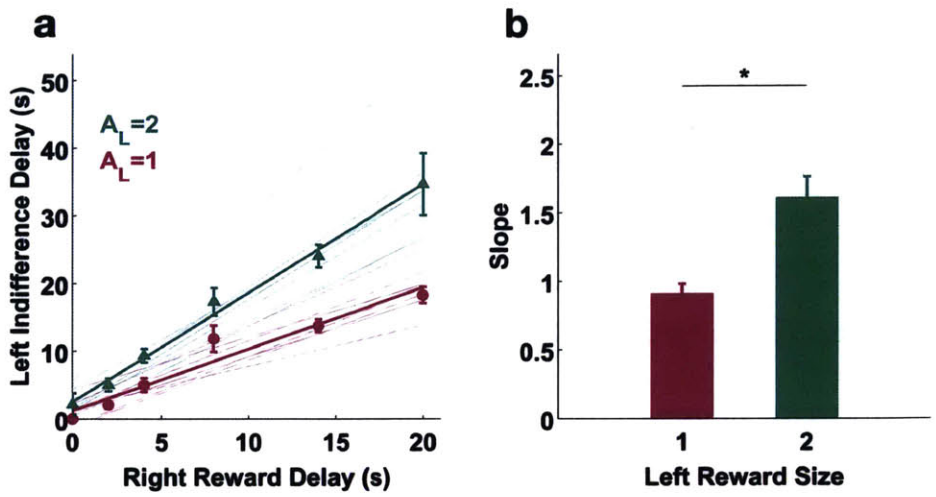

Figure 2-4: Indifference function of the sample group. ***a.*** *Indifference functions for different left reward sizes. Data points show mean indifference points for various right reward delays, error bars indicating standard error of population mean (Triangles: left reward size was 2. Dots: left reward size was 1.) Thin straight lines represent indifference function for individual subjects. Thick straight lines indicate the mean population indifference function.* ***b.*** *Gradients of the indifference functions are significantly different between the two left reward sizes (Wilcoxon Signed-rank Test, $p<0.05$).*

2.2.6 Hyperbolic Discounting of Reward Value in the Present Task

Indifference large delays can be estimated by finding the large reward delay when the subject prefers either option equally, i.e. when the preference curve crosses the (percent large choice = 50%) line. The resulting indifference points can be found for each right reward delay. Exponential and hyperbolic discounting both predict that the indifference points vary linearly with right reward delay (See derivation in Appendix A). Fig. 2-4a shows the linear fitting of the indifference function (for $A_L = A_R$ mean Pearson's correlation coefficient was 0.951, mean p-value was 0.006; for $A_L = 2\text{x}A_R$ mean Pearson's correlation coefficient was 0.971, mean p-value was 0.001). If the mice were discounting reward exponentially, the gradients will be the same between the two conditions with different left reward sizes. Hyperbolic discounting, on the other hand, predicts that the slope of the indifference function is sensitive to the ratio between the two reward sizes (See Appendix A). Fig. 2-4b shows that the indifference function

of the subjects is a function of reward sizes. The ratio between the slopes from the two indifferent functions has a mean of 1.86s and a standard error of 0.26s. This ratio approaches 2. We conclude that mice discounted reward hyperbolically in the OGIC task. The discount factor K estimated from the indifference function has a mean of 0.35s with a standard error of 0.097s.

2.2.7 Gross Motor Aspects of Behavior

In this section I characterize the task further by quantifying aspects of the behavior other than the choice. I mainly look at two measures, sampling time and transit time. Sampling time is defined as the period of time between the entry into the center port and the exit from the center port. Transit time is the period of time between the exit from the center port to the entry into one of the side ports. Sampling times were not significantly modulated by the delays of reward options (fig. 2-5a and b). This results were not surprising since the task required the subjects to make a long poke before the trial could be initiated, considerably longer than needed for the subjects to understand what the cue entailed [24]. After extensive training, the subjects knew the cues very well, and were fully informed of the delay contingencies when the odor cues were presented. Transit times were positively correlated to the delay of either options offered (fig. 2-5c and d). It is possible that in trials where cues predicted options with long delays, the subjects were less motivated to make the choice, and therefore spend longer moving to the side port for a commitment.

2.3 OGIC Task is a Good Assay for Intertemporal Choice

In conclusion, mice can learn to perform intertemporal choice by learning to respond to delay-predicting odor cues in an automated task. Their choice is both sensitive to the delay of the upcoming reward options and the sizes of the reward options offered.

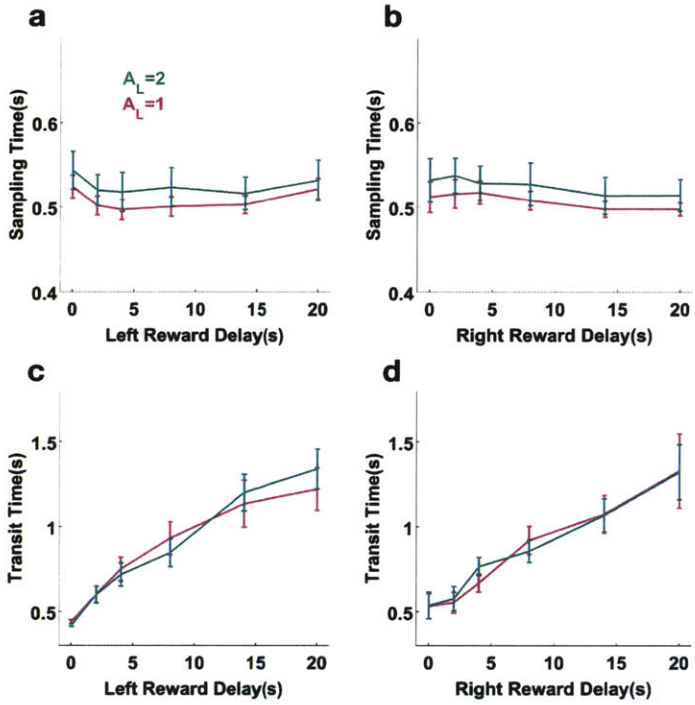

Figure 2-5: Cue sampling time and reaction time. ***a.*** *Cue sampling time as a function of left reward delay.* ***b.*** *Cue sampling time as a function of the right reward delay.* ***c.*** *Transit time as a function of left reward delay.* ***d.*** *Transit time as a function of right reward delay.*

Chapter 3

Introduction to Dorsal Raphe Serotonergic Neurons

Serotonin has been suggested to play a regulatory role in temporal discounting. In this chapter I provide a brief background of serotonin, aspects of its biology and an account of the different hypotheses that have been proposed to explain serotonin function in behavior, mostly in the context of intertemporal choice.

3.1 Serotonin Synthesis and Cellular Features

Serotonin is one of the oldest and most mysterious neuromodulators. Of all the serotonin produced in the body, only a very small proportion is made in the brain (90% of the body's serotonin production happens in the gut, where serotonin regulates gut motility [30]). Since serotonin does not cross the blood-brain barrier, the only source of brain serotonin is conversion of brain tryptophan by the brain-specific tryptophan hydroxylase isozyme 2 (TPH2) [31]. The synthesis enzyme TPH2 acts as an important molecular marker of serotonergic neurons. TPH2 is a low-affinity enzyme and therefore is rate-limiting, allowing fast responses to increases in tryptophan levels. This allows the use of tryptophan loading as an effective and non-invasive way of manipulating serotonin levels in the brain. Apart from TPH2, serotonergic neurons also express serotonin transporter (SERT), another molecular marker frequently used

to target serotonergic neurons [32]. SERT can be blocked with selective serotonin reuptake inhibitors (SSRIs), which also proved to be a very effective way of increasing synaptic serotonin levels and manipulating serotonin transmission.

3.2 Anatomy of the Dorsal Raphe Nuclei

Almost all the serotonin-containing neurons are situated in the brain stem in a few relatively clustered nuclei. The dorsal raphe nucleus (DR) is a major source of brain serotonin [33], containing about 40% of the serotonin producing neurons in the brain [34]. In addition to serotonergic neurons, the nucleus also contains dopaminergic, GABAergic and glutamatergic neurons. Some serotonergic neurons also coexpress glutamate. Most of the serotonergic neurons are situated along the midline, with two lateral wings extending outward laterally.

In the rat [35] and the mouse [36], the projections from DR serotonergic neurons innervate diverse brain regions including many components of the mesolimbic pathway (the ventrotegmental area, ventral striatum and prefrontal cortices), as well as the amygdala and hypothalamus, but rarely the hippocampal formation, dorsal striatum or substantia nigra. These projections are anatomically distinct from those originating from the median raphe nucleus [35] [36], another major aggregation of serotonergic neurons.

Recent genetic studies have shown the dorsal raphe nucleus to be divided into functionally distinct subnuclei [37]. However, for this thesis, the dorsal raphe nucleus is considered as a whole and targeted with a serotonin transporter specific cre-recombinase mouse line (Sert-cre [32]).

3.3 Serotonin Receptors

Serotonin signals are transduced by seven families of receptors [38]. All but one are G-protein-coupled receptors, the exception being 5HT3, an ion channel. Serotonin receptors are widely distributed throughout the brain. 5HT1A acts as a presynaptic

autoreceptor, and can inhibit serotonin release.

3.4 Physiology of Serotonergic Neurons in Behavior

Serotonergic neurons are known to fire tonically and phasically in a manner that is dependent on behavioral state. During quiet waking, serotonergic neurons fire tonically between 1Hz and 5Hz in a clock-like fashion. When animals are aroused by external stimuli, these neurons fire at a higher rate transiently in response to the stimuli. Because serotonergic neuronal firing rate is modulated by arousal state, serotonin is believed to regulate arousal and sleep [39]. Since they are phasically modulated by external events, these neurons are believed to encode a variety of things such as salience, noxious stimuli [40] and reward [41]. Serotonergic neurons have also been observed to fire in a manner time-locked to hippocampal theta rhythm [42], leading to speculations about their role in learning and memory.

3.5 Hypotheses about Serotonin Function in Decision-Making

As we saw above, serotonin has been observed to be important to an extraordinary array of behaviors in animals, including aggression [43] [44], feeding [45], learning [46] [47] and decision-making [48] [49]. Pharmacological interventions have produced alterations to many of these behaviors. However, precisely because of the diversity of its function, it has been difficult to synthesize a unified function for serotonin. Serotonin's role in decision-making is one that has been studied extensively, with a focus on its role in intertemporal choice. Serotonin is thought to suppress impulsive choice and promote patience. I summarize below a few of the recent hypotheses for a general function of serotonin that might shed light on our understanding of serotonin's role in intertemporal choice.

3.5.1 The Negative Prediction Error Hypothesis (The Dopamine-Serotonin Opponency Theory)

Daw and Dayan (2002) articulated the hypothesis that phasic serotonin activity acts as a prediction error signal for punishment, opposing dopamine, which acts as a prediction error signal for reward [50]. This theory was largely rooted in pharmacological evidence pointing towards opposing interactions between dopamine and serotonin and a need for a neuromodulator to fill the opponent role in a proposed symmetrical system. The account, though, ignored studies which reported synergistic effects of serotonin manipulations and dopamine release [51], and those which reported enhancement of self-stimulation by serotonin microinjection [52]. Furthermore, it is difficult to explain serotonin's contribution to curbing impulsive choice with the negative prediction error hypothesis, apart from the possible suppression of dopamine response to a cue that predicts immediate reward.

