Dissociable Cost and Benefit Encoding of Future Rewards by Mesolimbic Dopamine

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Abstract

Dissociable Cost and Benefit Encoding of Future Rewards by Mesolimbic Dopamine

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Dopamine is a neural substrate implicated in the complex computation of weighing the costs and benefits of future actions. Midbrain dopamine neurons encode fundamental economic parameters pertaining to predicted rewards in their subsecond firing pattern and innervate areas that have been implicated in economic decision-making such as the nucleus accumbens. Disruptions of dopamine in the nucleus accumbens core (NAcc) diminish animals' ability to respond to cues and overcome effortful response costs. However, electrolytic and chemical depletions of dopamine disrupt more than just the subsecond dopamine signal shown to encode economic parameters. Thus, to deconstruct the nature of the signal transmitted by phasic dopamine to the NAcc, new technology to detect dopamine at a subsecond time scale viable over months needs be developed. With this new technology we may characterized how subsecond dopamine accounts for behaviors by determining how it reacts to changes in anticipated costs and benefits.

Part I of this thesis will discuss dopamine in general. I will discuss dopamine as a neurochemical, anatomy of the dopaminergic systems involved

with decision making, and dopamine neuron firing patterns. I will briefly review some theories of dopamine's role in behavior, with attention to dopamine's role in decision making.

In Part II, I discuss the development and characterization of chronically implantable fast-scan cyclic voltammetry (FSCV) microsensors. Though *in vitro* validation of these electrodes was performed, I will primarily describe the *in vivo* validation of these electrodes in this thesis. With the advent of these electrodes, we extend the ability to detect subsecond dopamine transmission in awake, behaving animals from a handful of recordings to a multitude of recordings across months.

In Part III, we examine the valuation signal transmitted by phasic dopamine in the NAcc. Animals implanted with chronic FSCV microsensors were asked to distinguish cues that predicted either differing amounts of food reward or differing efforts (amounts of lever presses). We found that dopamine release in rat nucleus accumbens encodes anticipated benefits, but not effort-based response costs unless they are atypically low. This neural separation of costs and benefits indicates that mesolimbic dopamine scales with the value of pending rewards, but does not encode the net utility of the action to obtain them.

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Acknowledgments

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Dedication

This dissertation is dedicated to Mike Garelick, who is my husband, my best friend, and the best doggy parent a pug could ever want.

PART I: INTRODUCTION

For many animals, the ability to make optimal decisions is a matter of life and death. To make these decisions, animals must weigh the benefits of an action versus the required costs. To do so, an animal must have neural mechanisms that utilize previous experiences to predict likely outcomes of different actions. These predictions not only have to be adaptive to changing environments, but must also be quantitative to discriminate between various outcomes. These predictions must also be able to modify animals' behavior. One neural system with the ability to represent predictions and modify behavior is the mesocorticolimbic dopamine system. Manipulations to this system result in alterations in animals' behavior consistent with a change in cost-benefit decisions. But, while this system is important in an animal's ability to make cost-benefit decisions, its precise role in the process is unclear.

Cellular effects of dopamine

The importance of dopamine as a chemical neurotransmitter in its own right and its function in motivation and reinforcement was only realized during the second half of the 20th century (Carlsson, Lindqvist, & Magnusson, 1957; Wise, 1978). Dopamine is a modulatory neurotransmitter, classically thought to modulate coincident glutamatergic input in neighboring terminals. Whereas glutamatergic neurons make asymmetric synapses upon the heads of dendritic

spines, dopaminergic neurons synapse symmetrically on dendritic shafts and the necks of spines (Sesack & Pickel, 1990). In fact, the dopamine innervation of the striatum is so dense that it is thought that every structure in the striatum will be within range of a concentration of dopamine sufficient to stimulate both low- and high-affinity receptors following activation of dopamine neurons (Moss & Bolam, 2010).

Dopamine acts upon a family of G-protein coupled receptors classified as either D1-like (D1 and D5) or D2-like (D2, D3, D4). These receptors regulate intracellular signaling cascades in a cyclic adenosine monophosphate (cAMP)-dependent manner where D1-like receptors increase and D2-like receptors decrease cAMP production (Neve, Seamans, & Trantham-Davidson, 2004). D2-like receptors are expressed both pre- and postsynaptially whereas D1-like-receptor expression is limited to postsynaptic locations. In addition to their action on cAMP production, which in itself can change conductivity through ERK/MAPK signaling (Greengard, Jen, Nairn, & Stevens, 1991), D2-like receptors regulate ion-channel conductance through the G-protein βγ complex, generally reducing cell excitability; and, as shown recently, participate in β-arrestin-2-dependent cell signaling using the protein-kinase-B/glycogen-synthase-kinase-3 pathway (Beaulieu, Gainetdinov, & Caron, 2007). While D1-like receptors are generally considered to be excitatory and D2-like receptors inhibitory, these inferences are oversimplifications.

Anatomy of the dopamine system

The majority of dopamine neurons arise from ventroanterior midbrain nuclei, which include the substantia nigra pars compacta (SNc: areas A8 and A9) and VTA (area A10). Afferent inputs into the VTA include glutamatergic input from many parts of the brain (Geisler, Derst, Veh, & Zahm, 2007), including prefrontal cortex, amygdala, lateral hypothalamus, superior colliculus, along with the adjacent pedunculopontine tegmental nucleus (PPTg) and laterodorsal tegmental nucleus (LDT) (Fields, Hjelmstad, Margolis, & Nicola, 2007). The PPTg and LDT also send cholinergic and GABAergic projections to the VTA (Semba & Fibiger, 1992). Other GABAergic projections to the VTA originate from the ventral pallidum, the NAcc, the rostromedial tegmentum, as well as from local-circuit connections within the VTA. Additionally, the VTA receives serotonergic input from the dorsal raphe and noradrenergic input from the locus coeruleus (Fields, et al., 2007). Unlike the VTA, the major inputs to the SNc are inhibitory, consisting of GABAergic innervation from the striatum, globus pallidus, ventral pallidum, and the substantia nigra pars reticulata (SNr) (Misgeld, 2004). Excitatory inputs, though in the minority, arise from the subthalamic nucleus, amygdala and PPTg (Misgeld, 2004; Oakman, Faris, Kerr, Cozzari, & Hartman, 1995).

Dopaminergic projections from these nuclei comprise three main projection pathways, i.e. the nigrostriatal, the mesolimbic and the mesocortical pathway (Fig 1.1). The nigrostriatal and mesolimbic dopaminergic pathways

heavily but differentially innervate the striatum. SNc A8 dopaminergic neurons of the nigrostriatal pathway mainly innervate the dorsolateral striatum while mesolimbic VTA neurons mainly innervate the ventral striatum, including NAcc. Other neurons of the nigrostriatal pathway originating from A9 innervate a broad, intermediate area primarily in the dorsolateral striatum but reaching areas considered in the ventromedial striatum (Joel & Weiner, 2000; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). This anatomical gradient from the dorsolateral to ventromedial striatum mirrors a functional differentiation demonstrated by both recording and interference studies across mammalian species: while dorsolateral striatum is implicated in a range of sensorimotor functions, ventromedial striatum has a more direct connection with rewards and motivated behavior.

Physiology of dopamine

Electrophysiological recordings from the cell bodies of midbrain dopamine nuclei demonstrate a phasic response to rewards as well as reward-predicting stimuli (Schultz, 1997). Presentation of a reward --- an unconditioned stimulus (UCS) --- elicits a phasic dopaminergic response if unpredicted. In animals that are classically (Pavlovian) conditioned with visual or auditory stimuli predicting a reward (CS), the phasic dopaminergic response shifts from the delivery of reward to the onset of the CS (Fig 1.2). The dopaminergic response at the UCS then behaves akin to a prediction error described by learning

models (Rescorla & Wagner, 1972). In trained animals, if the amount of reward is in compliance with the CS-predicted value, there is no phasic dopaminergic activity at the UCS. Strikingly, greater-than predicted reward elicits a phasic response to the UCS and less-than predicted reward is marked with a brief cessation of dopaminergic cell firing at the time of the UCS (Schultz, 1997). Thus, DA neurons can reflect in their firing a prediction error that can be defined as the actual value minus the predicted value (Fig 1.2).

Recent studies have begun to characterize the determinants of dopaminergic activation and adaptation. Since dopamine cell firing acts as a prediction error, this implies that the amplitude of the DA response to the CS and the UCS may be useful as well. According to learning and prediction error models, the phasic dopamine response encodes a valuation signal reflective of an expected value. Expected value, in economic terms, is the sum of all possible reward magnitude weighted by the probability of the reward occurring. Tobler et al. found that in monkeys, the dopaminergic response at an unexpected UCS increased with reward magnitude (volume of juice) delivered. The monkeys were then trained to distinguish between distinct visual stimuli (CS) that predicted a range of reward magnitudes and probabilities. Dopaminergic cell firing to the stimulus presentation increased as expected values predicted by the CS increased. This scaling of the dopamine response to expected utility was found to be sensitive to changes in reward magnitude as well as probability (Fiorillo, Tobler, & Schultz, 2003; Tobler, Fiorillo, & Schultz, 2005).

For rewards predicted by a CS, the dopamine response at the UCS represented the reward prediction error, i.e., the delivered reward magnitude minus the expected value. When the CS predicted a probabilistic reward, there was always a prediction error at the UCS, since on the occasions when reward was omitted, the expected value was higher than the reward magnitude and on occasions where the reward was delivered the expected value was lower than the reward magnitude (Fiorillo, et al., 2003). For instance, for a CS predicting 0.2 ml of juice fifty percent of the time, the expected value would be 0.1 ml (0.2 ml x 50 %) for all trials, but the reward magnitude would be 0 ml on half of the trials and 0.2 ml on the others. Further experiments showed that the same reward magnitude elicits dissimilar prediction errors depending upon whether the stimulus predicted a larger or smaller average reward. Though this result implied that dopamine responses shifted relative to predicted reward magnitude, the investigators proceeded to further characterize the adaptive coding of the valuation signal. By varying the range between the larger and smaller reward, they found that the magnitude of the prediction error was equivalent though the difference between the volumes of reward varied up to a factor of 10 (Tobler, et al., 2005). These results imply that the midbrain dopamine neurons have the capacity to relay information regarding reward that is both quantitative and adaptive to the environment.

Brief activation of VTA neurons can elicit reward-seeking behavior (Phillips & Wightman, 2003) whereas inactivation inhibits cue-induced reward

seeking (Blackburn, Pfaus, & Phillips, 1992; Di Ciano, Cardinal, Cowell, Little, & Everitt, 2001; Wakabayashi, Fields, & Nicola, 2004). Animals will also electrically self-stimulate when stimulation elicited dopamine release. If the intracranial self-stimulating (ICSS) electrode did not elicit dopamine release, animals did not learn the ICSS behavior (Garris et al., 1999). The question then becomes how dopaminergic reward signals are utilized in areas such as the nucleus accumbens and the prefrontal cortex to change behavior.

Theories of dopamine's role in behavior

Theories abound as to mesocorticolimbic dopamine's role in behavior. One influential though controversial theory is the anhedonia hypothesis. The anhedonia hypothesis posits that dopamine integrates sensory information into "hedonic messages we experience as pleasure, euphoria or 'yumminess'" based on neuroleptics attenuating animal behavior towards positive reinforcers (Wise & Bozarth, 1982; Wise, Spindler, deWit, & Gerberg, 1978). Though numerous fMRI studies support the hedonia hypothesis (Volkow et al., 1999), other evidence calls its validity into question. Down-regulation of dopamine, such as in dopamine deficient (DD) mice, does not alter preference of sucrose over water compared to wild-type animals (Cannon & Palmiter, 2003). Nor does indirect agonism of DA by systemic amphetamine injections (Wyvell & Berridge, 2000) or NAcc microinjections (Tindell, Berridge, Zhang, Pecina, & Aldridge, 2005)

accentuate pleasure. Also, Parkinson's patients do not perceive pleasantness of sweet foods differently than healthy individuals (Sienkiewicz-Jarosz et al., 2005).

Alternatively, studies in Parkinsonian patients reveal a correlation between DA transmission and "wanting" a drug reward as opposed to a "liking" (Evans et al., 2006). Additionally, increasing extracellular DA concentrations by conditional knockdown of dopamine transporters induces higher breakpoints and diminishes latency to achieve rewards due to presentation of distracting cues (Cagniard et al., 2006; Pecina, Cagniard, Berridge, Aldridge, & Zhuang, 2003; Yin, Zhuang, & Balleine, 2006), which can be interpreted as an increase in "wanting" (Berridge, 2007). This "wanting" of reward has been formalized into the incentive salience theory in which reward is a construct composed of wanting, learning and liking. DA is thought to mediate the "wanting" but not the learning or liking of reward (Berridge, 2007).

Other theories of dopamine function center upon DA's possible role in learning. Some psychological theories use DA function to relate stimuli (e.g. UCS) with other stimuli (e.g. CS) or responses (e.g. lever-press). Since many drugs of abuse increase dopamine levels, a simple theory of DA function is to facilitate the association of the CS to the UCS (Di Chiara, 2002). In essence, DA "stamps in" a CS-UCS relationship (Wise, 2004). A more rigorous theory posits that DA enhances the CS-UCS relationship to form habits (Everitt, Dickinson, & Robbins, 2001). Like "stamping in" theories, "habit" enhancement theories are based upon animals' reactions to drugs of abuse such as enhancement of

amphetamine-induced stereotypy and DA-mediated modulation of repetitive grooming behaviors (Cromwell & Berridge, 1996). More computational theories, corroborated by electrophysiological studies (Schultz, 2002), posit that DA relays prediction errors important in learning (Schultz, 1997). The Rescorla-Wagner model of learning ($\Delta V = \alpha\beta(\lambda - V)$) posits that the strength of association (ΔV) is dependent upon the difference between predicted value of the CS (λ) and actual reward value (V) (Rescorla & Wagner, 1972). The temporal difference (TD) model attaches a time component to prediction errors by discounting rewards away from the present (Bayer & Glimcher, 2005; Montague, Hyman, & Cohen, 2004). Both the TD and Rescorla-Wagner model imply that DA transmission is a mechanism to review and then to guide reward learning.

DA function has also been hypothesized to mediate sensorimotor gating and activation of behavior (Brooks, 1987; Salamone, Correa, Farrar, & Mingote, 2007b). Electrical stimulation delivered to parts of the limbic forebrain causes an animal to repeat that action that results in the stimulation (Olds & Milner, 1954). This intracranial self-stimulation (ICSS) acts as such a potent positive reinforcer that animals would overcome electric shocks or would even forgo food when starving to achieve the stimulation (Olds, 1969). More recently, it has been argued that stimulation parameters can be titrated so that animals will trade off stimulation for other positive reinforcers such as sucrose or saline dependent on their internal state (Shizgal, 1997). ICSS sites included parts of the cortex,

hippocampus, lateral hypothalamus, NAcc, and "as far back as the tegmentum" (Olds & Milner, 1954).