3.5.2 The Discount Factor Hypothesis

The discount factor hypothesis focuses on serotonin's influence on an animal's ability to evaluate future rewards. Serotonin has long been implicated in suppressing impulsive behavior. Low serotonin levels are associated with aggression [44], and premature responses [53] [54]. Results from delay discounting experiments suggest that serotonin is also involved in suppressing impulsive choice. In an adjusting delay task [13] (see also section 1.3.1), raphe serotonin lesion caused rats to choose the large reward less often [14] and forebrain serotonin depletion resulted in higher discount factors (more impulsive behavior) in rats [55]. Schweighofer *et al.*(2007) proposed that serotonin controls the discount factor in evaluating future rewards, and therefore controls choice impulsivity. Two further studies in waiting illustrate that optogenetic stimulation of serotonin neurons promotes waiting for an uncertainly delayed reward [28] [44].

3.5.3 Reward-Encoding Hypothesis

A series of electrophysiological studies revealed reward-related activities in serotonergic neurons [41] [56] [57] [58] in behaving animals performing operant or Pavlovian tasks. These findings suggest that putative serotonergic neurons respond to many reward-related events in a transient manner. Most interestingly, Cohen *et al.* (2015) recorded optically tagged serotonergic neurons in a pavlovian task, and showed that they fired transiently to punishment (air puffs) and reward-predicting cues. Liu *et al.* (2014) showed that optogenetic stimulation of serotonergic neurons caused self-stimulation, further arguing that at least some serotonergic neurons encode reward [59]. Serotonergic projections (some coreleasing glutamate) in VTA have also been shown to induce self-stimluation [60] [61].

3.6 Conflicts between the Hypotheses

All the studies which correlate serotonergic neuronal activities with reward-related events argue against the negative prediction error hypothesis. In Cohen *et al.* (2015), most serotonergic neurons responded to aversive stimuli, but they never acquired punishment-predicting cue activities. This result is incompatible with the idea that serotonin can act as a signal for negative prediction error. The authors also discussed the possibility of the punishment-responsive neurons as encoding pain relief after short-lived air puffs. It seems that at least some serotonergic neurons contain reward prediction signal.

Until Cohen *et al.* (2015) showed value-encoding activities in the cue response in sertonergic neurons, it was not clear if serotonin was acting during the unconditioned stimulus (US) or the conditioned stimulus (CS). Liu *et al.* (2014) suggested that serotonergic neurons can encode unconditioned reward events, which could be reconciled with Cohen *et al.* (2015): it is possible that serotonergic neurons go through a process of encoding reward events first, and then acquire a representation of reward-predicting cues over training, like dopamine neurons. The experimental results, however, cannot be explained by the discount factor hypothesis. The stimulation in question consti-

tuted a fictitious reward and any actual natural reward was absent, therefore there was a lack of substrate for discounting to act on. Furthermore, almost all delay discounting experiments with serotonin manipulations were long time-scale manipulations, including pharmacological interventions and lesions. This makes it impossible to distinguish serotonin's role in CS encoding from its role in US encoding. Many choices we make about time happen before the options realize, and have distinct choice points. I reason that if serotonin in fact controls reward delay discounting by encoding predictive values related to reward delay, then manipulating serotonergic neuronal activities at the CS should affect choice about the delay.

Almost all the delay discounting experiments with serotonin manipulation are adjusting tasks. Given that 5,7-dihydroxytryptamine lesion in orbitofrontal cortex has been shown to impair reversal learning, there is a possibility that these effects in delay discounting could be learning effects [47]. If serotonin encodes rewarding US, these learning effects can be explained. This is something that my odor-guided intertemporal choice task addresses. Since reward contingencies are randomized and cued at the beginning, there is minimal need to track a changing environment.

Chapter 4

Raphe Serotonergic Neurons Suppress Impulsive Choice in Difficult Trade-offs in the OGIC Task

4.1 Aim

The aim of the experiment is to investigate whether activity of serotonin neurons drives intertemporal decision-making by suppressing impulsive choice. I tested this hypothesis by optogenetically silencing or augmenting serotonergic neuronal activity specifically at the decision point in the OGIC task and assessing any change in the preference of the subjects.

4.2 Materials and Methods

4.2.1 Subjects and Training

Sert-cre mice [32] aged between two and six months were trained as described in Chapter 2.2.

4.2.2 Viral Transfection and Optical Fiber Implantation

Sert-cre mice were anesthetized with avertine (250 mg/Kg) and then mounted on a stereotactic setup. A small craniotomy was made over dorsal raphe. For the Arch experiments, mice were injected in the dorsal raphe nucleus (AP: -4.6 mm, DV: -3 mm, ML: 0 mm) with AAV9-ef1a-DIO-Arch3.0:YFP, or a control virus containing YFP, diluted to a titer of $1x10^{11}$ particles/ml, in a pulled micropipette needle attached to a microinjector. For the ChR2 experiments, mice were injected in the dorsal raphe nucleus (AP: -4.6 mm, DV: -3 mm, ML: 0 mm) with AAVrh8-hsyn-DIO-ChR2, or a control virus containing GFP. A single optical fiber ($\oslash$ $200um$) was implanted over the dorsal raphe nucleus, and secured with dental cement fitted with the top segment of a black eppendorf tube. For further light shielding, any dental cement not covered by the eppendorf tube was painted over with black nail polish. Mice were allowed to recover over a period of two weeks before being water-restricted again.

4.2.3 Behavior Testing

Implanted mice performed the OGIC task daily with laser delivery through patch cords attached to the optical implants by ceramic sleeves. The patch cords were attached to a rotary joint to allow rotations and to free the mice for movement within the operant chamber. Between 10% and 20% of the trials were light-on trials, in which the laser was turned on when a valid center poke was made, concurrent with the odor onset, and turned off when a valid side poke was made, committing the choice. The lasers (CNI, Jilin, China; Optoengine, Utah, USA) were triggered via a TTL pulse issued from the state machine that also controls the behavior apparatus. For the Arch experiment, a constant pulse of 3-5 mW 532 nm light was used. For the ChR2 experiment, a train of 473 nm light was used at 10 Hz and 5 ms pulse width, power measured to be 10mW at constant output.

4.2.4 Data Analysis

Port entry timestamps were logged by computers with a customized behavior control program. For each subject, trial-by-trial data arrays were constructed using Matlab. The preferences were tabulated for each combination of left and right delays. Sampling time and reaction time were calculated and tabulate as well. The data was first sorted by relative reward delays: whether left reward delay was longer than, equal to or shorter than the right reward delay. Average preference for each scenario was calculated and compared between stimulation conditions. To produce a preference curve for each small reward delay, preference data was sorted by right (small) reward delays, and fitted with a generalized linear model with *comploglog* link function. Indifference large delays were defined as the left (large) reward delay when the preference curve crossed the y=50 % line. To produce an indifference function, the indifference large delays were plotted against the corresponding small reward delay, and then fitted with a straight line.

4.3 Results

4.3.1 Optogenetic Manipulations of DR serotonergic Cell Bodies Altered Subjects' Delay Preference in OGIC Task

Water restricted Sert-cre mice (n=6 per group) first trained on the OGIC task and then injected with AAV9-ef1a-DIO-Arch3.0:YFP (Fig. 4-1c) were implanted with a single optical fiber above the dorsal raphe nucleus (Fig. 4-1a). They then performed the OGIC task with the left reward spout delivering two rewards and the right reward spout delivering one reward for all the trials. A 532 nm laser was used to deliver green light in a constant pulse between cue onset and choice commitment (Fig. 4-1b). This light pulse was effective in suppressing multiunit activities in the dorsal raphe neurons recorded in an *in vivo* anesthetized preparation (Fig. 4-1d), see also Appendix C. First, preference data was assessed for three broad categories of trials: ones in which the large reward was more delayed than the small reward, ones in which the large reward

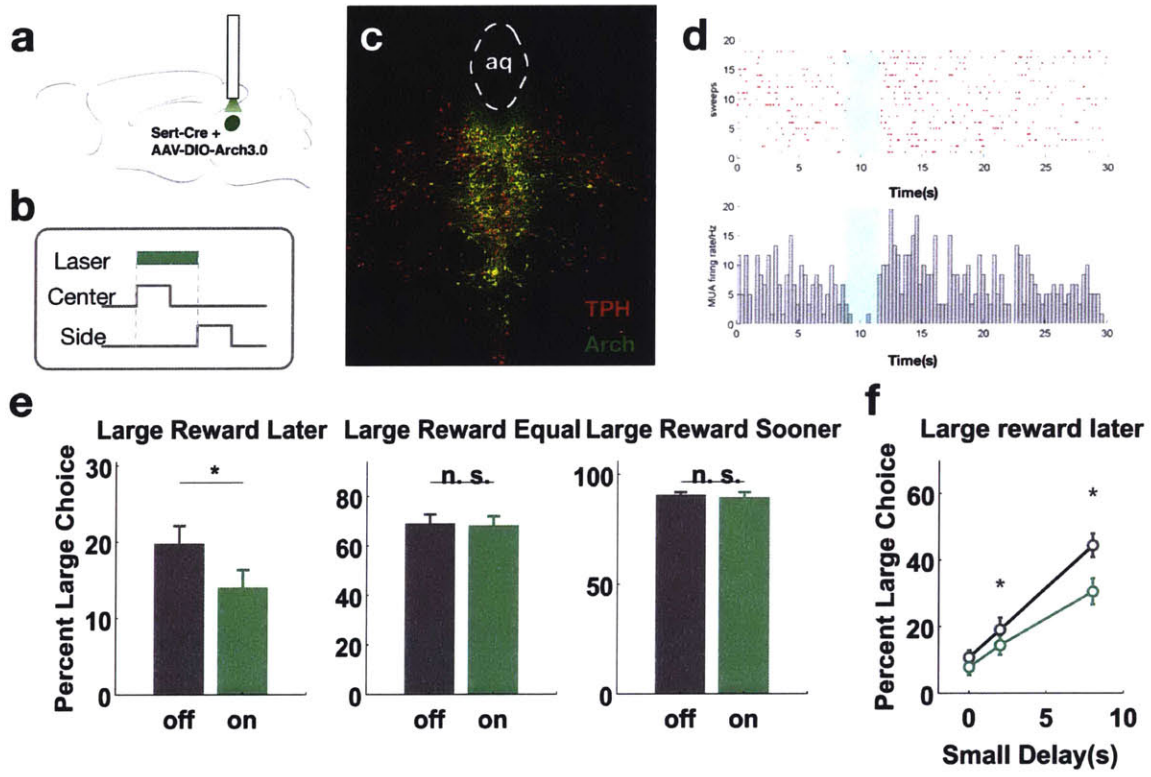

Figure 4-1: Supression of Arch-expressing DR sertonergic neurons suppresses impulsive choice. ***a*** *Schematic for the viral injection and fiber implant in a sagittal view.* ***b*** *Timing of light delivery.* ***c*** *Immunohistochemistry showing the extent of viral transfection.* ***d*** *Multiunit recordings of spontaneous firing in serotonergic neurons in an in vivo anesthetized preparation show suppression of neuronal activities by light.* ***e*** *Preference data categorized by relative delays.* ***f*** *Preference data for trials where the large reward delay was longer than the small reward delay, as grouped by small reward delays.*

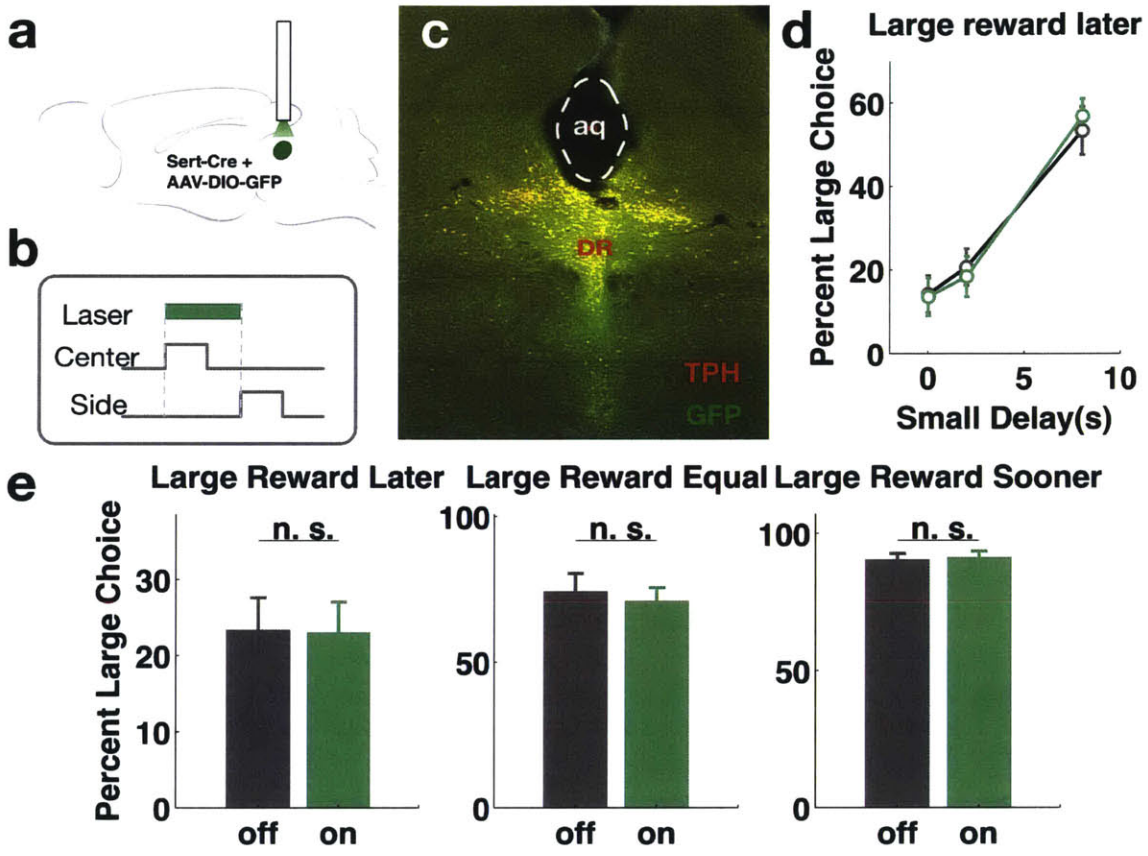

Figure 4-2: Light delivery to GFP-expressing DR serotonergic cell bodies did not altered subjects' delay preference in OGIC task. ***a*** *Schematic for the viral injection and fiber implant in a sagittal view.* ***b*** *Timing of light delivery.* ***c*** *Immunohistochemistry showing the extent of viral transfection.* ***d*** *Multiunit recordings of spontaneous firing in serotonergic neurons in an in vivo anesthetized preparation show suppression of neuronal activities by light.* ***e*** *Preference data categorized by relative delays.* ***f*** *Preference data for trials where the large reward delay was longer than the small reward delay, as grouped by small reward delays.*

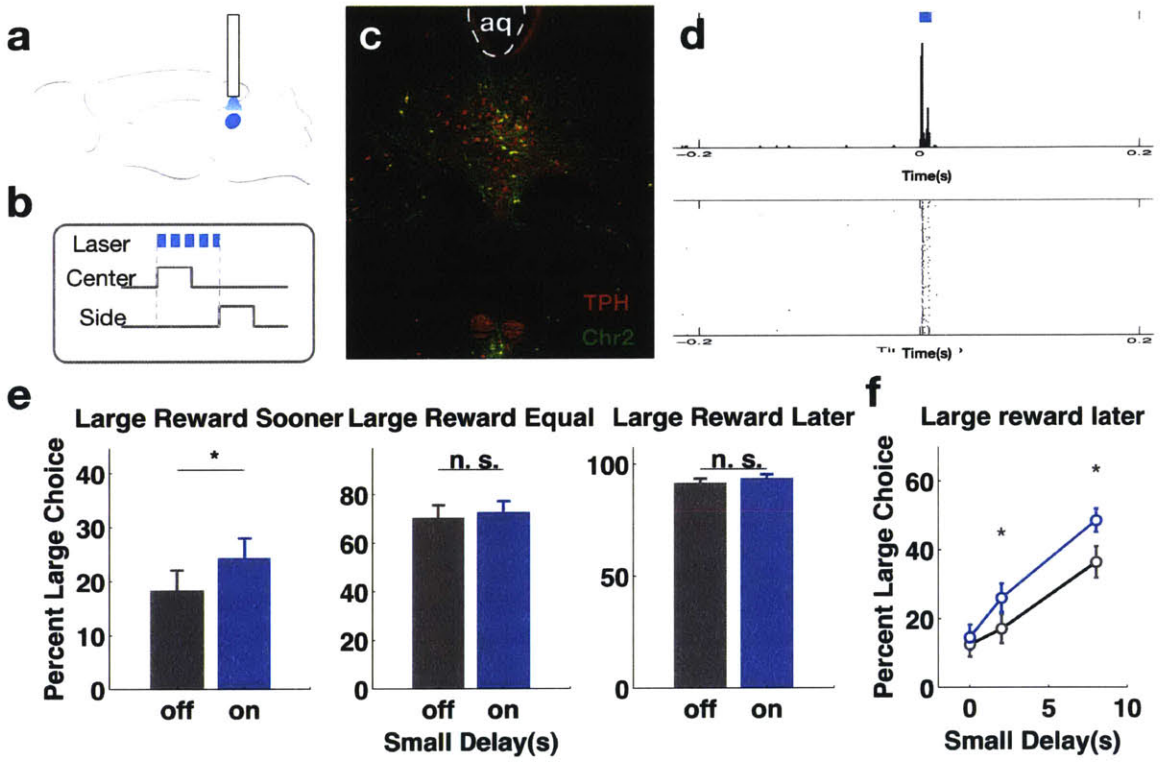

Figure 4-3: Activation of ChR2-expressing DR sertonergic neurons suppresses impulsive choice. ***a*** *Schematic for the viral injection and fiber implant in a sagittal view.* ***b*** *Timing of light delivery.* ***c*** *Immunohistochemistry showing the extent of viral transfection.* ***d*** *Multiunit recordings of spontaneous firing in serotonergic neurons in an in vivo anesthetized preparation show suppression of neuronal activities by light.* ***e*** *Preference data categorized by relative delays.* ***f*** *Preference data for trials where the large reward delay was longer than the small reward delay, as grouped by small reward delays.*

was equally delayed as the small reward and ones in which the large reward was less delayed than the small reward. I hypothesized that if DR serotonergic neurons were involved in the suppression of impulsive choice, then serotonergic neuron suppression should cause the subjects to prefer the large but more delayed reward less often. The preference for a large reward that was equally delayed as the small reward, or one that was sooner than the small reward, should not be affected. This was the case. The subjects chose the large but more delayed reward less often in the light-on trials than in the light-off trials. Their preference was not altered by light when the large reward was equally delayed as the small reward or less delayed than the small reward (Fig. 4-1e, Wilcoxon Signed Rank Test, $p<0.017$, significant after correcting for multiple comparisons). A closer look at the preference for the large, more delayed reward reveals that the effect was more prominent as the delay to the small reward increased (Fig. 4-1f). This result suggests that when subjects were deciding between two delayed options, and the large reward was more delayed than the small reward, DR serotonergic neurons were necessary for choosing the larger, more delayed reward. Green light did not cause any change in preference in control subjects (Fig. 4-2).

Based on the loss-of-function results, I hypothesized that augmenting the activity of DR serotonergic neurons should increase the choice for the large, more delayed reward. Therefore, I performed a gain-of-function experiment in a largely similar arrangement. Sert-cre mice (n=7) were similarly trained and implanted (Fig.4-3a), but injected with a AAVrh8-hsyn-DIO-ChR2 virus (Fig. 4-3c). A 473 nm laser was used for the activation experiments (Fig. 4-3b). A short pulse of blue light was able to reliably trigger spiking in the ChR2-infected serotonergic neurons (Fig. 4-3d). As hypothesized, blue light increased the subjects' preference for the large, more delayed reward (Fig. 4-3e, Wilcoxon Signed Rank Test, $p<0.017$, significant after correcting for multiple comparisons), but did not significantly change the preference for the large reward that was similarly delayed or was sooner than the small reward. Fig. 4-1f shows that when small reward delay was non-zero, the preference for large reward was significantly higher in light-on trials than light-off trials. This result suggests that a transient bout of activation of DR serotonergic neurons was sufficient to increase

subjects' preference for the large, more delayed reward when faced with two delayed rewards.

Taken together, these results suggest that manipulating the predictive activity of DR serotonergic neurons bidirectionally drove intertemporal choice.

4.3.2 Optogenetic Manipulations of Serotonergic Cell Bodies Were Sensitive to Reward Magnitude

Mazur (1987) showed that with hyperbolic discounting, the indifference large delay is a linear function of the small delay. The y-intercept is a function of the discount factor K, and the gradient indicates the subject's sensitivity to the ratio between the magnitudes of the two reward options (See Appendix A).

Indifference large delays were calculated for both the light-on and light-off trials (Fig. 4-5) as described in Section 2.2.6. Green light reduced the gradient of the indifference function of the DR-Arch subjects and blue light increased the gradient of the indifference function of the DR-ChR2 subjects (Fig. 4-7). These results suggest that optogenetic manipulation of serotonergic neurons was sensitive to magnitude.

Discount factor K was also computed for both experiments (Fig. 4-7). Curiously, green light reduced the discount factor of the DR-Arch subjects, and blue light did not significantly change the discount factor of the DR-ChR2 subjects.

Taken together, these results suggest that serotonin manipulations were sensitive to reward magnitude, and altered the subjects' choice impulsivity by making them more or less discerning between the reward size of the future rewards.