Dopaminergic innervations relevant to sensorimotor gating

The midbrain dopamine neurons send their projections to areas in the prefrontal cortex such as the anterior cingulate and orbitofrontal cortex as well as limbic areas including the basolateral amygdala, and ventral striatum (Sesack & Pickel, 1990) (Fig 1.2). One particularly interesting area within the ventral striatum that receives dopaminergic input from the VTA is the nucleus accumbens (NAcc) (Yun, Nicola, & Fields, 2004). The NAcc consists primarily of GABAergic neurons onto which glutamatergic input from regions such as the cortex, amygdala and hippocampus synapse. Dopamine modulates the relationship between the presynaptic glutamate and postsynaptic GABA neurons (Sesack & Pickel, 1990). With these glutamatergic and dopaminergic inputs, the NAcc is in an anatomically favorable position to integrate information in cost-benefit analyses. Multiple studies have also implicated the nucleus accumbens in mediating some functions of reward. Low dose DA antagonism can actually increases food consumption (Clifton, 2000) when little or no effort is required to obtain the food (Aberman, Ward, & Salamone, 1998; Rusk & Cooper, 1994). In contrast, when the required effort is increased, low dose DA antagonism suppresses effort-related tasks such as lever pressing or lengthy maze traversal (Aberman & Salamone, 1999; Caine & Koob, 1994). High dose regimens and

lesions to the NAcc also suppress effort-related tasks without altering choices or responses at lower effort schedules (Correa, Carlson, Wisniecki, & Salamone, 2002; Mingote, Weber, Ishiwari, Correa, & Salamone, 2005). Electrophysiological studies support the role of VTA efferents in mediating this alteration in NAcc function (Garris, et al., 1999; Yun, et al., 2004). These pharmacological manipulations highlight a possible role of dopamine in bridging the internal calculations of reward and the actions required to attain them.

Yet while there is evidence to corroborate each theory of DA function, there exists a host of evidence questioning each theory. Revisions to the hedonic hypothesis such as incentive salience do not explain why dopamine deficient (DD) mice retain preference for sucrose over water (Cannon & Palmiter, 2003). Nor does incentive salience account for modulation of effort by dopamine antagonism (Salamone, et al., 2007b). DA's role in learning is also unclear since animals have the ability to learn about rewards with a systemic absence of dopamine (S. Robinson, Sandstrom, Denenberg, & Palmiter, 2005).

Neuroeconomic approach to dopamine function

In light of the discrepancies and controversies, new avenues have been opened to attempt to explain DA's role in behavior. The field of neuroeconomics incorporates economic concepts into neuroscience and psychology. As previously described, DA activation has been shown to monotonically increase with reward amount as well as react as a prediction error (Tobler, et al., 2005).

However, the perceived reward may be "framed" by individual- and scenario-specific parameters such as history, prior knowledge, emotions, biases and internal state (e.g. hunger, thirst, stress). Consequently, "utility" of the reward, or the subjective value weighted by internal states, may be a better descriptor of DA activity. Also, since most electrophysiological characterizations of dopamine have been during Pavlovian tasks, it is not known whether cost is factored into DA activity. If DA cell firing is modified by the cost associated with rewards, another neuroeconomic concept that could be represented by DA is "net utility" (cost-subtracted utility). Furthermore, determining which of these economic principles may be encoded in dopamine transmission to target regions may focus our understanding of dopamine function.

Neuroeconomic descriptions of dopamine function complement sensorimotor gating theories. That ICSS stimulation parameters are sensitive to internal state suggests that ICSS might be acting as a "payoff" signal in a computation of the overall subjective utility of the available options (Shizgal, 1997). Addiction (e.g. drug abuse, pathological gambling, etc), which include behaviors shown to be sensitive to dopamine manipulation (De Wit & Wise, 1977), can be described as issues in risk preferences due to problems of impulse control (Dalley et al., 2007; Deminiere, Piazza, Le Moal, & Simon, 1989; Nader, Czoty, Gould, & Riddick, 2008). Increasing the difficulty of a speed-accuracy trade-off task also renders Parkinson's disease patients less able to perform the

previously workable task, implying a change in the calculation of costs and benefits (Niv & Rivlin-Etzion, 2007).

Detection of dopamine at subsecond timescale

Regardless of overarching theories of dopamine function, empirical testing has, until relatively recently, been limited due to technological difficulties. Changes in phasic DA may not lead to the same behavior as changes in tonic DA and different brain regions may integrate dopamine signals differently. Electrophysiology can detect phasic activity but lacks the ability to differentiate where dopamine neuron project to. Since not all (~70%) VTA neurons phasically activate when presented with reward-stimuli (Schultz, Dayan, & Montague, 1997) and activation may differ depending on the target brain region, electrophysiology is limited in its ability to characterize DA. Additionally, discrete characterization of tonic and phasic DA action in key brain regions is important to fully understand dopamine's role in behaviors. Consequently, to test DA's role in behavior, levels must be assessed at the terminal regions at different timescales.

A great number of studies have used microdialysis to assess tonic DA levels (Di Chiara, 2002; Lapish et al., 2009). Detecting phasic dopamine release, on the other hand, has been limited by timescale and substrate specificity; especially in awake, behaving animals (Salamone, 1996). Fast-scan cyclic voltammetry (FSCV) has, within the last decade or so, enhanced analyte

specificity (Phillips, Robinson, Stuber, Carelli, & Wightman, 2003) and has been adapted to animals performing behaviors by acutely implanting a carbon fiber microsensor into the region of interest (Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Roitman, Stuber, Phillips, Wightman, & Carelli, 2004; Stuber, Roitman, Phillips, Carelli, & Wightman, 2005). However, the acute implantation of a voltammetric probe into the brain via a microdrive before each experimental day limited the ability to track longitudinal changes in neurotransmitter dynamics. Nor did acute implantations allow for detection of neurotransmitter release at the same terminal location across days.

To overcome this limitation, Part II will describe the development and characterization of chronically implanted microsensors capable of detecting release of specific analytes at brain regions of interest. With this technology, Part III will determine the economic principle that best describes the transmission of dopamine in the nucleus accumbens. We hypothesize that dopaminergic transmission to the nucleus accumbens will not encode net utility. Instead, it will encode information regarding benefit without taking account the cost to attain rewards.

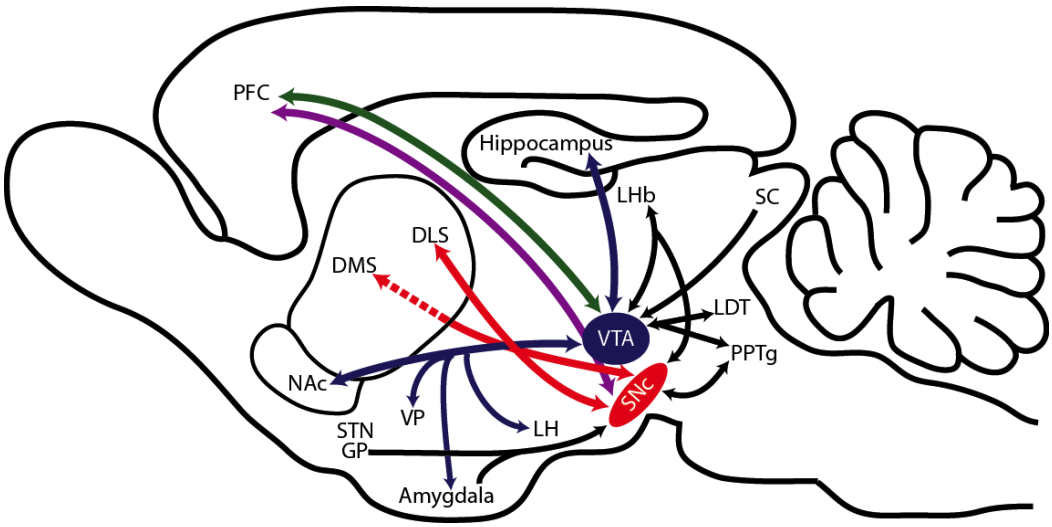

Fig 1.1 Dopaminergic nuclei and major innervations required for decision making

The primary midbrain dopaminergic nuclei, the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc), Innervations to midbrain dopaminergic nuclei include those from the hippocampus, lateral habenula (LHb), superior colliculus (SC), the lateral dorsal tegmentum (LDT), pedunculo-pontine nucleus (PPTg), amygdala, globus pallidus (GP), subthalamic nucleus (STN). Dopamine neurons in the subtantia nigra innervate the dorsolateral (DLS) and dorsomedial (DMS) striatum and the prefrontal cortex (PFC). The Ventral tegmental area innervates the nucleus accumbens (NAcc) and also the prefrontal cortex.

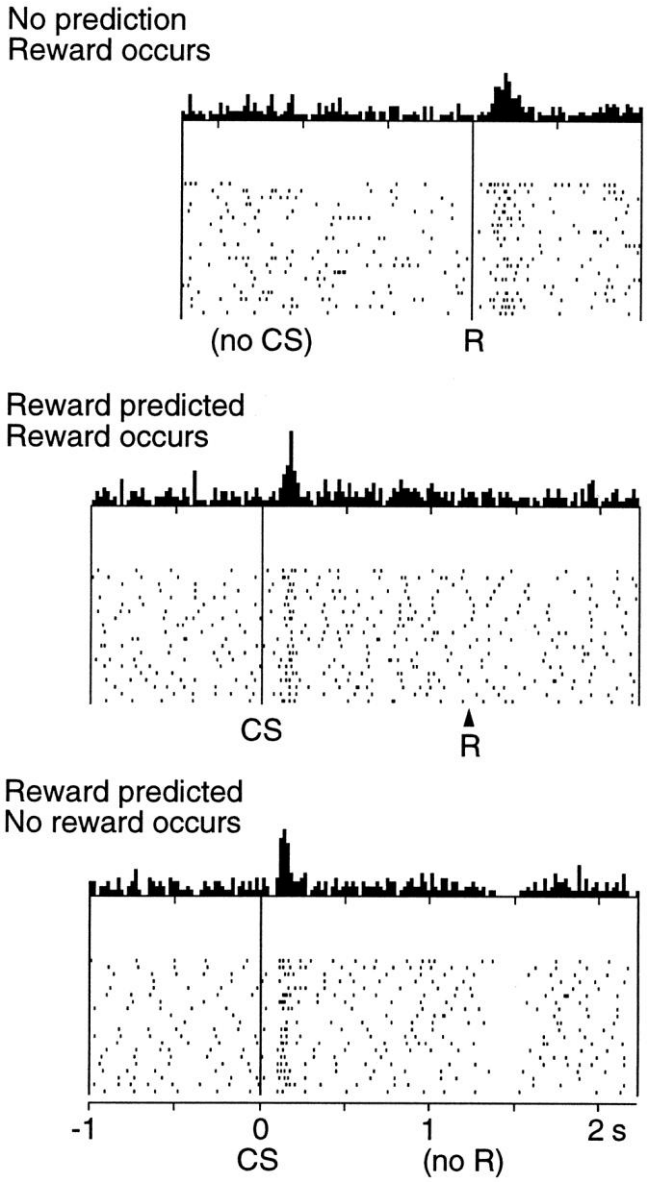

Figure 1.2 Dopaminergic neuron response to stimuli

Electrophysiological activity of dopamine neurons to rewards (R), conditioned stimuli that predict reward (CS). Presentation of primary rewards (top) elicit dopamine neuron activity/. After animals learn that a stimulus predicts the reward, dopaminergic neuron activity shifts to the presentation of the stimulus (middle). If conditioned stimulus is presented and subsequent reward is withheld, there is a brief cessation of tonic dopamine cell firing. (Schultz, 1997)

PART II: Development of Chronic Microsensors to Detect Sub-second Dopamine Transmission in Behaving Animals

Summary

Dopamine neurotransmission has been implicated in the neural mechanisms underlying cost-benefit analyses. Previous applications of fast-scan cyclic voltammetry (FSCV) to detect dopamine transmission relied on daily implantation of a carbon fiber microsensor via microdrive. While capable of detecting behaviorally-evoked dopamine, daily implantation limited the number of times an animal could be recorded from. To overcome this, we developed a chronically implantable microsensor. In this chapter, I will describe the development of a chronically implantable microsensor capable of detecting dopamine over a period of months.

Introduction

The neurochemical dopamine has been implicated in normal cognitive processes such as motor control, motivation and reinforcement learning (Schultz, Dayan, and Montague, 1997; Wise, 2004). Perturbations to dopaminergic systems have been thought to underlie neurological and neuropsychiatric disorders such as Parkinson's disease, schizophrenia, substance abuse, and depression[3-5]. But, though dopamine's importance to neural function cannot be denied, limitations in technology have hampered the characterization of dopamine in the brain.

One aspect of dopamine function that makes it difficult to characterize fully is that it has purported functions on multiple timescales. Dopamine neurons can (i) be hyperpolarized and quiescent, (ii) fire action potentials in a pacemaker-like fashion (2–10 Hz) or (iii) fire action potentials in bursts up to 15–30 Hz. The pacemaker dopamine neuron firing is thought to give rise to the 'tonic' levels of dopamine with concentrations ranging from 5–20 nM, while the burst firing is thought to give rise to 'phasic' elevated dopamine levels which can reach as high as 1 μM (Grace & Bunney, 1983; A. A. Grace & B. S. Bunney, 1984; A.A. Grace & B.S. Bunney, 1984; Martinelli, Rudick, Hu, & White, 2006; Shi, 2005; Wanat, Willuhn, Clark, & Phillips, 2009). The third firing pattern is especially fascinating as these short-latency bursts encode, as described with electrophysiology, properties of reward-related stimuli. But, since not all (~70%) VTA neurons phasically activate when presented with reward-stimuli and

activation may differ depending on the target brain region, electrophysiology cannot fully characterize dopaminergic signaling. Thus, to understand dopamine's role in animal behavior, detection of dopamine at the terminal brain regions is necessary.

Classic neurochemical techniques, such as microdialysis, are ideal for measuring tonic baseline levels of neurotransmitters such as dopamine, but have low sampling rates (minutes) that cannot temporally resolve the rapid changes in extracellular dopamine concentration predicted by the neurophysiological data. Electrochemical techniques, such as fast-scan cyclic voltammetry (FSCV), provide both high temporal resolution and an electrochemical signature of the analyte conferring the necessary chemical selectivity to discriminate dopamine from other electroactive species in the brain. This methodology has been used to detect subsecond changes in behaviorally-evoked dopamine after the presentation of salient stimuli and during behavioral tasks (Phillips, Robinson, et al., 2003; Phillips, Stuber, et al., 2003) (D. L. Robinson, Heien, & Wightman, 2002) (Day, Roitman, Wightman, & Carelli, 2007; Roitman, et al., 2004). However, existing approaches have been constrained by the requirement for acute implantation of a voltammetric probe into the brain via a microdrive for each experiment (Stuber, et al., 2005), limiting the ability to track longitudinal changes in neurotransmitter dynamics over the course of animal behavior.