4.3.3 Optogenetic Manipulations of Serotonergic Cell Bodies Did Not Affect Sampling Time or Transit Time

Since serotonergic neuronal manipulations have been shown to affect waiting, which could result in different amount of cue sampling, and therefore might explain the choice effect, I looked at the sampling time under light on and light off conditions. The subjects sampled the cues for a similar amount of time at the odor port (Fig. 4-8a

$$V = \frac{A}{1+KD}$$

$$V_R = V_L$$

$$\frac{A_R}{1+KD_R} = \frac{A_L}{1+KD_L}$$

$$D_L = \frac{A_L - A_R}{A_R K} + \frac{A_L}{A_R} D_R$$

V_L value of left reward **A_R size of right reward**

V_R value of right reward **A_L size of left reward**

D_L delay of left reward **K discount factor**

D_R delay of right reward

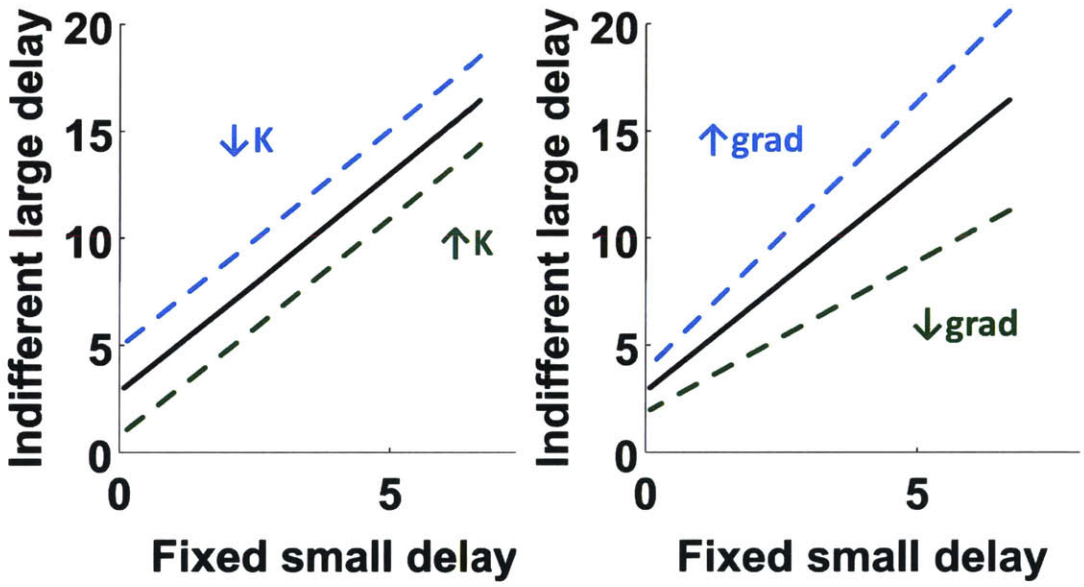

Figure 4-4: Illustrations for Indifference Function Parameter Changes. If subject discounts future reward less or more steeply, but is similarly sensitive to the relative magnitude of the reward options, the indifference function shifts up and down parallel to the original function. If the subject becomes more or less sensitive to the magnitude, the gradient of the indifference function increases or decreases respectively. Both effect could result in a change in the preference for the large but more delayed reward.

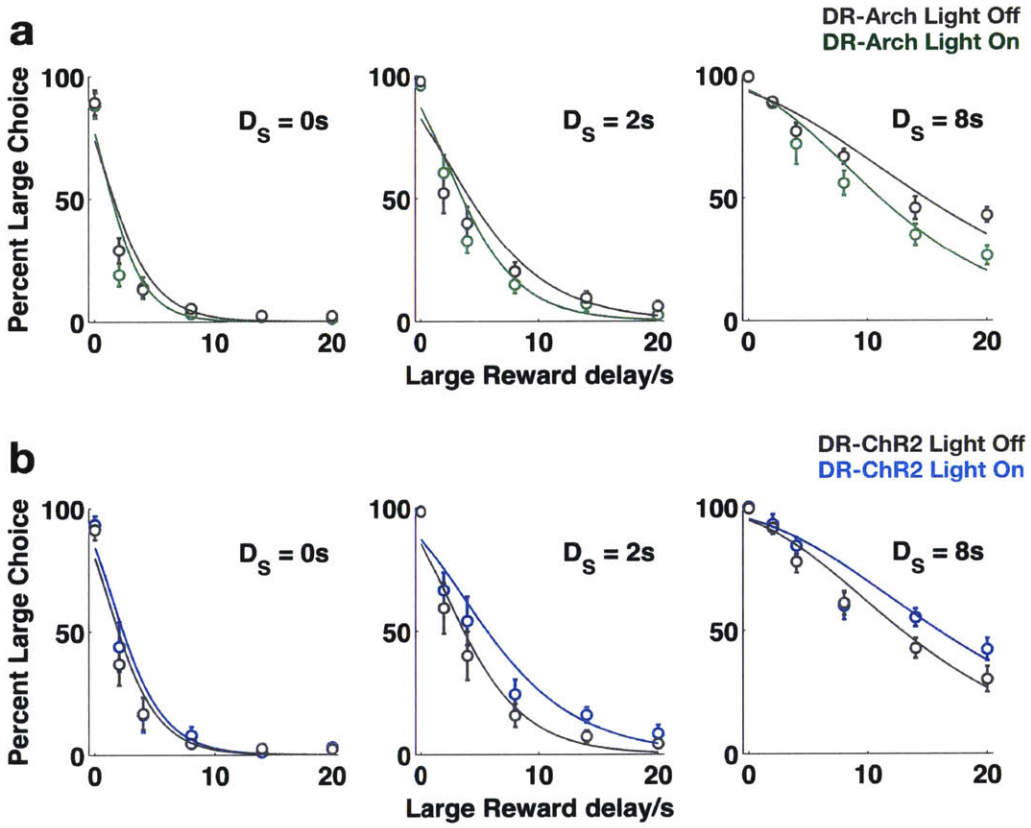

Figure 4-5: Psychometric curves for the dorsal raphe activation and inactivation experiments. Error bars indicate standard error for the population mean.

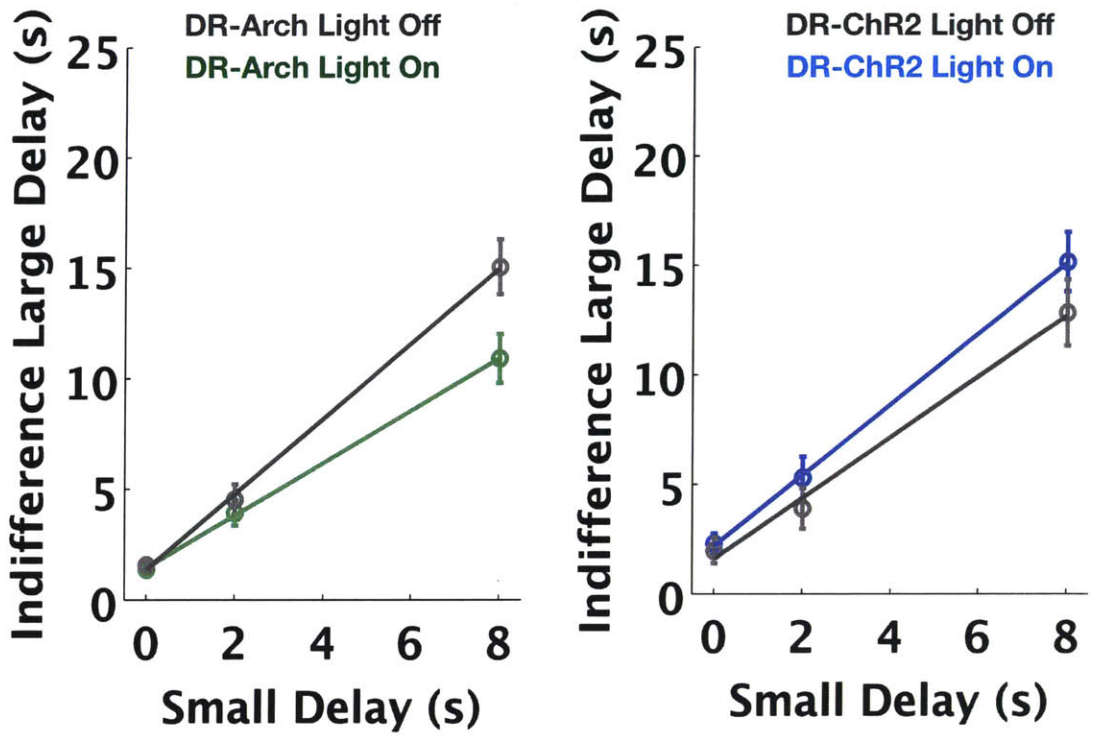

Figure 4-6: Indifference function for dorsal raphe activation and inactivation experiments. Effect was magnitude-sensitive.

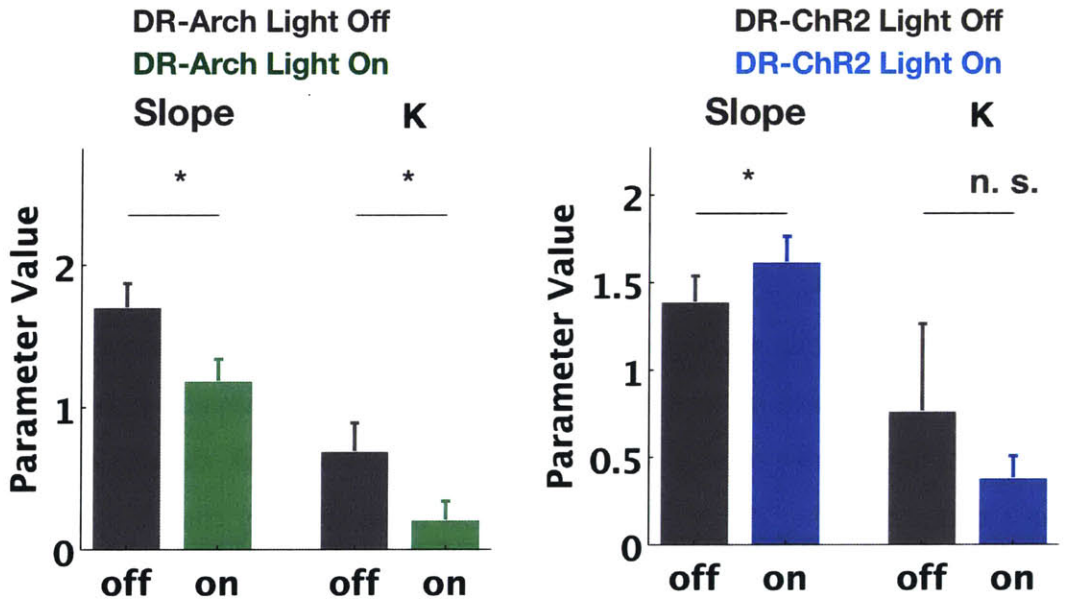

Figure 4-7: Estimates for the gradient of the indifference function and discount factor K. Each panel shows the estimation of the slope of the indifference function and the discount factor K.

and b) in the *large reward later* type of trials, where their choice was affected by the light manipulations, indicating that the subjects got similar amounts of odor mixture regardless of light delivery. To check for motivation levels of the subjects, I examined transit time from the center port to the side port. Transit time wasn't significantly affected either (Fig. 4-8c and d), suggesting that light delivery did not significantly alter the motivation level. In conclusion, gross motor aspects of the behavior was not affected by the optogenetic manipulations of serotonergic cell bodies.