Thus, to examine dopamine signaling across epochs relevant to many behaviors, chronically implanted electrodes are necessary. Previous attempts to

use chronically implanted electrodes to make long-term electrochemical measurements have discovered that the fidelity of chemical recordings can be severely impaired by perturbation of the microenvironment through physical tissue disruption and/or neuroinflammation, emphasizing that size and biocompatibility are critical considerations in the design of chronic devices for *in situ* neurochemistry.

Here we outline the development of a biocompatible voltammetric microsensor that can be chronically implanted into the targets of midbrain dopamine systems where they can detect subsecond dopamine dynamics from day to day over periods of months (Clark et al., 2010). Validation of long-term biocompatibility of these chronically implantable microsensors was assessed both *in vitro* and *in vivo*. Discussed in this thesis will be *in vivo* validations including microsensor survival curves for both electrically- and behaviorally-evoked dopamine sensitivity.

Methods

Rats

Young adult male Sprague-Dawley rats (Charles River, Hollister, CA) were used in this study. Rats had free access to food and water and were kept on a 12 h/12 h light/dark cycle at a temperature of 25 °C. Rats were group housed for at least 1 week before training or implantation to reduce behavioral variability. Post-surgery, animals were singly housed to prevent equipment failure. All experiments were conducted according to Institutional Animal Care and Use Committee guidelines and regulations.

Fabrication of electrode

The microsensor consists of a 7-μm diameter carbon fiber housed in a 90-μm diameter polyimide-covered fused-silica capillary (Fig 2.1). Microsensor fabrication began with the insertion of a single carbon fiber (34-700, Goodfellow Corporation) into a 10-15-mm length of fused silica (Polymicro technologies) by floating fibers and silica in 2-propanol. The sensing end of the microsensor was then sealed with Devcon two-component epoxy (IWT Performance Polymers, Riviera Beach, FL) and allowed to dry. A silver connector (Newark, Chicago, IL) was secured in contact with the carbon fiber on the non-sensing end of the silica with silver epoxy (8331; MG Chemicals, Surrey, BC, Canada), allowed to cure overnight, then insulated with a layer of two component epoxy. After an additional twelve hours of drying, the fabrication of the chronic microsensor was

finalized by trimming the carbon fiber on the sensing end to desired length (150-200 μm).

Electrochemichal instrumentation.

In fast-scan cyclic voltammetry a voltage ramp is applied to the working electrode and resulting current is measured. The working electrode (WE) is connected to the inverting input of an operational amplifier (current-to-voltage transducer) where the signal is amplified and converted to voltage. The operational amplifier has a feedback resistor (R_f) determining amplification of measured signal, with a capacitor (C_f) in parallel with R_f for filtering. The applied potential is fed into the non-inverting input (+) and is referenced to a Ag/AgCl reference electrode (RE), which is connected to ground. The power supply to the operational amplifier is denoted as V+ and V-. The output of the operational amplifier is connected to an analog to digital converter. During all experimental sessions, the chronically implanted microsensors were connected to a head-mounted voltammetric amplifier for dopamine detection by fast-scan cyclic voltammetry where the redox current associated with an electroactive analyte is converted to voltage. The circuit for the voltammetric amplifier consists of an operational amplifier with a feedback resistor (R_f). The current-to-voltage 'gain' is determined by the value of R_f (following Ohm's Law: $V_{out} = I_{in} \times R_f$). In addition, a capacitor (C_f) is wired in parallel with the feedback resistor to filter high frequencies, while other capacitors bridge each of the power sources

(V+ and V-) to ground to filter operational amplifier noise. These components were laid out on a miniaturized printed-circuit board in house. Voltage signals from the voltammetric amplifier were transmitted to a PC-driven data acquisition system (National Instruments, Austin, TX) through an electrical swivel (Crist Instrument Co., Hagerstown, MD) mounted above the recording chamber. For all voltammetric recordings in this paper, the applied potential at the working electrode was held at -0.4 V vs Ag/AgCl between voltammetric scans, and then ramped to +1.3 V and back at 400 V/s during the scan (8.5-ms total scan time). Voltammetric scans were repeated every 100 ms to obtain a sampling rate of 10 Hz.

Voltammetry Surgery

All animal procedures presented in this paper followed the University of Washington Institutional Animal Care and Use Committee guidelines. Surgical preparation for in vivo voltammetry used an aseptic technique. Male rats weighing between 300g and 350g (Charles River, Hollister, CA) were anesthetized with isoflurane and placed in a stereotaxic frame. The scalp was swabbed with 10% povidone iodine, bathed with a mixture of lidocaine (0.5 mg/kg) and bupivicaine (0.5 mg/kg), and incised to expose the cranium. Holes were drilled and cleared of dura mater above the nucleus accumbens core (1.3 mm lateral and 1.3 mm rostral from bregma) the dorsolateral striatum (4.3 mm lateral and 1.2 mm rostral from bregma), and/or the nucleus accumbens shell (0.8

mm lateral and 1.2 rostral from bregma) for microsensors, above the midbrain (1.0 mm lateral and 5.2 mm caudal from bregma) for a stimulating electrode in some animals, and at convenient locations for a reference electrode and three anchor screws. The reference electrode and anchor screws were positioned and secured with cranioplastic cement, leaving the stimulating electrode and working electrode holes exposed. Once the cement cured, the microsensors were attached to the voltammetric amplifier and lowered into the target recording regions (the core/shell of the nucleus accumbens, 7.0 mm ventral of dura mater, and the dorsolateral striatum, 4.0 mm ventral of dura mater). For animals in which a stimulating electrode was to be implanted, the voltammetric waveform was switched on and dopamine monitored. Next, the stimulating electrode (Plastics One, VA) was lowered to 7.0 mm below dura mater and an electrical stimulation (60 biphasic pulses, 60 Hz, ±120 μA, 2 ms/phase) was applied via an optically-isolated, constant current stimulator (A-M Systems, Carlsborg, WA). If an evoked change in dopamine concentration was not observed at the working electrode, the stimulating electrode was positioned 0.2 mm more ventral. This was repeated until dopamine efflux was detected following stimulation. It was then lowered further in 0.1 mm increments until dopamine release was maximal. This is usually when the stimulating electrode is 8.4 mm ventral from dura mater. Finally, cranioplastic cement was applied to the part of the cranium that is still exposed to secure the stimulating electrode.

Recording sessions.

Testing was carried out in operant chambers (30.5 x 24.1 x 29.2 cm; Med Associates, VT, USA) with sloped inserts between the floor and walls (63° towards the levers and magazine, and the back wall, 52° towards the sides). Each chamber was housed within a custom-built sound- attenuating cabinet ventilated with a fan. Each chamber was fitted with two retractable levers on either side of an extra-tall food magazine into which 45-mg food pellets (Bioserv, NJ, USA) could be dispensed. Above each lever was a stimulus light, which could act as a visual cue, and the chamber could be illuminated by a 2.8-W house light located at the top of the wall opposite the levers and food magazine. The food magazine was fitted with an infrared beam that could signal when animals entered the receptacle and could also be illuminated by an internal light. All behavior was video recorded to DVD via an infrared ('night vision') camera and indexed to electrochemical information with a video character generator (Decade Engineering, OR). Animals were connected to the FSCV electronics and the electrode was allowed to condition for about one hour. Animals were then presented with an unexpected food pellet or with an electrical stimulation to the VTA (12 biphasic pulses, 60 Hz, ±120 μA, 2 ms/phase) applied via an optically-isolated, constant current stimulator (A-M Systems, Carlsborg, WA). Microsensor survival curves were based on whether they detected behaviorally evoked dopamine.

Electrochemical isolation of dopamine.

Electrochemical data was analyzed using software written in LabVIEW (National Instruments, Austin, TX). All statistical analyses were carried out using Prism (GraphPad Software, La Jolla, CA). The chemical signature of behaviorally evoked phasic dopamine release events was statistically compared to a template obtained from stimulated release (or unexpected pellet evoked release) prior to each session. Behaviorally and electrically evoked changes in signal are attributable to dopamine if the cyclic voltammogram is closely correlated with that of stimulated release ($r^2 \geq 0.75$).

Results

As reported in Clark et al 2010, we have developed a chronically implantable biocompatible microsensor capable of detecting dopamine for months. Though in vitro validation was performed on these electrodes, of greater importance was their viability *in vivo*.

The functionality of a voltammetry electrode can be assessed by measuring fluctuations in dopamine release after electrical stimulation of the ventral tegmental area (VTA), substantia nigra pars compacta (SNc), or the medial forebrain bundle fiber tract (Phillips, Robinson, et al., 2003). Using the chronically implanted microsensor, electrically-evoked dopamine release in the nucleus accumbens (NA) was detected and was comparable with that obtained with acute voltammetry electrodes. However, it was noted that there was more temporal distortion of the response from the chronically implanted microsensor compared to an acutely implanted electrode (Venton, Troyer, & Wightman, 2002). Indeed, FSCV is known to confer temporal distortion to *in vivo* dopamine signals (Venton, et al., 2002). While this does not impair the detection of changes in phasic signaling amplitude during learning or in disease models, it renders it less ideal for detailed kinetic analysis (Venton, et al., 2002).

Electrically-evoked neurochemical signals provide a reliable means to assess the functionality of specific recording electrodes; however behaviorally-evoked signals, such as reward presentation, are often the most experimentally relevant. Delivery of natural rewards has been shown to increase the firing of

dopamine neurons in the VTA and SNc in monkeys (Hollerman & Schultz, 1998) and elicits dopamine release in the NA in rats (Phillips, Robinson, et al., 2003). The chronic microsensor is also effective at detecting dopamine release to this type of stimulus. The CV corresponding to the food-evoked signal is significantly correlated with an electrically-evoked signal from the same animal ($r^2 \geq 0.75$; Fig 2.2), indicating that the behaviorally-evoked signal is reliably identified as dopamine by established signal identification methods (CV analysis). In addition, a comparison of CVs obtained *in vitro* and electrically and behaviorally evoked dopamine *in vivo* revealed a significant correlation for all observations ($r^2 \geq 0.75$). Importantly, this relationship was similar to that for acute electrode preparations.

To assess the longevity of dopamine detection with this approach, twenty microsensors were chronically implanted and repeatedly tested for their ability to detect behaviorally-evoked dopamine release, verified by CV analysis (Fig. 2.3). The voltammetric signal from four microsensors did not meet criterion for dopamine detection. The remaining sixteen microsensors were capable of detecting dopamine for a period ranging from one and a half to four months post-surgery. Glial scarring and debris from acutely implanting electrodes rendered animals unusable within a handful of recordings.

Discussion

The need for repeated, long-term measurements is crucial to the evaluation of learning, memory and neuropathological processes (Martin, Grimwood, & Morris, 2000; Tolias et al., 2007). This challenging objective is further complicated by the need for measurement on a physiologically relevant timescale. However, with the development of a chronically-implanted carbon-fiber microsensor coupled to FSCV, we attain this goal and demonstrate the measurement of behaviorally-evoked dopamine release with subsecond temporal resolution from day to day over months.

Presented in this thesis are aspects of the development and *in vivo* validation of these microsensors. In contrast to previous FSCV assemblies, the carbon fiber is encased in fused silica tubing overlaid with biocompatible polyimide polymer. Epoxy is used to seal the carbon fiber into place and a connector attached to the non-sensing end of the silica. After curing of all epoxy, the carbon fiber is trimmed to an appropriate length.

To determine viability of the microsensor for long-term neurochemical recording, we implanted the microsensors into the NAcc of a cohort of animals. Animals were routinely tested to determine sensitivity to dopamine transmission by either electrical stimulation or by presentation of an unexpected food reward. Analyte specificity, as established by correlation of cyclic voltammogram, demonstrated that dopamine could be detected in some cases up to four months

post-surgery. Thus, chronically implantable microsensors were able to detect dopamine from between weeks to months.

Though not part of this thesis, immunofluorescence studies demonstrated that activated microglia was present only along the shaft of the microsensor but not in the area surrounding the carbon-fiber. Nor was there glial encapsulation around the carbon-fiber that might have impeded dopamine diffusion to the microsensor. Sensitivity to dopamine was examined in vitro pre- and post-implantation. When compared to un-implanted microsensors, sensitivity was not significantly altered after one-, two- and four-month implantations. The behaviorally-evoked signal was further validated by demonstrating that it could be attenuated by inactivation of the VTA, the primary dopamine innervation to the NAcc. The diminutive size of the microsensor also allowed for multiple recording sites within an animal as well as implantation into smaller animals such as mice (Clark, et al., 2010).

Clearly, as a system that has been implicated in multiple functions (e.g. learning, decision making, movement), dopamine must have a certain flexibility and dynamism. With the advent of these microsensors, longitudinal studies may examine dynamic changes implicit in dopamine signaling. The maintained fidelity *in vitro* and *in vivo* demonstrates that microsensor sensitivity is stable over months after implantation, allowing for a more complete temporal description of dopamine's role in behaviors such as Pavlovian learning and, as

discussed in the ensuing chapter, changes in dopamine signaling due to behavioral history.

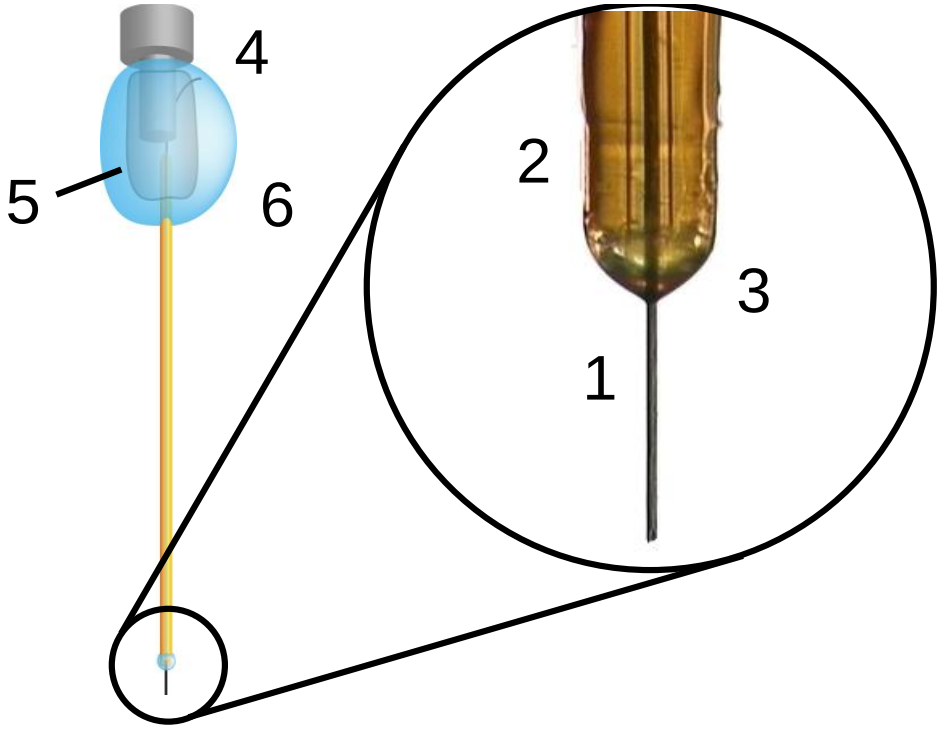

Figure 2.1 Construction of chronic voltammetric microsensors

All chronic microsensors used in these experiments consisted of a carbon fiber (1) encased in a polyimide fused silica (2). In order to ensure electric insulation, a two-component epoxy (3) was applied to the fused silica carbon fiber interface. At the opposite end, a female pin connector (4) was electrically connected to the carbon fiber with silver epoxy (5). Finally, two-component epoxy (6) was used to coat the connector for electrical insulation and structural integrity.