4.4 Discussion

The set of experiments in this chapter are among the first attempts at manipulating a decision-making task with a transient intervention at a symmetrical decision point. The observation that manipulations of the serotonergic activity at the decision point were able to drive the subjects' choice in a bi-direction manner suggests that serotonergic neurons contain information, or modulate neurons that contain information, about the upcoming delay and size contingencies. This is consistent with the finding that serotonergic neurons fire transiently in proportion to the value-predicting power of a conditioned stimulus [58] and is consistent with the hypothesis that serotonergic neurons encode reward value. These present results highlight the prospecting nature of serotonin encoding. Hypotheses about serotonin functioning as a punishment cannot explain the choice effect.

In contrast to previous waiting experiments with optgenetic serotonergic manipulations [28] [29], subjects in the present experiments were not already in a waiting state when the light onset occurs. After the light delivery started, the subjects had to make a choice in either direction. This excludes the possibility that activation of serotonergic neurons promote patience by reducing motor impulsivity, or that the observed results were simply due to a confounding motor arrest. It had also not been demonstrated previously that a transient loss-of-function in serotonin transmission underlies a gain in impulsivity. The present experiments demonstrated that serotonergic neurons cue activities were specifically involved in impulsive choice.

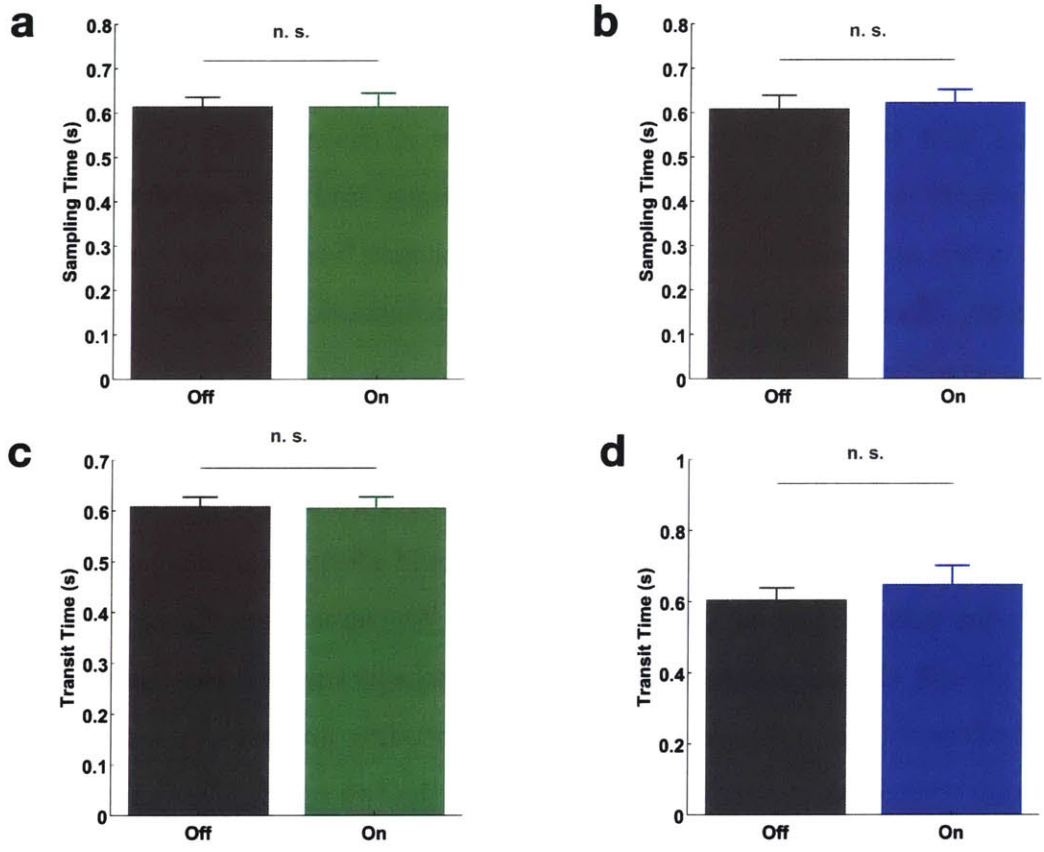

Figure 4-8: Optogenetic manipulations did not affect reaction times for trials where the large reward was more delayed than the small reward. ***a*** *and* ***c*** *show reaction times for DR-Arch subjects and* ***b*** *and* ***d*** *for DR-ChR2 subjects. Neither kind of reaction time was affected by either manipulation.*

There was no manipulation effect when either reward was immediate. This is in contrast with the literature in rats. It is also possible that serotonergic neurons are not important for *easier* choices involving an immediate option, but are more important when a trade-off is difficult. Alternatively, the manipulations might have disproportionately affected choice about reward options that are further into the future, where a longer range prediction was required. If serotonergic neurons did encode a prediction about the value of options available to choose from, then their activity might be a function of both the reward size and reward delays of the options, and altering their activity might affect certain cases of choices. Difficult trade-offs and long-delayed rewards are both low value situations, and may entail threshold firing rate in the serotonergic neurons, which may in turn be especially sensitive to manipulations. Electrophysiological recording the serotonergic neurons in task may clarify the speculations.

Since serotonergic neurons are prominently implicated in a range of physiological processes such as feeding [45] and carbohydrate matabolism [62], it is possible that they code for thirst, hunger or other factors that could affect motivation, which in turn affects the decision-making process about time. We cannot directly exclude this possibility, though the manipulations did not affect transit time for the trials where choice was affected (Fig. 4-8c and d). Since value encoding and motivational states are intimately connected, it may be difficult to tease the two apart. Characterization of the upstream and downstream connections to the dorsal raphe nucleus may clarify this concern to some extent. Presence or absence of neuronal projections from or to the thirst centers such as hypothalamic supraoptic nuclei may indicate such a modulation.

Optogenetic manipulations of DR cell bodies seem to be sensitive to the relative magnitude of the reward options. This differs from the results of Mobini *et al.* (2000), where serotonin depletion was found to change the discount factor but was insensitive to magnitude. The discrepancy might be explained by a variety of differences between the experimental setups. Mobini *et al.* used an adjusting delay task, whereas the present experiments varied delay contingencies systematically and randomly. Mobini

et al. used a manipulation of longer time-scale and therefore could have affected US responses and tonic firing, whereas the present experiment only examined the effect of serotonin manipulation on reward predicting cues.

Paradoxically, transient manipulations of the seoronergic neurons did not alter discount factor in a way that was consistent with long-term manipulation experiments in the literature [55] [63]. The method used in this section to estimate discount factor makes one important assumption, that the discount factor is constant across time course. It is possible that discount factor is inconsistently affected in time by the serotonergic manipulations, which makes the estimate less meaningful. More systematic experiments could clarify this point.

There is a concern that global optogenetic manipulations such as used in the present experiments could affect the odor perception of the mouse. Serotonin is an important modulator of the olfactory bulb and the piriform cortex and manipulations of serotonin signaling may cause a perceptual confound. It is unlikely the effect I observed was due to an odor perception confound because the manipulations produced no effects in trials where the large reward was less delayed or equally delayed. Sampling time was not significantly affected by the light manipulations either (Fig. 4-8a and b). In other words, the performance of the task was not affected by the manipulations. Furthermore, a recent study found that optogenetic activation of DR serotonergic neurons did not influence odor-evoked activity in the piriform cortex [64], indicating that in the present experiments odor perception was probably intact.

Many serotonergic neurons also corelease glutamate [60] [61][59], therefore the effects in the current experiments could be due to glutematergic transmission. Further studies are needed to clarify this component.

Chapter 5

Nucleus Accumbens and Intertemporal Choice

In this chapter, I review the involvement of the nucleus accumbens (NAc) in intertemporal choice. This is a key candidate target site for serotonergic neurons to act on.

5.1 Nucleus Accumbens

Central to the mesolimbic pathway and a major component of the ventral striatum, the nucleus accumbens is an important node in reward processing. The nucleus comprises a core region (NAcc) and a surrounding shell (NAcSh) [65]. The core and shell regions have different efferents and afferents, and exhibit different expression levels of key proteins. NAcSh preferentially receive neuromodulator innervations from areas such as VTA and DR [66]. Behaviorally, NAc has been suggested to play an important role in reward processing, and hence to be involved in locomotion [67], impulsivity [68], feeding [69], sexual motivation [70] and social reward [71]. Physiologically, NAc is thought to encode motivational values for both reward and punishment [72] [73] [74]. It follows naturally that NAc is a target for intense investigation around the topic of impulsivity.

5.2 Nucleus Accumbens and Impulsive Choice and Involvement of Dopamine and Serotonin

Animal studies of NAc were mostly carried out in rats. NAcc and NAcSh likely play opposite roles in controlling impulsivity: microstimulation in NAcc decreased impulsivity whereas microstmulation in NAcSh increased it [75]. NAcc lesions and NAcSh lesions produced contrasting effects on amphetamine-induced motor impulsivity [76]. NAcc lesions resulted in increased choice impulsivity [77], whereas NAcSh lesions produced no such effect [78]. Combined lesions of NAcc and NAcSh decreased choice impulsivity [79]. Monoamines have been found to play a role in NAc control of impulsivity [80]. In rats selected to have high trait impulsivity, NAc exhibited markedly lower availability of D2/3 receptors [81]. Serotonin may be an upstream regulator of dopamine in NAc for impulse control. Blocking serotonin transmission in NAc using autoreceptor 5HT1A agonist 8-OH-DPAT increased choice impulsivity, but not when dopamine input to NAc was lesioned [82]. Interestingly 8-OH-DPAT also seemed to have blunted the animal's sensitivity to the large reward even when both reward options were immediate, suggesting a magnitude-related effect.

In humans, fMRI studies have shown a correlation between ventral striatum (which contains NAc) activity and preference for small and immediate rewards [83] [84]. Serotonin depletion increased choice impulsivity [63], and this has been shown to correspond with ventral striatum activity at low serotonin levels [22].

In my hands, the DR-NAc projection could be traced both anterogradely and retrogradely (See Appendix B). Given these findings, I hypothesized that NAc was a potential innervation target that could mediate the influence of serotonergic projections on suppressing choice impulsivity. I tested this hypothesis in Chapter 6.

Chapter 6

Nucleus Accumbens as a Site for Serotonin Suppression of Impulsive Choice

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6.1 Aim

The aim of the experiments in this chapter is to examine NAc as a target area that mediates serotonergic control of intertemporal choice.

6.2 Materials and Methods

6.2.1 Subjects and Training

Sert-cre mice aged between 2 and 6 months were trained as described in Chapter 2.2.