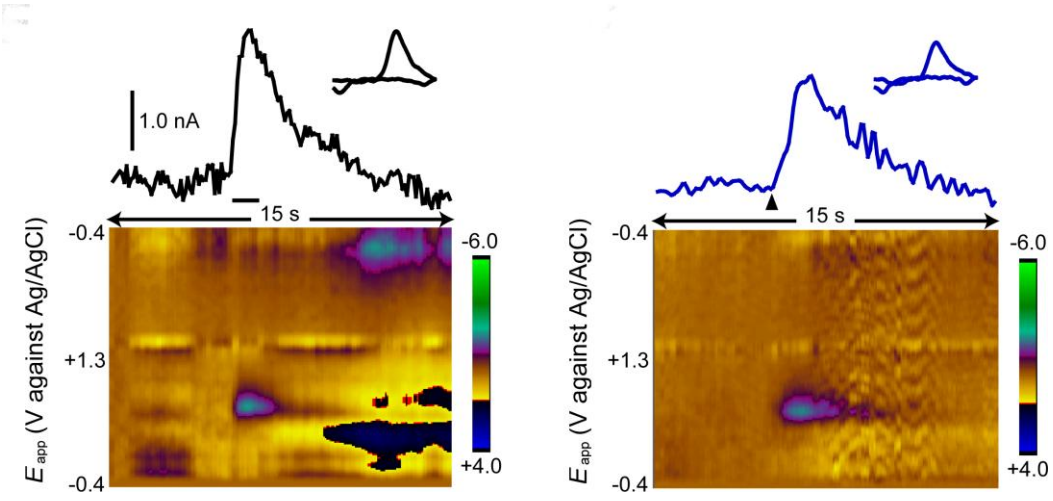

Fig 2.2 Stimulated and behaviorally evoked release of dopamine four months post-implantation

An stimulating electrode was lowered into the ventral tegmental area where electrical pulses (60 Hz, 24 pulses at 120 μA) elicited dopamine release at the recording location in the nucleus accumbens (left). The trace depicts electrically-evoked dopamine release recorded one month after surgical implantation and the corresponding background-subtracted cyclic voltammogram (inset). The trace shows a voltammetric signal in response to reward delivery in the same animal and on the same day as electrically-evoked release (right) and the corresponding background-subtracted cyclic voltammogram (inset).

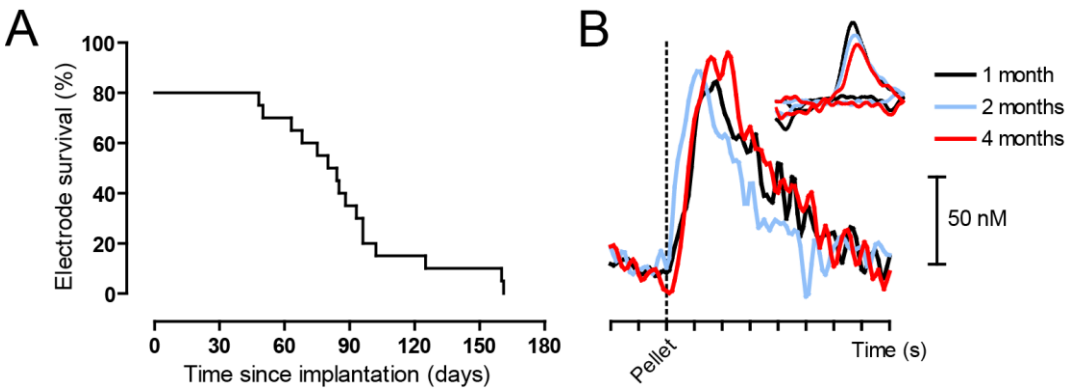

Figure 2.3 Microsensor longevity

(A) A survival curve depicting the attrition rate of chronic microsensors as a function of time since implantation. A microsensor was classified as "viable" if an electrochemical signal elicited by reward delivery was statistically consistent with a background subtracted cyclic voltammogram from electrically-evoked dopamine ($r^2 \geq 0.75$). (B) Voltammetric signals in response to the reward delivery one (black), two (blue) and four (red) months post-implantation. Background-subtracted cyclic voltammograms are all consistent with the electrochemical signature for dopamine (inset).

Part III: Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine

Summary

Reward-predicting cues evoke activity in midbrain dopamine neurons that encodes fundamental attributes of economic value, including reward magnitude, delay and uncertainty. We found that dopamine release in rat nucleus accumbens encodes anticipated benefits, but not effort-based response costs unless they are atypically low. This neural separation of costs and benefits indicates that mesolimbic dopamine scales with the value of pending rewards, but does not encode the net utility of the action to obtain them.

Introduction

For individuals to prosper in diverse environments, they need to use predictive sensory information to optimize outcomes in a flexible manner. Decision-making processes weigh the benefits of a reward with the cost of obtaining it to determine the overall subjective value (utility) of the transaction (Thorndike, 1933; Walton, Kennerley, Bannerman, Phillips, & Rushworth, 2006). Dopamine is a neural substrate that has been heavily implicated in this valuation process. Midbrain dopamine neurons encode fundamental economic parameters pertaining to predicted rewards (magnitude, probability, delay and uncertainty) in their firing rate (Fiorillo, Tobler, & Schultz, 2005; Kobayashi & Schultz, 2008; Morris, Nevet, Arkadir, Vaadia, & Bergman, 2006; Roesch, Calu, & Schoenbaum, 2007b) and innervate areas that have been implicated in economic decision-making (prefrontal cortex, amygdala, dorsal striatum and nucleus accumbens) (Floresco, Onge, Ghods-Sharifi, & Winstanley, 2008; Glimcher, Dorris, & Bayer, 2005; Knutson, Delgado, & Phillips, 2008). Moreover, dopamine in the nucleus accumbens core (NAcc) enables animals to respond to cues and overcome effortful response costs (Fields, et al., 2007; Salamone, et al., 2007b).

However, to fully understand decision-making computations encoded by the mesoaccumbens dopamine pathway, we need to deconstruct the nature of the valuation signal: specifically, how it accounts for changes in anticipated costs and benefits. We employed fast-scan cyclic voltammetry to record phasic

dopamine transmission in NAcc while rats performed decision-making tasks that independently manipulated either benefits or cost. Rats were trained to select between a reference option (16 lever presses for 1 food pellet) and an alternative that differed in either the reward magnitude (4 or 0 food pellets, benefit conditions) or response requirement (2 or 32 lever presses, cost conditions). Though several behavioral metrics indirectly address whether the changes in costs impart similar utility as changes in reward, we directly assessed this issue by asking the animals to choose between the high reward option (4 food pellets for 16 lever presses) and the low effort option (1 food pellet for 2 lever presses). Animals chose indifferently between the high reward and low effort options, inferring that animals consider them of equal value. Thus, we are able to treat changes in cost and changes in reward as they conferred the same amount of utility. We then compared dopamine transmission to cues that predicted different changes in benefit or changes in cost and found that though dopamine transmission faithfully scaled with predicted reward magnitude, dopamine transmission to predicted costs were either insensitive or became increasingly insensitive with more previous experience.

As we found a behavioral history dependence in animals that had experienced the alternative option less than nine sessions before the test session, an additional two sets of animals received extended training (>9 sessions of experience). Dopamine transmission still scaled with reward magnitude but no longer was sensitive to changes in predicted costs.

Methods

Animals

Twenty-seven naïve male Sprague-Dawley rats (Charles River, CA, 3-8 months old during testing) were used for this experiment. Sixteen animals contributed to the data reported here while the others failed to meet criterion for dopamine detection (see *Recording Sessions* for details). Animals were maintained on a twelve-hour light/dark cycle (lights on 0700) and were group housed during initial habituation and training but individually housed following surgery. All testing was carried out during the light phase. During the training and testing periods, access to food was restricted to a total of ~12-16 g per day, consisting of the reward pellets gained during testing supplemented by lab chow given at the end of the day, such that rats' weights were kept at 85-90% of their free-feeding weight. Water was available *ad libitum* while animals were in their home cages. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

Behavioral training

Testing was carried out in operant chambers (30.5 x 24.1 x 29.2 cm; Med Associates, VT, USA) with sloped inserts between the floor and walls (63° towards the levers and magazine, and the back wall, 52° towards the sides). Each chamber was housed within a custom-built sound- attenuating cabinet ventilated with a fan. Each chamber was fitted with two retractable levers on either side of

an extra-tall food magazine into which 45-mg food pellets (Bioserv, NJ, USA) could be dispensed. Above each lever was a stimulus light, which could act as a visual cue, and the chamber could be illuminated by a 2.8-W house light located at the top of the wall opposite the levers and food magazine. The food magazine was fitted with an infrared beam that could signal when animals entered the receptacle and could also be illuminated by an internal light.

Habituation and training were comparable to that performed in previous studies of operant cost-benefit decision making[1,2]. In brief, following initial habituation to the chambers, rats experienced a 60-minute session in which a single reward, cued by the magazine light, was dispensed under a variable interval schedule (every 40-80s with a 60s mean). On the following sessions, animals were trained to lever press for reward on a fixed ratio (FR) 1 schedule. The house light was illuminated throughout, and either the left or right lever (counterbalanced across animals) was extended and its associated cue light illuminated throughout the session. To facilitate responding in some animals, a few food pellets were placed behind the extended lever such that their odor was evident but the pellets themselves were unobtainable.

Once animals reliably responded on both levers, the paradigm was changed so completing the response requirement caused the lever to retract and the associated cue light to extinguish. At the same time, reward was delivered and the magazine-light was illuminated. Six seconds after food delivery, the magazine-light was extinguished and the intertrial-interval (ITI) began. The start

of a subsequent trial was signaled by illumination of one of the two cue lights and simultaneous extension the associated lever. In these "forced" trials (where only one of the two response options was available), the response cost was increased on each lever across sessions up to a maximum of sixteen lever presses for a single pellet. This response cost (16 lever presses) and reward (1 pellet) is subsequently referred to throughout as the "reference" option. Once animals responded on both levers with the reference response requirement across 80 trial sessions, they subsequently underwent surgery to allow for *in vivo* voltammetric recording.

Following recovery from surgery, rats were reintroduced to the behavioral task described above. Once pre-surgery levels of performance were achieved, the animals were introduced to new contingencies where the benefit or the cost was altered from the reference (16 lever presses for 1 food pellet). These contingencies consisted of four or zero food pellets for sixteen lever presses (benefit manipulations) or one food pellet for two or thirty-two lever presses (cost manipulations). In each session, the altered contingency was assigned to one lever with the reference assigned to the other and remained fixed for the entire session. To avoid side-biased habit formation, the lever assigned to the high-value option was reversed at the start of each session.

Decision-making sessions

Reference and alternative options were presented independently in "forced" trials

or concurrently in “choice” trials. Forced trials ensured that the animal experienced both the preferred and non-preferred contingencies throughout the session while choice trials permitted assessment of the animal’s subjective preference. Sessions were comprised of repeating blocks of four forced trials (each option presented twice in pseudo-random order) followed by four choice trials (Fig 3.1).

The start of each trial (forced or choice) was signaled by the illumination of the house light, presentation of the lever(s) and illumination of the associated cue light(s). During choice trials, the first lever press caused the other lever to retract and its cue light to extinguish, eliminating the unselected option for that trial. Completion of the response requirement on the selected lever resulted in reward delivery. At this time, the lever was retracted, the cue light was extinguished, the magazine light was illuminated, and the appropriate reward magnitude was delivered to the magazine. After six seconds, the house and magazine lights were extinguished and an inter-trial interval commenced. The inter-trial interval was sixty seconds minus the time taken to complete the response requirement for the completed trial, ensuring that the overall rate of reward delivery throughout the session was independent of choice and response rates. If animals did not make a lever-press response within ten seconds from the start of a trial, all lights were extinguished for a “time out” of sixty seconds.

On each session animals learn the assignment of the contingencies to the levers, as evidenced by development of a preference for one lever during choice

trials. Preference is inferred when a behavioral criterion was reached, defined as choosing one option ≥75% of the last twelve choice trials. For example, an animal reached the behavioral criterion when it chooses 4 pellets over 1 pellet in nine out of the last twelve choice trials. Decision-making sessions continued for 6-8 blocks after animals reached this criterion or a maximum of 120 trials. No additional training was provided to teach the animals to choose between the alternatives. However, for each condition, all animals completed at least two (side-counterbalanced) decision-making sessions to criterion while tethered to the voltammetry recording equipment prior to the first session of voltammetric data acquisition.

To prevent our results from being influenced by the order of testing, half of the animals started by performing a benefit condition and the other half, a cost condition. The order (alternative option = higher/lower utility than the reference option) and side (reference option = left or right lever) of the cost-benefit contingencies was counterbalanced across animals.

Test of utility equivalence between the high-benefit and low-cost contingencies

Both the high-benefit (4 pellets for 16 presses) and low-cost (1 pellet for 2 presses) options were preferred over the reference option (1 pellet for 16 presses) (see Results). However, these data do not tell us the relative utility of these options compared to each other. To test whether the utility conferred by the increased benefit was equivalent to that conferred by the decreased cost, eight

rats took part in further cost-benefit behavioral experiments where the high-benefit and low-cost options were compared directly. The high-benefit and low-cost contingencies were assigned to the left and right levers counterbalanced across animals for a first session and reversed on a second session. During these sessions, animals were tethered to the voltammetry recording equipment during testing to mimic the conditions during recording sessions, although electrochemical data were not acquired.

Assignment of a behavioral criterion to assess a learned preference for one option was not pertinent in this experiment because it was reasonable that a strong preference to one contingency would not prevail. Therefore, animals were pre-trained with 16 forced trials (8 for each contingency) to provide experience with the pairing comparable to that for the pre-criterion trials of a decision-making session where one contingency is paired with the reference option. Thirty minutes after pre-training, animals were tested in a session consisting of blocks of trials similar to those previously described, up to a maximum of 56 trials. Animals were tested on this utility equivalence experiment after either ≤9 training sessions (n=5) or extended training of >9

sessions (n=5) of experience with the high-benefit or low-cost contingencies (in separate sessions paired with the reference option).

Surgical procedures

Following habituation and initial operant training, animals underwent surgical preparation for in vivo voltammetry using an aseptic technique, following the University of Washington Institutional Animal Care and Use Committee guidelines. All rats were anesthetized with ~5% isoflurane and maintained during surgery with ~2-3%isoflurane. They were placed in a stereotaxic frame, the scalp was swabbed with 10% iodine, bathed with a mixture of lidocaine (0.5 mg/kg) and bupivicaine (0.5 mg/kg), and an incision with made over the midline to expose the cranium. After the head was leveled between bregma and lambda, holes were drilled for 3 anchor screws and a reference electrode, along with 2 others bilaterally above the NAcc (at +1.3 mm anterior and ±1.3 mm lateral to bregma). The NAcc was targeted (rather than the adjacent shell region) as this has been suggested to be the critical site where dopamine allows animals to overcome effort constraints. In-house constructed carbon fiber microsensors for long-term chronic recordings were lowered into position (+6.8-7.0 mm ventral to dura), and these, along with an Ag/AgCl reference electrode, were attached to a voltammetric amplifier. Voltammetric components along with a headpost were secured with cranioplastic cement. Rats were given an injection of 5mg/kg carprofen mixed in with 3ml ringer's solution immediately following surgery and again 12 hours later. The animals were allowed between 7-14 days to

recover with food and water freely available before being food deprived again prior to further behavioral training and testing.

Recording sessions

During experimental recording sessions, the chronically-implanted carbon-fiber microsensors were connected to a head-mounted voltammetric amplifier for dopamine detection by fast-scan cyclic voltammetry as described in the previous chapter[5]. In brief, the potential applied to the carbon fiber was ramped from -0.4 V (vs Ag/AgCl) to +1.3 V and back at a rate of 400 V/s during a voltammetric scan and held at -0.4 V between scans. Scans were repeated at a frequency of 10 Hz throughout the session. The application of this triangular waveform causes redox reactions in electrochemically active species at the carbon fiber (including dopamine: ~+0.7 V and -0.3 V peak oxidation and reduction potentials respectively) that can be measured as changes in current. Signals were background subtracted by subtracting the average current in the second prior to trial initiation and cue onset from the signal (Schultz, 2007).

To ensure that recording electrodes were able to reliably detect behaviorally-evoked dopamine, we measured the neurochemical response to a food pellet delivered to the magazine without forewarning at the start and end of each session. This procedure has been shown to consistently increase burst firing in midbrain dopaminergic neurons (Garris, Collins, Jones, & Wightman, 1993) and also to elicit dopamine release in the nucleus accumbens (Phillips &

Wightman, 2003; Schultz, 2007) (Fig 3.2). The inclusion criterion for neurochemical recording sessions was electrochemically verifiable dopamine release for unexpected food-pellet delivery both before and after the session. This verification was achieved by obtaining high correlation of the cyclic voltammogram (electrochemical signature) to that of a dopamine standard (correlation coefficient $r^2 \geq 0.75$ by linear regression). The only other analyte known to closely approximate the chemical signature of dopamine is norepinephrine. However, the norepinephrine tissue content in the NAcc is only 2-20% of that for dopamine (Tobler, et al., 2005; Walton et al., 2009) and electrode sensitivity to norepinephrine is approximately half of its sensitivity to dopamine (Roitman, et al., 2004). Furthermore, in genetic dopamine-deficient mice that have a fully intact norepinephrine system, no catecholamine release is detected in slices of NAcc by fast-scan cyclic voltammetry following intense local stimulation, using detection parameters identical to those in the current work (Morris, et al., 2006). Therefore, it highly unlikely that norepinephrine contributes to any signals observed in the current experiment.

Data analysis

Animals included in the study contributed two side-counterbalanced recording sessions for a given cost-benefit contingency (e.g., 4 pellets assigned to left lever, 1 pellet assigned to right lever in one session, and 4 pellets assignment to right lever, 1 pellet assigned to left lever in another). These sessions were

treated as a within-subjects repeated measure. All other factors were treated as between-subjects measures, even though in seven rats, the same animals contributed to the data from separate cost-benefit contingencies. Analysis of extracellular dopamine concentration was restricted to the period of 2 seconds following cue onset, prior to reward delivery, on postcriterion forced trials. Dopamine signals on trials where no lever-press response was made within the 10 second response window were excluded to ensure that the data only reflected trials where animals had perceived the cues.

Voltammetric data analysis was carried out using software written in LabVIEW. Electrochemical signals were low-pass filtered at 2,000Hz. Individual cyclic voltammograms (electrochemical current-voltage plots) were used for chemical identification. The current at the peak dopamine oxidation potential across successive voltammograms was used for dopamine quantification. Any noise spikes of >±1.5 nA greater than the signal in both 100ms time-bins before and after the time point were manually removed, and the data were smoothed using a 0.5-s moving average.

Estimation of dopamine concentration

The main statistical tests in this work were within-session comparisons and so are unaffected by determination of the absolute concentration of dopamine. Nonetheless, it is more intuitive to present these data as estimated dopamine concentrations rather than raw voltammetric currents. For histological

verification of recording sites, electrolytic lesions were made via the recording electrode as described above. This procedure renders electrodes unsuitable for post-implantation assessment of sensitivity. Thus, electrode sensitivity was estimated by extrapolation from a cohort of electrodes (matched to background current) through which a lesion was not made. Control electrodes (n=15) were implanted for an equivalent period to experimental electrodes and underwent post-implantation assessment of sensitivity *in vitro*. Electrode background currents generated during recording sessions were used to verify comparability to those obtained during electrode calibration. Notably, conversion to dopamine concentration did not change any of the reported effects, either within or between sessions.

Histology

Following completion of the experimental sessions, animals were anaesthetized with ketamine/xylazine (100 mg/kg) and the recording site was marked by making a small electrolytic lesion at the electrode tip by passing a current (~70µA) through the carbon fiber microsensor for twenty seconds. Animals were subsequently perfused transcardially with physiological saline and then with 4% paraformaldehyde in phosphate-buffered saline, before the brains were removed and post-fixed in a paraformaldehyde solution. The brains were then placed in 30% sucrose solution in phosphate-buffered saline for 48 h, flash

frozen, and sectioned coronally (30 μm). All sections were mounted and stained with cresyl violet.

Results

Behavioral results under standard training protocols

Three behavioral metrics were analyzed from recording sessions: (i) number of trials to criterion, (ii) post-criterion choice allocation and (iii) response latencies on post-criterion forced trials. All three measures demonstrated that animals reliably preferred the option with greater benefits or lower cost in each condition. There was no significant difference in the number of trials to behavioral criterion between the two cost conditions or the high-benefit condition (Mann- Whitney test: all comparisons $p>0.3$, n=10-12 sessions; Fig 3.3a). However, rats took significantly fewer trials to reach criterion when the reward was reduced to zero ($p<0.05$ versus other conditions, n=10; Fig 3.3a). All animals continued to allocate their choices preferentially to the option with the higher benefit or lower cost in post-criterion trials (Fig 3.3b). There was no difference in choice performance between either cost condition or the high-benefit condition but the preference was strongest in the low-benefit condition (main effect of group: $F_{3,17}=5.37$, $p=0.01$; post-hoc tests, $p<0.05$ for all comparisons of lower benefit session with the other sessions; all other comparisons $p>0.16$). Choice performance can also be reliably indexed by reaction times on forced trials (Walton, et al., 2006). Post-criterion, rats were significantly faster to select the higher benefit or lower cost option in all conditions ($F_{1,17}=52.75$, $p<0.001$), though this difference was again particularly marked when reward on the alternative was reduced to zero ($F_{3,17}=5.22$, $p=0.01$)

with animals responding significantly slower to the cue predicting zero rewards ($p<0.01$; Fig 3.3c). Based on these three behavioral criteria, we conclude that the utility of reward options were modulated in both benefit and cost conditions (i.e. increased utility conferred to the option with greater benefit or lower cost).

Test of utility equivalence between high-benefit and low-cost contingencies

As demonstrated above, increased utility of a reward was conferred by increasing the benefit (number of food pellets) or decreasing the response cost (number of lever presses). These manipulations altered behavior in a comparable manner as assessed by learning rate, response latency and choice when presented concurrently with a reference reward. To test directly whether high-benefit and low-cost conditions yield equivalent utility, these two conditions were compared directly in a behavioral experiment. Animals that had ≤9 sessions of training in all conditions chose the higher-benefit at roughly the same rate as the lower-cost ($p=0.71$, Fig 3.4a). Similarly, extensively trained animals still chose either option at the same rate ($p=0.76$; Fig 3.4b). This indifference of choice leads us to believe that regardless of animals' experience and by extension, lever-pressing aptitude, the utility conferred by the two manipulations are approximately equal. Therefore, different patterns of dopamine release between the high-benefit and low-cost conditions are not a result of differences in conferred utility.

Dopamine responses to cues after standard training

Despite predictable behavior, cue-evoked NAcc dopamine release did not track utility under all conditions. Manipulating reward magnitude led to a corresponding increase (main effect of reward size, $F_{1,5}$ = 15.61, P = 0.01) or decrease ($F_{1,4}$ = 19.88, P = 0.01) in cue-evoked dopamine compared with the reference option (Fig 3.5). Manipulations of response cost, on the other hand, did not always alter dopamine release. When the response cost of the alternative was increased, there was no difference in dopamine release between the reference and alternative option (main effect of response cost, $F_{1,4}$ = 0.05, P = 0.84; Fig 3.5), despite the strong behavioral preference for the reference option. When the response cost was reduced, there was greater dopamine release to the low-cost cue than to the reference ($F_{1,4}$ = 25.38, P = 0.007), but this was only significant in the first of two counterbalanced sessions in each rat (session × option interaction, P = 0.03, $F_{1,4}$ = 10.92; Fig. 3.6). *Post hoc* tests indicated that this effect was driven by a reduction in dopamine release to the low-cost cue (P = 0.0006), but not the reference cue (P = 0.20), across sessions.

Effect of behavioral history on dopamine

To further investigate across-session effects, we performed regression analysis between utility encoding and experience with any alternative contingency before recording. Experience-related changes in cue-evoked

dopamine release were only observed in the reduced-cost condition, in which the preferential dopamine release for the low-cost cue diminished over time (Pearson's $r = –0.830$, $P = 0.005$, $n = 9$; Spearman's rho = –0.817, $P = 0.007$; Fig 3.7). Additional experimentation with a cohort of rats that were given more experience (>9 sessions) with the high-benefit option before recording verified that both behavioral preference and preferential encoding of the higher benefits was maintained with extended training ($P = 0.007$, $t = 4.08$, degrees of freedom = 6, $n = 7$ session; Fig 3.8a). Conversely, in a parallel experiment with the low-cost option, cue-evoked dopamine release did not preferentially encode the low-cost option after additional experience before recording ($P = 0.16$, $t = 1.55$, degrees of freedom = 8, $n = 9$ sessions), even though behavioral preference was preserved (Fig 3.8b). These data are consistent with the notion that, although preferential encoding of high benefit by dopamine release is stable over training, low costs are only preferentially encoded early in training.

Cue- versus response-evoked dopamine

There are several lines of evidence that negate the potential confound that dopamine release may be elicited by approach behavior rather than directly by the cue. First, there is temporal dissociation between the response latency and peak dopamine release, with responses for high-utility options at the dopamine peak, but responses for low-utility options arising after the dopamine peak. Second, peak dopamine concentration did not significantly correlate with

response latency (Fig. 3.9). Third, the dopamine signal is strongly time-locked to cue onset rather than the time of the initial response (Fig 3.9). Taken together, these data strongly suggest that dopamine evoked by the onset of cues was not altered by variations in responding in the different conditions.

Dopamine responses to delivery of food reward

Dopaminergic signaling to the onset of food delivery to animals with standard training was also examined (Fig 3.10). Prior to the animals reaching the pre-established behavioral criterion, dopamine transmission to reward receipt reflected the unexpected magnitude of the outcome, with peak dopamine signals significantly larger when animals received four food pellets than the reference reward of one pellet. These prediction error signals to receipt of the high reward (4 or 1 food pellets) were significantly reduced once animals had a stable preference for that option. By contrast, there was no consistent relationship between dopamine transmission at cue onset and animals' preference towards options requiring lower effort and no comparable prediction error signals at the time of reward delivery dependent on the effort requirement.

Contextual framing

To test whether the reference option was regarded differently based upon context (Roesch, et al., 2007b), dopamine release to the reference cue on post-criterion forced trials across all conditions were compared. Regardless of whether

the reference option conferred higher or lower utility, presentation of the cue predicting the reference elicited similar release of dopamine (main effect of condition: $F_{3,19}$=0.369, p=0.78; interaction between session x condition: $F_{3,19}$=0.203, p=0.89).

Forced versus choice trials

While the focus of our investigation was on post-criterion forced trials, voltammetric data were also recorded (i) on choice trials and (ii) while the animal was learning to choose between the cost-benefit contingencies. When comparing the peak amount of dopamine on the post- criterion choice trials (where the high utility option was subsequently chosen) against the peak dopamine on high utility forced trials, there was no statistical difference between the cue-evoked dopamine on forced and choice trials (main effect of trial type or interaction between trial type and group: $F<2.3$, $p>0.15$; Fig. 3.11). There were too few post-criterion low net value choice trials to gain a reliable estimate of changes in dopamine concentration. For the few trials in which the low utility option was chosen, the animal's motivation is unclear. Though the animal could have consciously chosen the low utility option, it is as likely that the animal made a mistake in his choice. So, while this rules out that cue-evoked dopamine release reflects the average value of all available options, this data set cannot arbitrate between models which advocate that dopamine signals the value of the chosen option or the highest available value option (Morris, et al., 2006; Roesch, et al.,

2007b).

Histology

The majority of recording locations were in the medial NAcc (Fig 3.12). The electrode for one animal was in the adjacent ventromedial shell and for another was on the boundary of the core and the shell, and both were therefore removed from the analyses. Nonetheless, their voltammetric data was similar to those from the NAcc and so their removal did not markedly alter the pattern of results described in the main text (data not shown).

Discussion

In making sound economic choices, one must consider a reasonable cost to obtain an outcome based on its perceived benefit. The mesolimbic dopamine system has been implicated in this type of decision making but understanding its role in the process has been hampered by an inability to characterize phasic dopamine while animals are awake and behaving. With the chronically implantable microsensors described in the previous chapter, we were able to longitudinally assess dopamine transmission while animals chose between options that differed in reward magnitude or effort required to attain the reward.