6.2.2 Viral Transfection and Optical Fiber Implantation

Sert-cre mice were anesthetized with avertine (250 mg/Kg) and then mounted on a stereotactic setup. A small craniotomy was made over dorsal raphe and two over

nucleus accumbens. For the Arch experiments, mice were injected in the dorsal raphe nucleus (AP: -4.6 mm, DV: -3 mm, ML: 0 mm) with AAV9-ef1a-DIO-Arch3.0:YFP, or a control virus containing YFP, diluted to a titer of $1x10^{11}$ particles/ml, in a pulled micropipette needle attached to a microinjetor. For the ChR2 experiments, mice were injected in the dorsal raphe nucleus (AP: -4.6 mm, DV: -3 mm, ML: 0 mm) with AAVrh8-hsyn-DIO-ChR2, or a control virus containing GFP. Optical fibers were implanted bilaterally into the left and right nucleus accumbens (AP: +1.35 mm, DV: -4 mm, ML: 0.7 mm), and secured with dental cement fitted with the top segment of a black eppendorf tube. For further light shielding, any dental cement uncovered by the eppendorf tube was painted over with black nail polish. Mice were allowed to recover over a period of two weeks before being water-restricted again.

6.2.3 Behavior Testing

Implanted mice performed the OGIC task daily with laser delivery through patch cords attached to the optical implants by ceramic sleeves. The patch cords were attached to a rotary joint to allow rotations and to free the mice for movement within the operant chamber. Between 10% and 20% of the trials were light on trials, in which the laser was turned on when a valid center poke was made, concurrent with the odor onset, and turned off when a valid side poke was made, committing the choice. The lasers (CNI, Jilin, China; Optoengine, Utah, USA) were triggered via a TTL pulse issued from the state machine that also controls the behavior apparatus. For the Arch experiment, a constant pulse of 3-5 mW 532 nm light was used. For the ChR2 experiment, a train of 473 nm light was used at 10 Hz and 5ms pulse width, power measured to be 10 mW at constant output.

6.2.4 Data Analysis

Port entry timestamps were logged by computers with a customized behavior control program. For each subject, trial-by-trial data arrays were constructed using Matlab. The preferences were tabulated for each combination of left and right delays. Sampling

time and reaction time were calculated and tabulate as well. The data was first sorted by kind of trial: whether left reward delay was longer than, equal to or shorter than the right reward delay. Average preference for each scenario was calculated and compared between stimulation conditions. To produce a preference curve for each small reward delay, preference data was sorted by right (small) reward delays, and fitted with a generalized linear model. Indifference large delays were defined as the left (large) reward delay when the preference curve crossed the y=50 %. To produce an indifference function, the indifference large delays were plotted against the corresponding small reward delay, and then fitted with a straight line.

6.3 Results

6.3.1 Optogenetic Manipulations of Serotonergic Projections in Nucleus Accumbens Altered Subjects' Preference in OGIC Task

Sert-cre mice (n=6) were similarly trained and virally tansfected with AAVrh8-hsyn-DIO-ChR2 as in 4.3, but they were implanted bilaterally above NAc. As hypothesized, blue light increased the subjects' preference for the large, more delayed reward(Fig. 6-1c, Wilcoxon Signed Rank Test, $p<0.05$), but did not significantly change the preference for the large reward that was similarly delayed or was sooner. Fig. 6-1d shows when small reward delay was non-zero, the preference for large reward was significantly higher in light-on trials than light-off trials. This result suggested that a transient bout of activation of DR serotonergic projections in NAc was sufficient in increasing the subjects' preference for the large, more delayed reward when faced with two delayed rewards.

I then performed the loss-of-function experiment with mice transfected with AAV9-ef1a-DIO-Arch3.0:YFP and implanted in NAc. Green laser delivery was as described in 4.3. The subjects chose the large, more delayed reward less often on the light-on trials compared to the light-off trials (Fig. 6-1g). The break down of these trials in

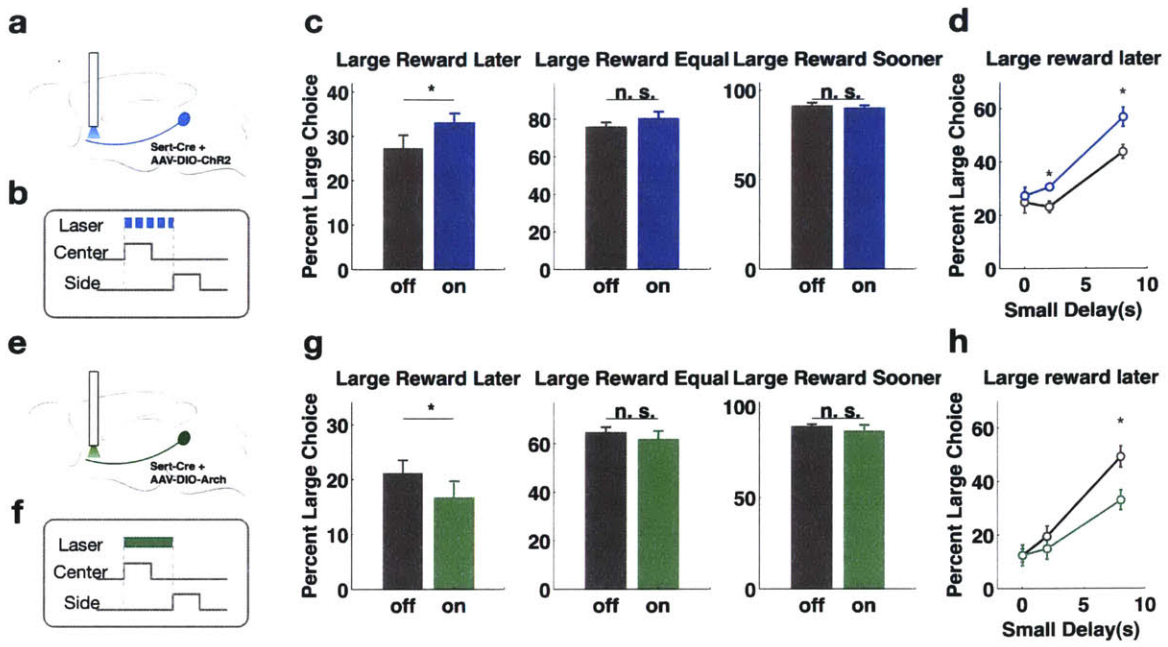

Figure 6-1: Inactivation of dorsal raphe sertonergic neurons promotes impulsive choice.

(Fig. 6-1h) shows that green light suppressed the subjects impulsive choice only when the small reward was very delayed, but it was not able to suppress the impulsive choice at no or short small reward delays.

Taken together, these results suggest that manipulations in the activity of the serotonergic projections in NAc causally shifts the subjects' preference for large, more delayed reward, and that NAc is a potentially important target for serotonin action in choice impulsivity.

6.3.2 Optogenetic Manipulations of Serotonergic Projections in Nucleus Accumbens Were Sensitive to Reward Magnitude

Manipulating sertonergic projections in NAc was similarly sensitive to magnitude (Fig. 6-2), consistent with the results in Chapter 4. Green light did not change the discount factor K but blue light increased it (Fig. 6-4).

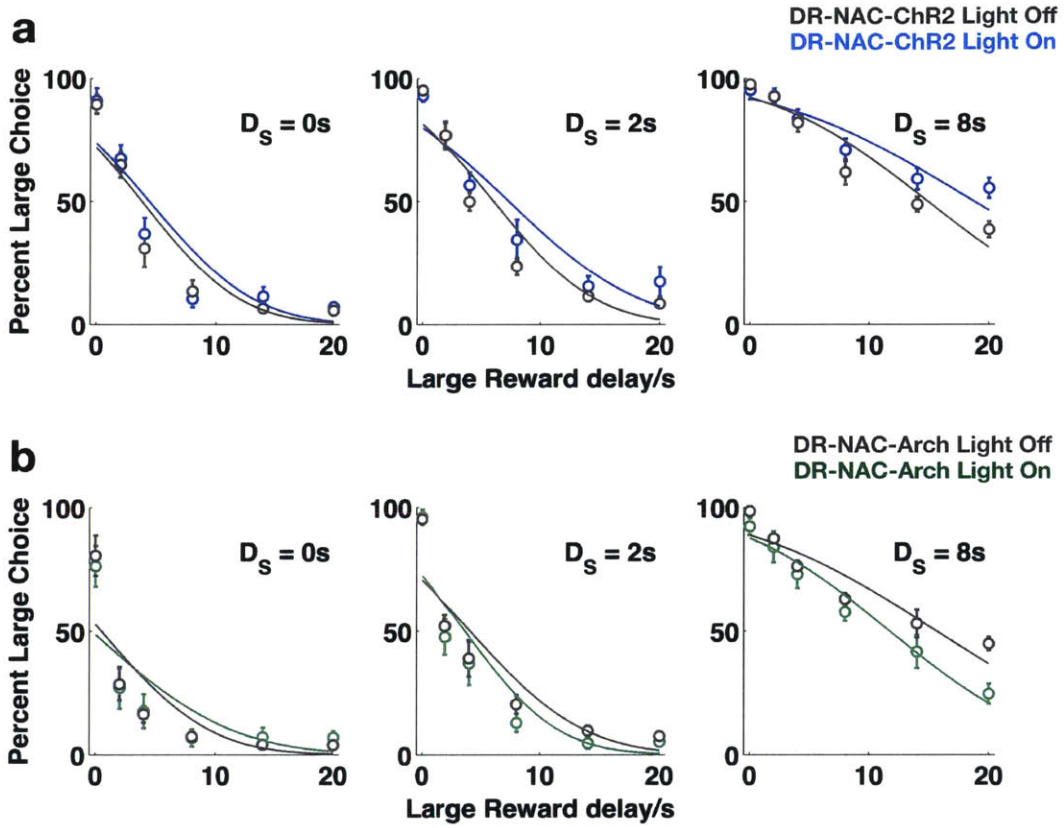

Figure 6-2: Psychometric Curves for the Dorsal Raphe to Nucleus Accumbens Projection Activation and Inactivation Experiments. Error bars indicate standard error for the population mean.

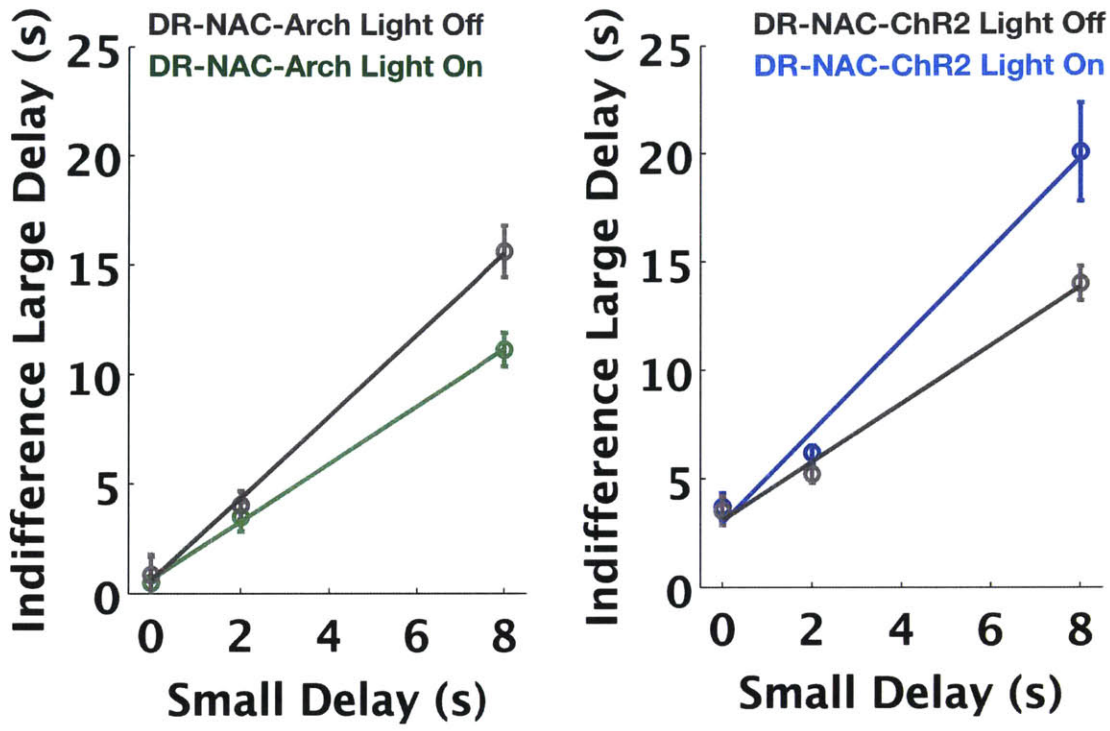

Figure 6-3: Indifference Function for Dorsal Raphe to Nucleus Accumbens Activation and Inactivation Experiments. Effect was magnitude-sensitive.