Animals developed a behavioral preference for the option that resulted in either more food reward (HR) or required less effort (LE) to attain. From this preference, we inferred that the HR/LE option had greater utility to the animal. The utility conferred by increasing the amount of food reward or lowering the effort requirement was equal as animals chose indifferently when empirically compared. But when we examined the phasic dopamine response to cues that predicted the rewards, we found that phasic NAcc dopamine transmission reliably reflected the magnitude of the benefit, but only correlated with effort-discounted utility in situations where the response cost was both novel and better than the reference. When animals experienced a standard training regimen (<9 sessions of experience with a particular contingency), dopamine responses to cues monotonically increased as reward magnitude increased. Cues predicting

greater than reference amount of effort did not elicit more dopamine than the cue predicting the reference contingency but cues predicting an atypically low amount of effort did elicit a greater amount of dopamine. This lack of encoding of upcoming response costs by NAcc phasic dopamine have also recently been observed in a more dynamic, progressive ratio paradigm where responses costs escalate as a function of the animals' past choices (Wanat, Kuhnen, & Phillips, 2010). After extended training (>9 sessions experience with each contingency) with the atypically low effort contingency, though, cues predicting low effort no longer elicited greater dopamine response than the reference. Also, while seldom representing the anticipated costs of a course of action at cue onset, phasic dopamine transmission did not signal cost prediction errors envisioned by learning models. Thus, we show dissociation between dopaminergic encoding of anticipated costs and benefits, demonstrating that, while dopamine release in the nucleus accumbens scales with the value of a pending reward, it is not sufficient to describe the net utility of the action to obtain it.

That dopamine transmission in the NAcc does not reflect net utility expands our understanding of dopamine function. Incorporating these findings with previous studies showing that dopamine enables effortful responses, we reason that representation of reward magnitude by phasic dopamine provides a threshold to determine worthwhile cost expenditures in familiar situations (Fields, et al., 2007; Phillips, Walton, & Jhou, 2007; Salamone, et al., 2007b). Moreover, in novel situations dopamine provides an additional opportunistic

mechanism for exploitation of low-cost rewards that become available unexpectedly (Phillips, et al., 2007; Redgrave & Gurney, 2006).

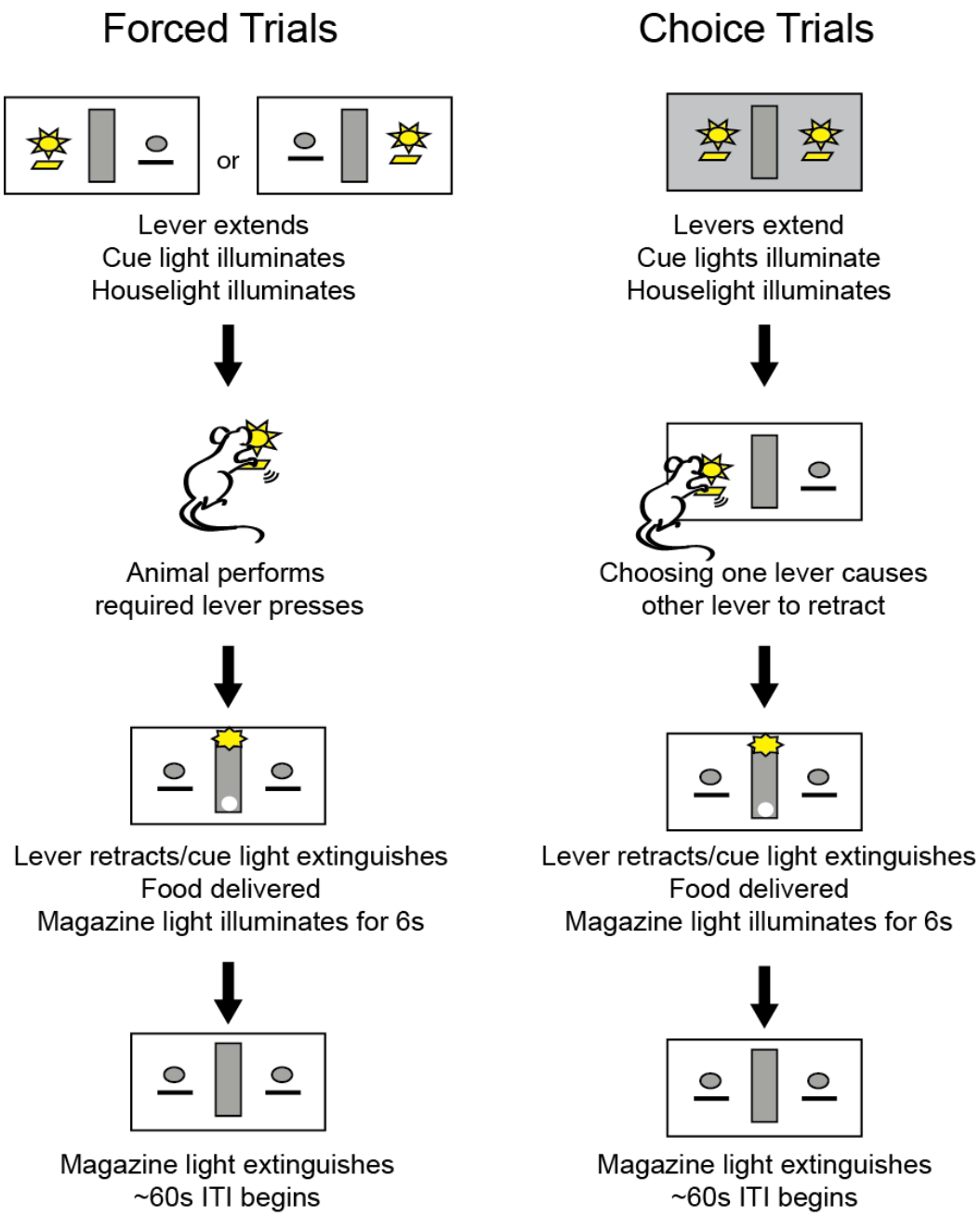

Fig 3.1 Economic decision making task

Schematic of a forced (left hand panel) or choice trial (right hand panel).

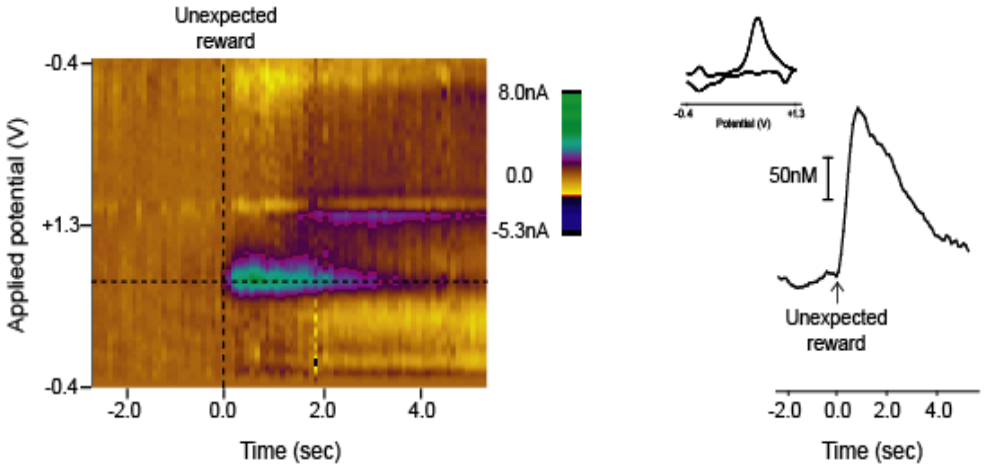

Figure 3.2 Example response following delivery of an unexpected food reward.

Left-hand panel shows the background-subtracted recorded current change time-locked to delivery of the reward. Color plot is a two-dimensional representation of a series of cyclic voltammograms across time. Dopamine oxidation is visualized as green peaks at the bottom third of the color plot with corresponding (though smaller) reduction visualized by yellow peaks at the top. Right-hand panel shows change in oxidative currents over time at the peak sensitivity to dopamine for this electrode (+0.71 V), converted to dopamine concentration using the calibration factor. The inset panel is the background subtracted cyclic voltammogram for this response (current versus applied potential) taken 0.8 s after reward delivery, which is consistent with the electrochemical signature for dopamine (r^2=0.95).

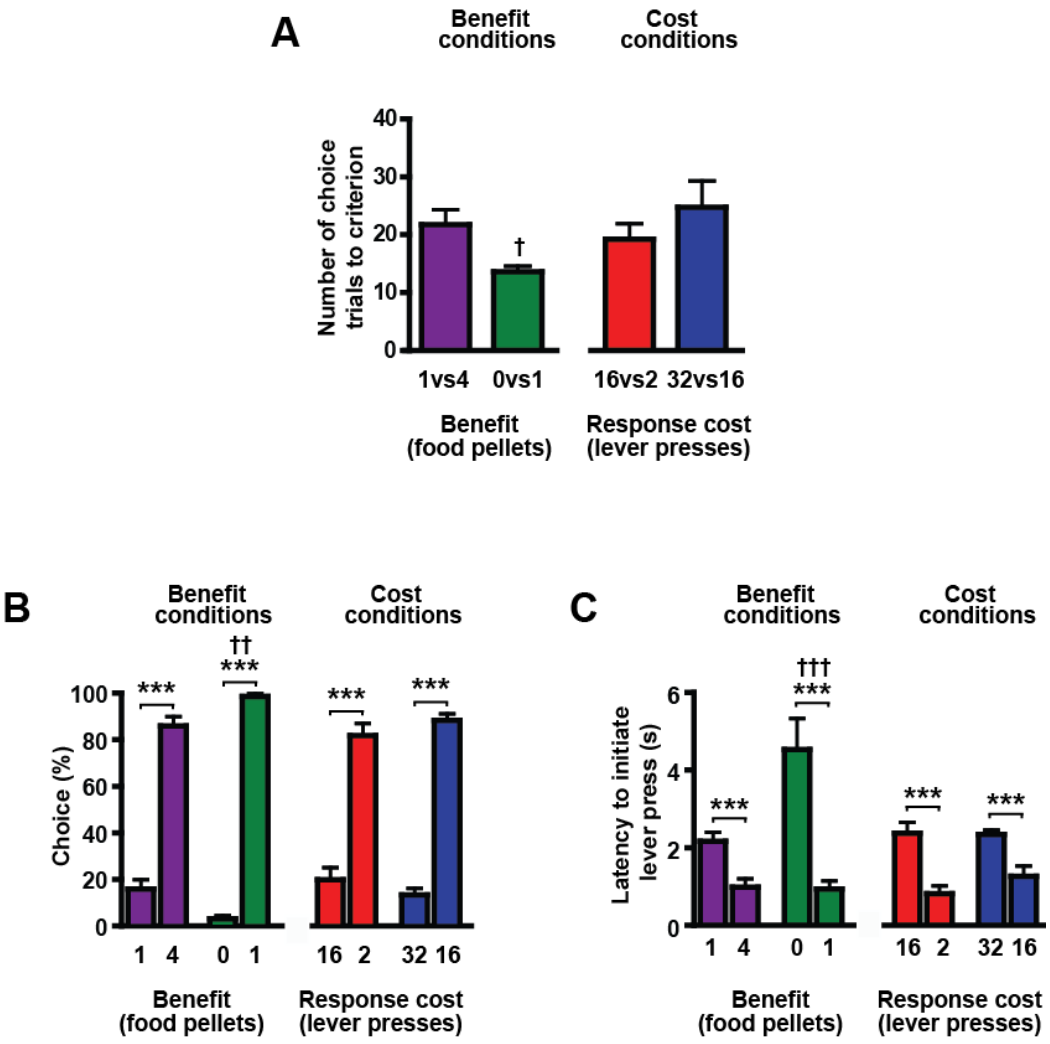

Fig 3.3 Behavioral results

(A) Number of choice trials required to attain the behavioral criterion. (B) Post-criterion choice behavior. (C) Latency to make an initial response on post-criterion forced trials. Data are mean ± s.e.m. * $P < 0.05$, *** $P < 0.0001$.

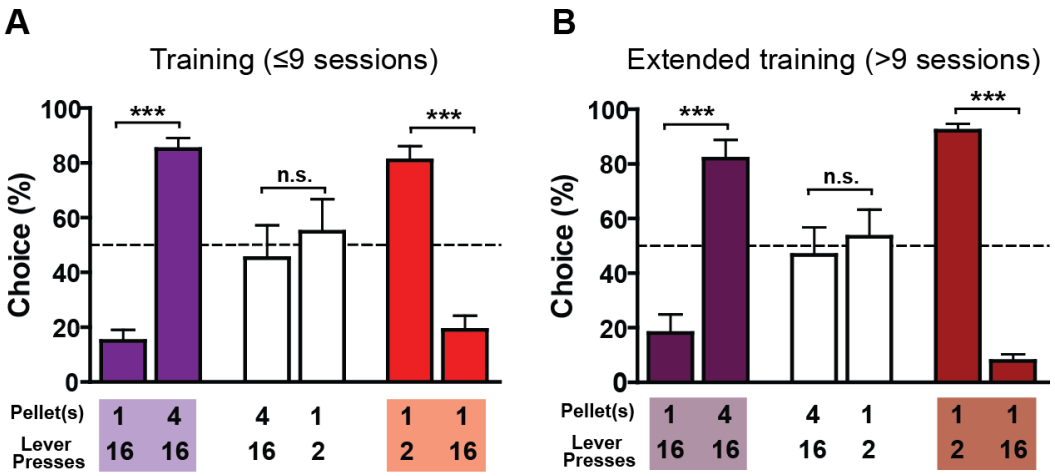

Fig 3.4 Utility equivalence between high-benefit and low-cost contingencies

(A) Choice behavior following training with ≤9 previous exposures to the contingencies. Bars represent percentage of choices allocated to: reference option vs higher reward (n=6), higher reward vs lower effort (n=5), lower effort vs reference effort (n=5). (B) Choice behavior following "extended" training regime which consisted of >9 previous exposures to one of the contingencies. Bars represent percentage of choices allocated to: reference option vs higher reward (n=3), higher reward vs lower effort (n=5), lower effort vs reference effort (n=4). (n.s., not significant; ***, $p<0.0001$).

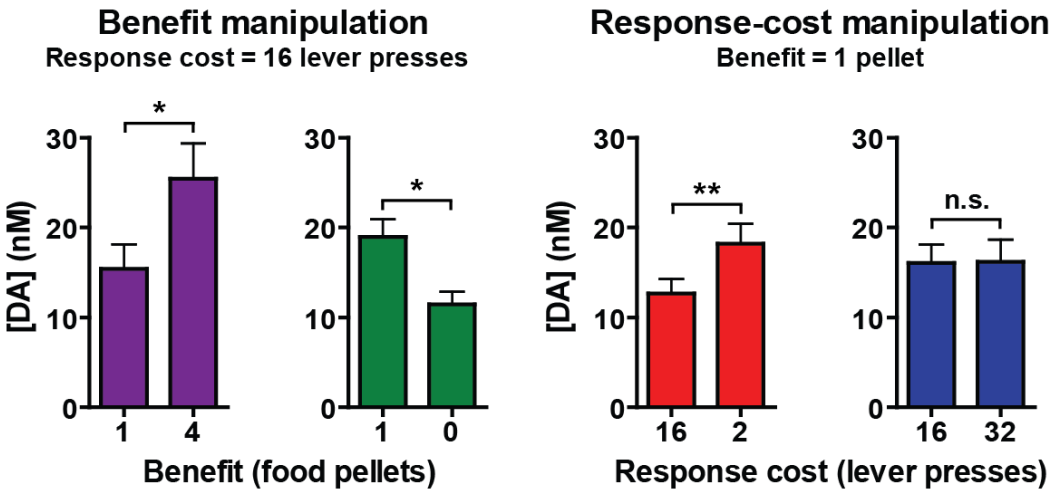

Fig 3.5 Post-criterion dopamine transmission to reward and effort predicting cues

Post-criterion cue-evoked dopamine release across sessions in benefit and cost conditions. Data are mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$. DA, dopamine.