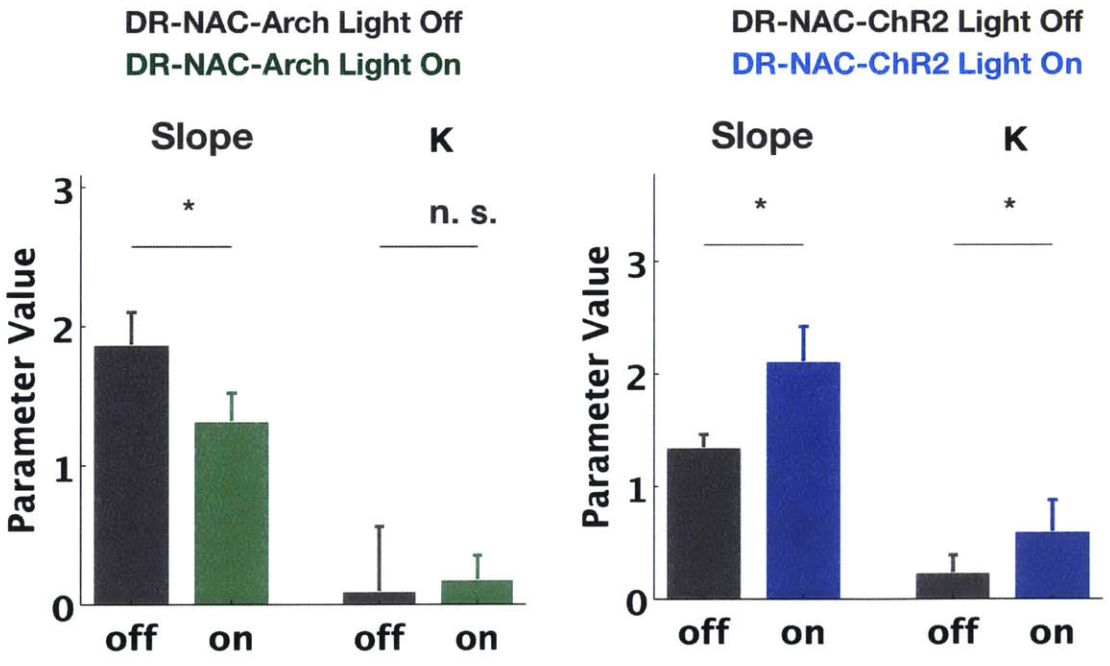

Figure 6-4: Estimation of the gradient of the indifference function and discount factor.

6.4 Discussion

The results in these chapter suggest that NAc could be an important site of action for serotonergic neurons to regulate intertemporal choice. Augmenting the activity of DR serotonergic projections increased choice for large, more delayed reward, and inactivating the activity of these projections suppresses such choice. It is possible that this happened either because DR sertonergic projections suppressed an NAc response associated with an immediate reward or because they potentiated an NAc response associated with a long-term reward. Since light did not affect the preferences in trials with at least one immediate option, the latter scenario is more likely.

Due to the way light spread in brain tissue, there was no way of restricting the activation or inactivation to NAcc or NAcSh. This makes interpreting the results difficult. As mentioned before in Section 5.2, the two components of NAc behave vastly differently. Serotonin could be activating NAcc medial spiny neurons via an excitatory receptor to promote patience or do the same by inactivating NAcSh medial spiny neurons via an inhibitory receptor. Alternatively, serotonergic projections could be acting on local inhibitory circuits via excitatory receptors and cause disinhibition of the medial spiny neurons. The second possibility is more probable since serotonergic neurons project more prominently to the shell (Fig. B-1), although this issue should be clarified with future studies. Finding a receptor subtype or monitoring neuronal activity in NAc during task can help immensely in interpreting the results.

Chapter 7

Conclusion and Future Directions

In this thesis, I present a novel behavioral paradigm that was designed to specifically investigate decision-making about waiting in mice. I found that transient manipulations of DR serotonergic neurons were able to alter the subjects preference in intertemporal choice. I conclude that the activity in serotonergic neurons is able drive decisions to delay gratification, specifically in situations in which there is difficult delay-size trade-off.

7.1 Main Findings

7.1.1 Mice Can Use Odor Cues to Perform a Cued Intertemporal Choice Task

The task described in this thesis communicates the delay contingencies to the mouse subjects via concentration of odor cues and allows the subjects to make informed choices about the reward delays. It is the first automated and cued intertemporal choice for rodent to date. In the present task, mice are found to discount reward hyperbolically, similar to other species in the literature. This task design brings rodent intertemporal choice research one step closer to human and non-human primate paradigms. It also expands the possibilities of odor-based rodent behavioral paradigms. Modifying primate behavioral tasks to suit rodents has immense translational values for psychiatric research and development.

7.1.2 Transient Activity of DR Serotonergic Neurons Causally Controls Delay-Size Trade-off in Intertemporal Choice

Short bouts of activation of DR Serotonergic Neurons caused the subjects to choose the delayed, large reward more often, and the inactivation of the same neurons caused the subjects to choose the delayed, large reward less often. These effects were only present in the cases where the subjects have to choose between two delayed rewards. I propose that serotonergic transmission is important for difficult trade-offs or decisions about rewards further into the future, but not involved in easy cases or decisions about rewards in the near future.

Unlike pharmacological or lesion methods often used in the literature, these results show that temporally precise manipulations of serotonin neurons at the reward-predicting cue drives choice in a meaningful way. They are novel for the following reasons: First, the results shed light on our understanding of serotonin's prospecting nature. Serotonergic neurons contain information about the future, and this information is able to drive behavior in the present time. Second, serotonergic neurons are active at a fast timescale in driving behavior. This is in contrast with long-held conception of neuromodulator neurons only firing at rather slow and tonic level. Third, these results are also one of the first cases where a manipulation at a symmetric choice point causes alteration to the choice in an asymmetrical manner. They suggest that serotonin activity acts as a decision variable for intertemporal choice. It is possible that serotonergic neurons encode information relevant to the context in which the choice is appropriate.

7.1.3 NAc Acts as a Target Structure for Serotonergic Influence in Intertemporal Choice

I found the nucleus accumbens mediate the control of interetemporal choice by serotonergic projections. These results are consistent with the literature in that NAc has been implicated as a center for impulse control. I suggest that serotonergic projections promote reward activities in NAc associated in long-term rewards.

7.2 Future Direction

The present study is wanting in several aspects. More work is needed to determine whether serotonergic neurons directly encodes reward values which affected intertemporal choice or it encodes thirst or other physiological states and modulate choice from upstream. It will be interesting to monitor neuronal activities in DR and NAc during task, especially during the choice point. Since the task is so designed as to allow collections of individual decision epochs, the activities during the epochs will reveal neural correlates of intertemporal choice. It will also be interesting to know what serotonin or glutamate receptor subtype act downstream of the serotonergic neurons, and their distribution in NAc. Such information will further our understanding of the mechanism by which DR-originating serotonin or glutamate acts. Finally, instead of a candidate area approach, a whole brain analysis of neuronal activation pattern for this task will be informative in finding other areas that are important.

Appendix A

Mazur's Derivation of Indifference Functions for Exponential and Hyperbolic Discounting

This section explains the logic for deriving indifference functions for an adjusting delay task, as adapted from Mazur(2009) [17].

A.1 Hyperbolic Discounting

The subjective value of a reward is defined as:

$$V = \frac{A}{1 + KD} \tag{A.1}$$

At indifference points, the value of the left option is the same as the value of the right option and therefore,

$$V_L = V_R \tag{A.2}$$

$$\frac{A_L}{1+KD_L} = \frac{A_R}{1+KD_R} \tag{A.3}$$

Rearranging terms gives

$$D_L = \frac{A_L - A_R}{A_R K} + \frac{A_L}{A_R} D_R \tag{A.4}$$

This would give an indifference function with a y-intercept that is sensitive to the discount factor K and a slope that is sensitive to the relative sizes $\frac{A_L}{A_R}$.

A.2 Exponential Discounting

The subjective value of a reward is defined as:

$$V = Ae^{(-KD)} \tag{A.5}$$

At indifference points, the value of the left option is the same as the value of the right option and therefore,

$$V_L = V_R \tag{A.6}$$

$$A_L e^{(-K_L D_L)} = A_R e^{(-K_R D_R)} \tag{A.7}$$

Rearranging terms gives

$$D_L = \frac{lnA_L - lnA_R}{K_L} + \frac{K_R}{K_L} D_R \tag{A.8}$$

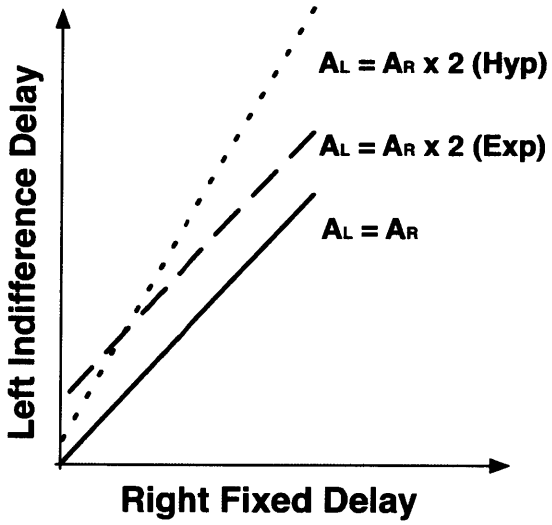

Figure A-1: Predictions about indifference functions for both hyperbolic and exponential discounting. Solid line indicates indifference function for the case where $A_L = A_R$. Dotted line indicates the predicted indifference function for the case where $A_L = 2xA_R$. Hyperbolic discounting predicts that the dotted line has a slope of $\frac{A_L}{A_R}$. The dashed line indicates the predicted indifference function for the same case by exponential discounting. The dashed line has the same slope as the original solid line.