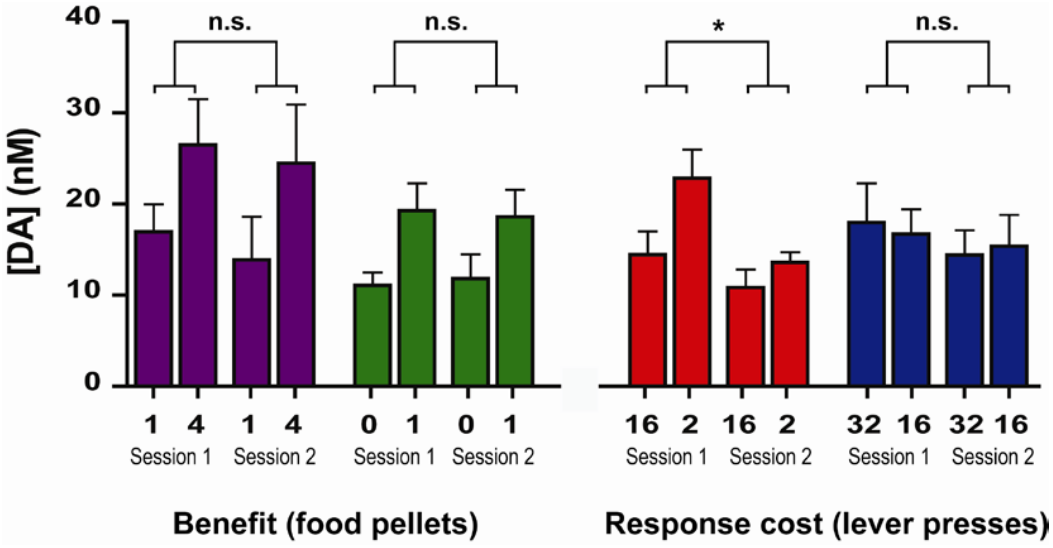

Fig 3.6 Dopamine transmission to cues by session

Average cue-evoked peak dopamine for the first and second of two contingency-counterbalanced sessions. (n.s., not significant; *, $p<0.05$).

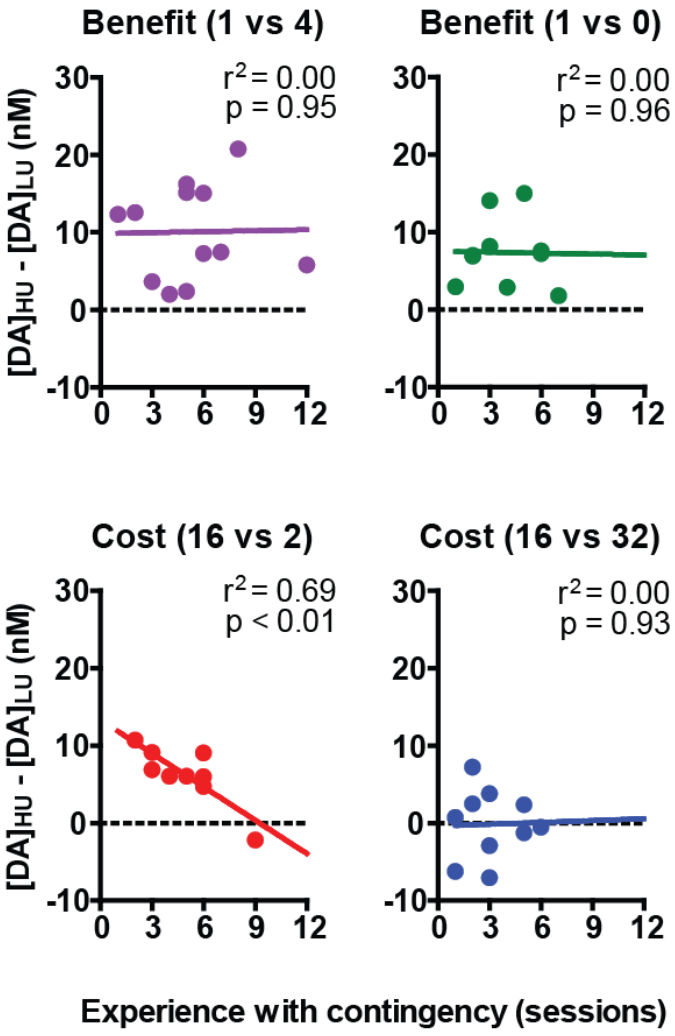

Fig 3.7 History dependence

Differences in cue-evoked dopamine release between the high- and low-utility options ($[DA]_{HU} - [DA]_{LU}$) against behavioral history.

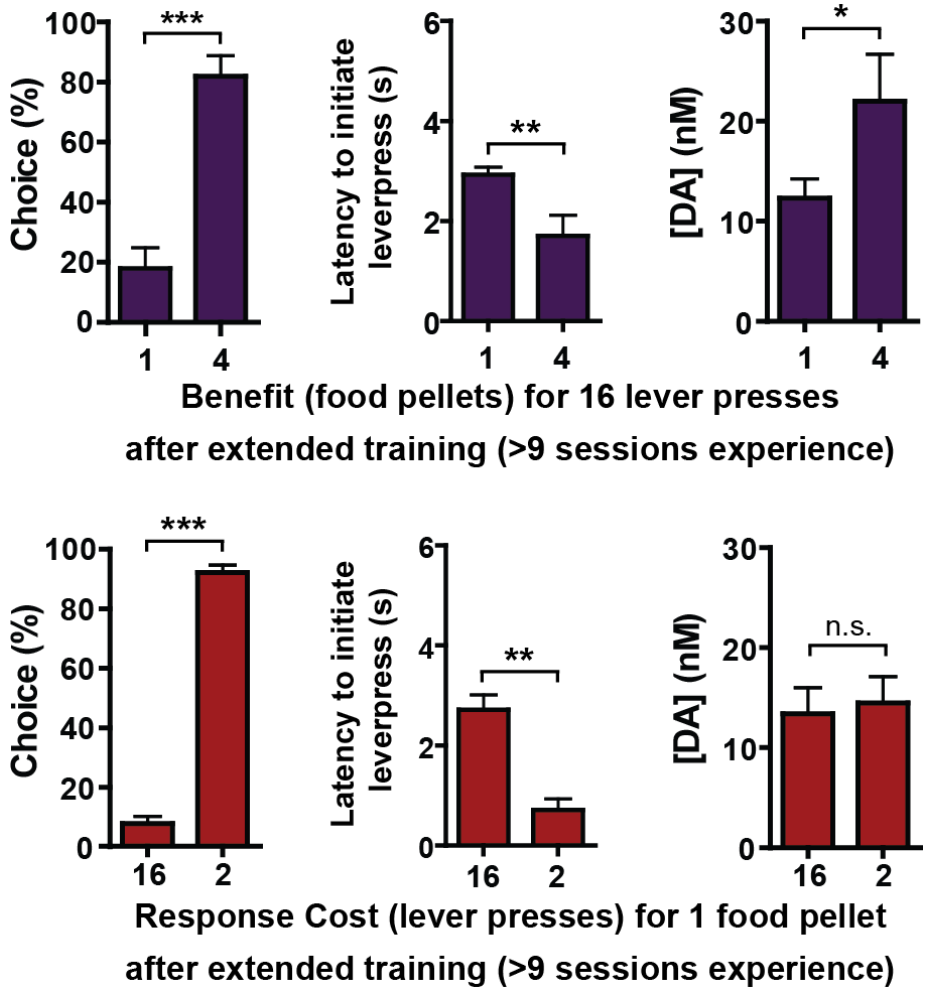

Fig 3.8 Behavior and dopamine to cues after extended training

Post-criterion choice behavior, latency to initiate lever press, and cue-evoked dopamine release for the high-benefit or low-cost option in rats given extended training (>9 sessions) with either contingency before testing. Data are mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$.

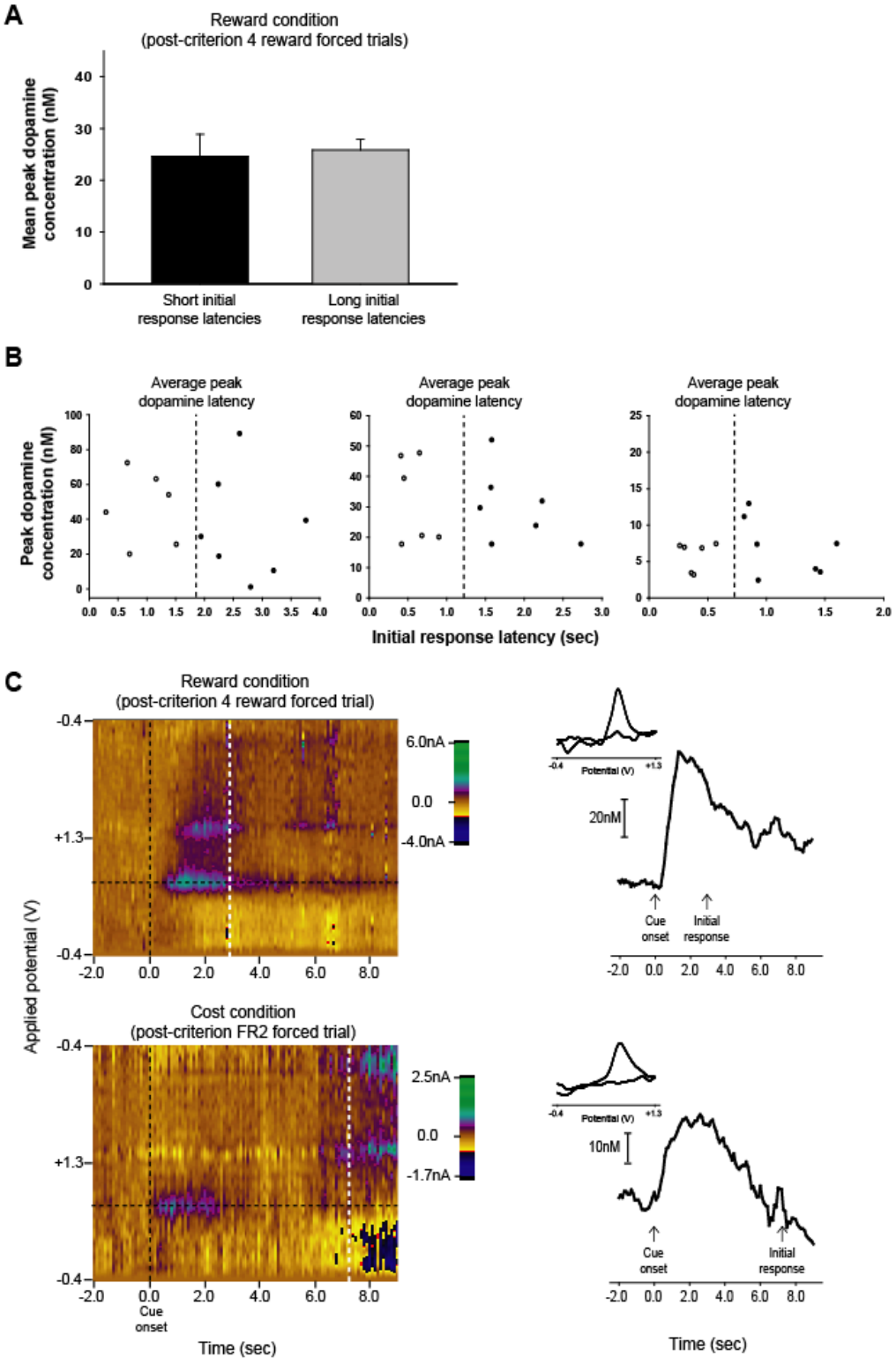
A
Reward condition
(post-criterion 4 reward forced trials)
Mean peak dopamine concentration (nM)
40
30
20
10
0
Short initial response latencies
Long initial response latencies
B
Average peak dopamine latency
Average peak dopamine latency
Average peak dopamine latency
Peak dopamine concentration (nM)
Initial response latency (sec)
C
Reward condition
(post-criterion 4 reward forced trial)
6.0nA
0.0
-4.0nA
Potential (V)
20nM
Cue onset
Initial response
Cost condition
(post-criterion FR2 forced trial)
2.5nA
0.0
-1.7nA
10nM
Applied potential (V)
Time (sec)

Fig 3.9 Dopamine response to cues versus movement

(A) Average increase in dopamine concentration to high utility cue on post-criterion trials with fast (<1.2 s, i.e., faster than the average time for dopamine release to peak) and slow (>1.5 s; i.e., slower than the average time for dopamine release to peak) initial response latencies in the 4 versus 1 food pellet condition. (B) Trial-by-trial comparison of high and low response latency in forced trials (dashed line represents the average time for dopamine release to peak) from three sessions where a similar number of trials fell before and after the average time for dopamine release to peak (n=3). (C) Two example long-latency post-criterion high utility forced trials: 4 versus 1 benefit condition (upper panel), 2 versus 16 cost condition (lower panel). Left-hand panels (color plots) represent background-subtracted recorded current change time-locked to cue onset (0 s; vertical black dashed line). Time of initial lever response is marked with a vertical dashed white line. Right-hand panels show change in dopamine concentration in these trials (at horizontal dashed black line in left-hand panels). Inset is the cyclic voltammogram taken 0.8 s after cue presentation for these cue-evoked signals, both of which are highly correlated with a dopamine template (r^2>0.90).