This would give an indifference function with a y-intercept that is sensitive to the relative sizes of the rewards but a slope that is insensitive to the relative sizes.

The prediction about indifference functions are illustrated in Fig. A-1.

Appendix B

Anterograde and Retrograde Tracing of Neuronal Projections from Dorsal Raphe to the Nucleus Accumbens

B.1 Anterograde Tracing of DR-NAc Projections

B.1.1 Materials and Methods

Sert-cre mice were anaesthetized by injection of avertin (250 mg/kg) and placed in the Stereotactic instrument with anaesthesia. 1ul of AAVrh8-hsyn-DIO-ChR2-EYFP virus (at titer around 10^11) was injected stereotatically into the dorsal raphe nucleus (AP: -4.6 mm, DV: -3 mm, ML: 0 mm). The virus was allowed to express for 3 months before the mice were sacrificed to check for expression in the nucleus accumbens. Sections were stained with a chicken anit-GFP antibody (1:2000) and alexa 488 anti-chicken secondary antibody. DAPI was used to label the nucleus. Sections mounted on to glass slides were examined with a Zeiss epifluorescence microscope.

B.1.2 Results

AAVrh8-hsyn-DIO-ChR2 expressed well after incubation. Green GFP labeled projections originating from DR was clearly visible in the nucleus accumbens (Fig. B-1). NAcSh were more prominently labeled than NAcc.

B.2 Retrograde Tracing of DR-NAc Projections

B.2.1 Materials and Methods

Mice were anaesthetized by injection of avertin (250 mg/kg) and placed in the Stereotactic instrument with anaesthesia. 200uL of cholera toxin subunit B conjugated with florescent dye Alexa 555 (Invitrogen) was injected stereotatically unilaterally into left nucleus accumbens (AP: +1.35 mm, DV: -4 mm, ML: - 0.7 mm). The dye was allowed to transfer for 4 weeks, and then mice were sacrificed to check for retrograde labeling. Sections were stained with a rabbit anit-TPH antibody (1:2000) and alexa 488 anti-rabbit secondary antibody. DAPI was used to label the nucleus. Sections mounted on to glass slides were examined with a Zeiss epifluorescence microscope.

B.2.2 Results

Figure B-1: Anterograde tracing from dorsal raphe to nucleus accumbens. Green: ChR2-EYFP expression from a DR AAVrh8-hsyn-DIO-ChR2-EYFP injection. Blue: DAPI.

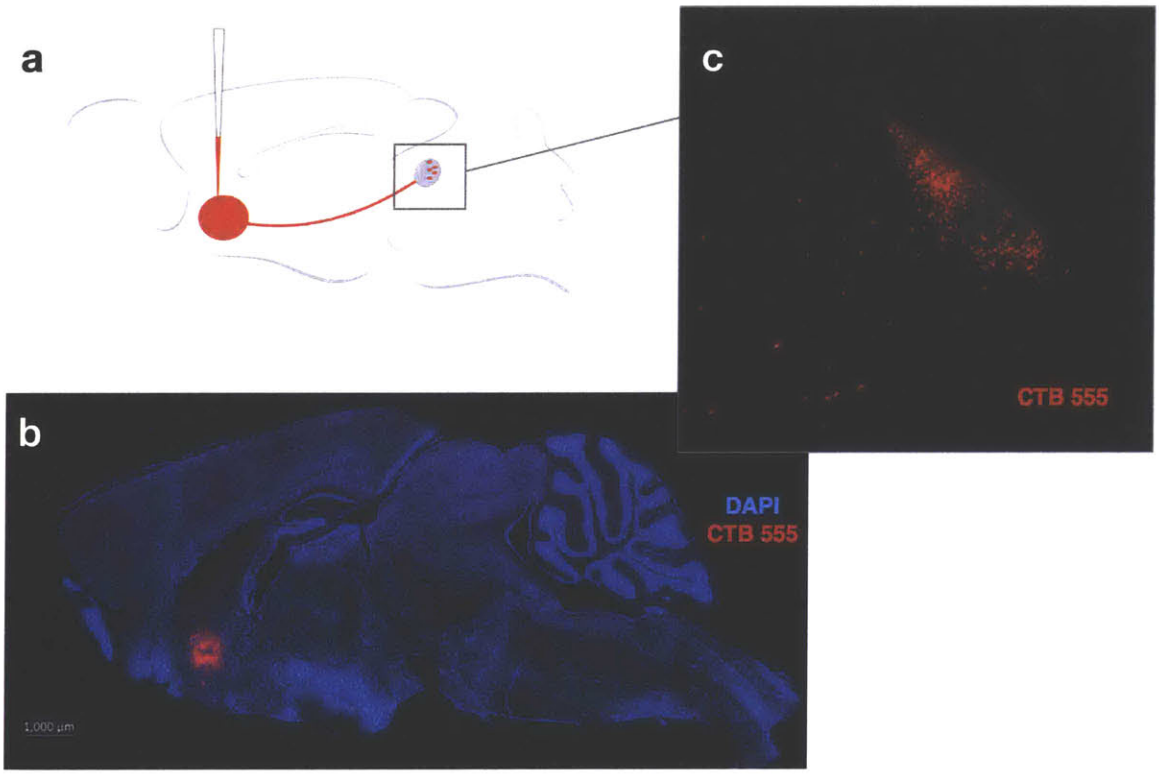

Figure B-2: Retrograde tracing from NAc with CTB 555. ***a.*** *Schematic for the injection.* ***b.*** *Sagital section of the injected brain, showing the injection site in accumbens.* ***c.*** *Sagital section showing cells retrogradely labeled in dorsal raphe, mostly distributed along the midline. Blue: DAPI.*

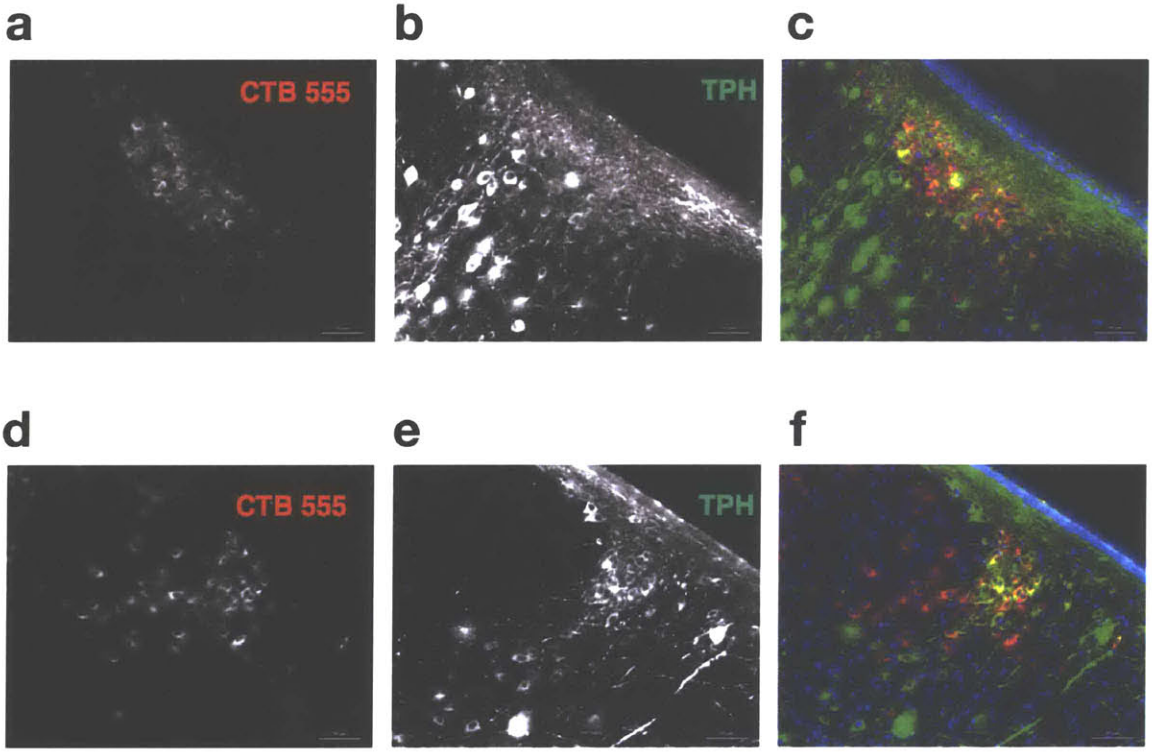

Figure B-3: Retrograde tracing from NAc with CTB 555 counterstained with TPH antibody. Two example sagittal sections containing CTB traced dorsal raphe cells. ***a, d*** *CTB 555 signal detected in raphe.* ***b, e*** *TPH staining labeling serotonin neurons.* ***c, f*** *Merged images. Blue: DAPI.*

Appendix C

Validation of Viral Transfection of Opsin Constructs into Sert-Cre Mice by *in vivo* Multiunit Recording in Anaesthetized Animals

C.1 Subjects

Sert-cre mice were injected with viruses containing opsin constructs as described in Section 4.2.2.

C.2 Materials and Methods

Mice were anaesthetized by injection of urethane (30 mg/kg) and placed in the Stereotactic instrument with anaesthesia. Body temperature was maintained by a pack of hand warmers. A diI-coated (Invitrogen) optrode consisting of a tungsten electrode (0.5 MΩ) attached to an optical fiber (200 m core diameter), with the tip of the optical fiber extending beyond the tip of the electrode by 100 μm, was used for simultaneous

optical stimulation and extracellular recordings. An optrode was slowly lowered to DR (AP -4.6 mm; ML 0 mm; DV -3 mm) using a hydraulic micropositioner at a speed of 50 μm per 5 -10 min. The optical fiber was connected to a 200 mW 473 nm blue laser or a 200 mW 561 nm green laser and controlled by a waveform generator. The power intensities of lights emitted from the optrode were calibrated to about 10 mW (blue laser) and 5 mW (green laser), which were consistent with the power intensities used in the behavioral assays. In the ChR2 experiments, 10 ms light pulses was used. In the eArch3.0 experiments, constant green light stimulation of 3 sec duration was used. Multiunit activity was band-pass filtered (500 Hz-5 kHz) and acquired with an Axon Digidata 1440A acquisition system running Clampex 10.2 software. Data were analyzed with custom software written in MATLAB. After recording, we confirmed that the electrode was in DR by histology.

Appendix D

A List of Abbreviations

DR	Dorsal Raphe Nucleus
VTA	Ventrotegmental Area
NAc	Nucleus Accumbens
NAcSh	Nucleus Accumbens Shell
NAcc	Nucleus Accumbens Core
OFC	Orbitofrontal Cortex
CS	Conditioned Stimulus
US	Unconditioned Stimulus
TPH	Tryptophan Hydroxylase
SSRI	Selective Serotonin Reuptake Inhibitor
GFP	Green Fluorescent Protein
EYFP	Enhanced Yellow Fluorescent Protein
AAV	Adeno-Associated Virus
ChR2	Channelrhodopsin 2
Arch	Archaerhodopsin
CTB	Cholera Toxin Subunit B
DAPI	4',6-Diamidino-2-Phenylindole
TTL	Transistor-Transistor Logic
LED	Light-Emitting Diode

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