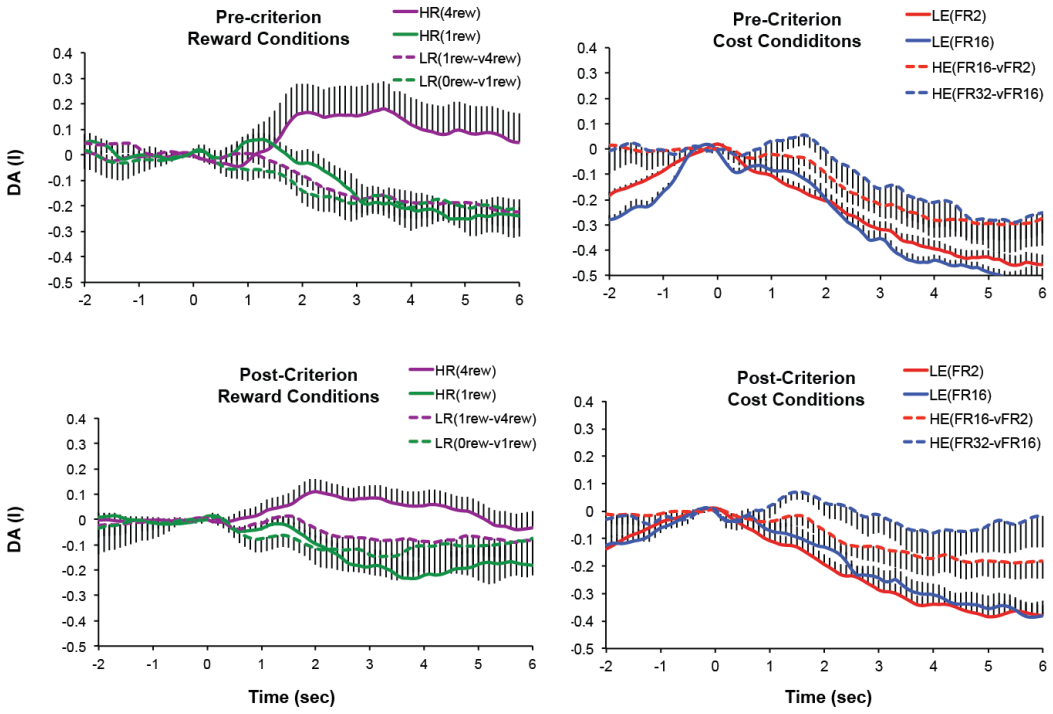

Figure 3.10 Prediction Errors

Dopamine transmission at the delivery of reward for benefit (left) and cost (right) conditions.

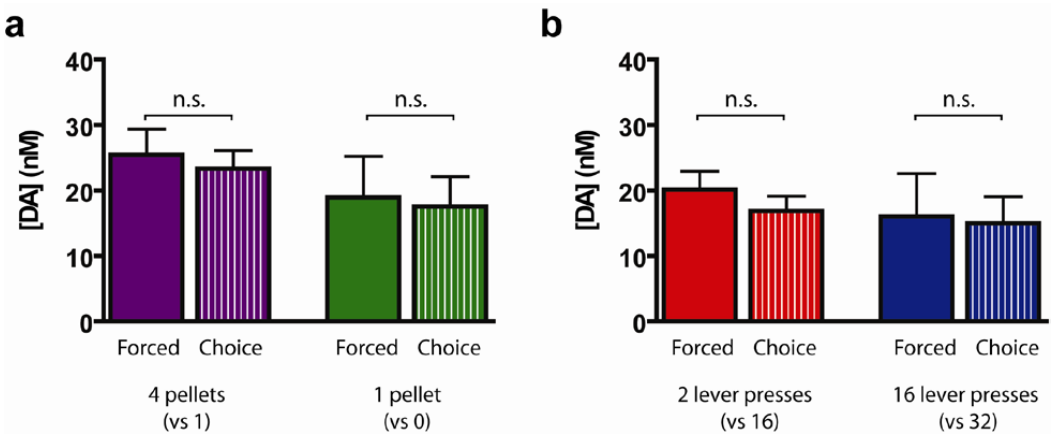

Fig. 3.11 Dopamine response during forced and choice trials

Comparison of post-criterion cue-evoked dopamine release on high utility forced trials and on choice trials where the high utility option was chosen in (A) benefit conditions and (B) cost conditions. There was no difference between the measured dopamine concentration on the forced and choice trials in any block (main effect of trial type: $F_{1,19}$=2.54, p=0.13) .

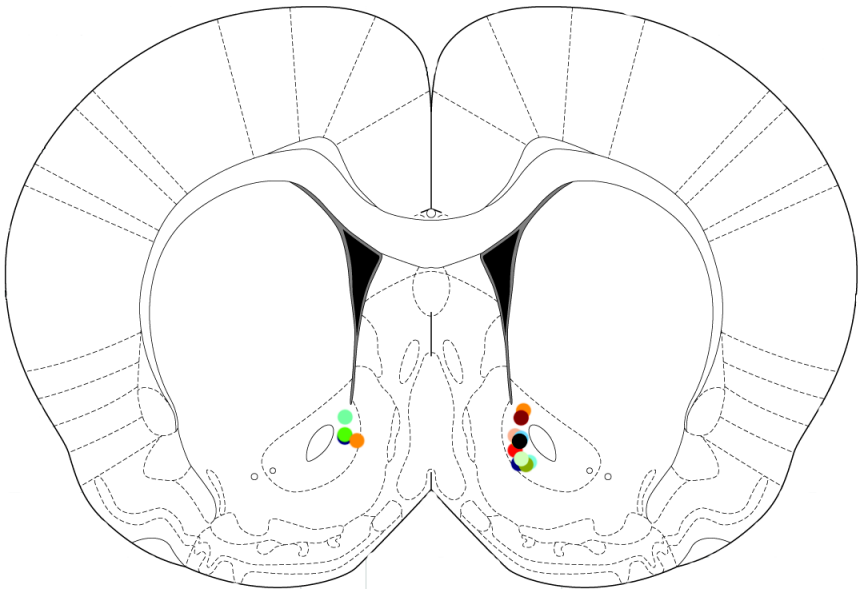

Figure 3.12 Histology

Locations of the carbon fiber recording electrodes within the NAcc.

Part IV: Conclusions and Future Directions

Immediate conclusions

The neural mechanisms underlying decision making in a normal animal requires dopamine. And while chemically and electrically induced perturbations hint at dopaminergic function, complete understanding of dopamine function requires characterization during normal behaviors. To this end, this thesis describes the development and validation of chronically implantable microsensors capable of detecting dopamine at a subsecond timescale across weeks (Part II). With this technique, we then characterized phasic dopamine transmission in the NAcc of animals performing a decision making task. We found that dopamine does not encode net utility as it is mostly insensitive to changes in predicted effort costs (Part III). These findings begin to characterize dopamine function in terms of neuroeconomic principles but also open further questions to be addressed in the future.

The need for repeated, long-term measurements is crucial to the evaluation of learning, memory and neuropathological processes (Martin, et al., 2000; Tolias, et al., 2007). This challenging objective is further complicated by the need for measurement on a physiologically relevant timescale. However, with the development of a chronically-implanted carbon-fiber microsensor coupled to FSCV, we attain this goal and demonstrate the measurement of behaviorally-evoked dopamine release with subsecond temporal resolution from day to day over months.

The advantage of these chronic microsensors lies in their stability. With these microsensors, not only do we gain ability to obtain multiple, repeated recordings in a single subject over a period of days, weeks or months but also the lack of movable parts required by microdrive-driven implantation allows for greater animal movement without as much movement-induced noise. The stability of the microsensor allows for the assessment of neurochemical correlates of psychological processes such as learning, research aimed at exploring dopamine in animal models of psychiatric or neurological disorders will benefit from the capacity to measure during all aspects of the modeled disorder. While the longitudinal data presented in this thesis were collected in rats, this microsensor has successfully been used for long-term, *in vivo* neurochemical measurements in mice (data not shown). The now widely used manipulation of the mouse genome makes this an attractive species for the study of disease processes (Gainetdinov et al., 1999). Finally, this approach also permits the simultaneous assessment of dopamine release in multiple brain nuclei within a single animal, a manipulation not possible with previous methodology. These additional applications have the potential to extend the already rich field of *in vivo* voltammetry and allow researchers to make new inquiries and approach long-standing questions in a novel way.

One such question revolves around phasic dopamine transmission's role in decision making. In order to make appropriate decisions, it is important not only to evaluate the potential benefits of a course of action, but also the costs,

such as the anticipated amount of work that will be required to obtain such payoffs. All other factors being equal, animals will usually prefer to pursue goals which require less effort to achieve (Solomon, 1948), and several lines of evidence demonstrate that animals' choices are weighted by both the costs and benefits of the available options, with animals tolerating increasing costs for preferable` rewards (Bautista, Tinbergen, & Kacelnik, 2001; Floresco, et al., 2008; Walton, et al., 2006). Lesion and pharmacology studies have implicated dopamine specifically in the NAcc in enabling an animal to overcome effort costs for more preferable rewards (Salamone, Correa, Farrar, & Mingote, 2007a). This is particularly prominent in tasks where the less beneficial outcome is a readily available primary reward (for instance, laboratory chow freely available in an operant box) whereas the availability of the larger reward at greater response cost is signaled by a conditioned stimulus (e.g., the presence of the lever in the operant box). Moreover, such cost-benefit decisions are influenced by the current motivational state of the animal, with food-deprived animals being more willing to put in work to achieve reward than those who have recently been given access to a meal (Floresco & Ghods-Sharifi, 2007). In neuroeconomic terms, this juxtaposition of internal state with cost-benefit analyses defines a transaction's utility.

Thus, dopamine's role in decision making may be in the calculation of the utility. To begin to address this question, we asked if utility is represented by phasic NAcc dopamine release. We tested animals on the two-option decision

making task, except that now the reward magnitude associated with each option was the same and value was instead manipulated by altering the number of lever presses required to obtain the reward (Gan, Walton, & Phillips, 2010). The cost parameters were set such that they had comparable motivating effects on choice behavior as the reward manipulation had, with animals rapidly learning to prefer the low cost option. Nonetheless, in spite of this preference, in most cases, dopamine did not encode an effort-discounted value signal. One exception was in situations where the response cost was unexpectedly lower than the reference cost; however, after repeated experience of the lower than reference cost, this scaling with net value disappeared. This lack of encoding of upcoming response costs by NAcc phasic dopamine have also recently been observed in a more dynamic, progressive ratio paradigm where response costs escalate as a function of the animals' past choices (Wanat, et al., 2010)

Insensitivity to costs seems contradictory to previous evidence of dopamine depletion affecting animals' tolerance of increased costs. However, this can be reconciled by considering such cost-benefit trade-offs in terms of utility curves depicting the amount of effort expenditure an animal would put in to obtain an expected future payoff given its current motivational state (Phillips, et al., 2007). In such as framework, mesolimbic dopamine might participate in encoding the availability of particular sizes of future payoffs with reference to the work required to reach these goals such that appropriate cost expenditures can be set. Somewhat paradoxically, to provide useful input to such a

computation, the phasic dopamine signal elicited by a predictive cue would itself have to be impartial to movement-related response costs. Moreover, this would allow for separate updating of predictions about the costs and benefits of a course of action when discrepancies are detected, something that would not be possible if dopamine signaled the overall net utility of a course of action.

Future directions

Outside of a scientific laboratory, choices are seldom presented separately. Nor do cues usually predict only one course of action. In reality, animals must make choices based on incomplete knowledge and unknown probabilities of reward and effort. The data presented in this thesis is, with the exception of that presented in figure 4.10, was from forced trials in which only one cue was presented at a time. In choice trials, animals preferred the option that resulted in higher reward or lower effort costs greater than 80% of trials. For the trials in which the animal chose the lower value or higher effort option, interpreting the dopaminergic response to the cues was confounded by our inability to assess whether the animal preferred that option or if that option was chosen by mistake. Several previous electrophysiological studies of animals choosing between two options suffer the same dilemma (Bayer & Glimcher, 2005; Roesch, Calu, & Schoenbaum, 2007a).

To properly examine dopamine transmission to different choices, we can use the fact that costs are not encoded by dopamine transmission in the NAcc.

The insensitivity of dopamine transmission in the NAcc to effort costs after significant training allows us to increase the effort requirement to obtain a higher reward to the point in which animals prefer the low reward option. To this end, we can ask animals to choose between a small reward incurring a standard effort costs (i.e. 1 food pellet for eight lever presses) and a larger reward with intolerably high effort costs (4 food pellets for ~48 lever presses). By changing the effort requirement, the option leading to less food becomes the preferred reward. This comparison will determine whether dopamine transmission in the NAcc reflects what the animal considers the 'better' option or reflects reward magnitude.

State of the field

While the findings in this thesis are steps towards understanding dopamine's role in decision making, there are numerous issues that have been sidestepped. First, what role does dopamine release at different timescales plays in these functions? We have concentrated here on phasic changes in dopamine-mediated activity and release. However, modulations in background tonic dopamine levels can be detected across minutes. Even within the phasic range, alterations in the firing rate of midbrain dopamine cells can happen as rapidly as 70-100 milliseconds following the presentation of a salient visual stimulus yet can also occur across several seconds during states such as uncertainty (Schultz, 2007). Moreover, it has recently been suggested that the dynamics of firing rates

within hundreds of seconds may convey different types of information, including salience, timing and value (Bromberg-Martin & Hikosaka, 2009; Nomoto, Schultz, Watanabe, & Sakagami, 2010). It will be important to determine how these different modes of transmission affect control of behavior.

Second, all the studies discussed have investigated how dopamine modulates animals' responses to positive reinforcers. However, it is evident that aversive events may also be strongly motivating. While it had been thought for a long time that dopamine neurons mainly coded positive prediction errors and were uniformly inhibited by negative prediction errors or aversive events (Schultz, 2007; Ungless, Magill, & Bolam, 2004), new evidence indicates that this may have been a simplification as dopamine cells, particularly more dorsolaterally within SNc, have been found to excited by stimuli associated with aversive consequences as well as the aversive airpuff itself (Matsumoto & Hikosaka, 2009).

This also relates to a third important area requiring consideration, namely how the modulatory role ascribed to the mesolimbic dopamine system relates to the functions of the nigrostriatal dopamine projection to dorsal parts of the striatum and to the mesocortical projection to thalamus and cortex. Do the same computational principles apply to each set of pathways, with the specific function of each being determined by the connectivity and local circuits of the terminal regions or is the information conveyed by each system markedly distinct? Does this separation relate in any way to the nature of the representations, in terms of

stimulus versus action values and goal-directed versus habitual response selection? While the answers to these are far from clear, it is apparent that the different dopamine systems interact to during learning and choice behavior to promote appropriate adaptive behavior (Ashby, Turner, & Horvitz, 2010; Belin, Jonkman, Dickinson, Robbins, & Everitt, 2009).

Manipulations of the mesolimbic dopamine pathways affect the motivation of humans and animals to act and the decisions that are ultimately taken. The firing patterns of midbrain dopamine neurons and dopamine release in the NAc reflect predictions of future benefits evoked by environmental stimuli. This appears to be important to prompt animals to seek rewards to satisfy their internal needs, particularly in situations where the structure of the environment remains unknown. Nonetheless, phasic dopamine release only appears indirectly related to the choices taken by an animal in instrumental situations. Instead, by signaling the benefits of pending payoffs separate to response costs, dopamine may provide a positive component to computations of the overall utility of a course of action to enable animals to overcome response costs. This may be crucial in uncertain environments to allow animals to explore novel options and to motivate animals to learn. However, in situations where the dopamine system fails to be appropriately regulated, such as certain neuropsychiatric disorders or through the effects of pharmacological agents, this may cause loss of control over behavior and an increase in impulsive choices (Buckholtz et al., 2010; Dalley, et al., 2007).

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