Neurophysiology and Neuropharmacology of

Decision Making

by

Arwen Long

Department of Neurobiology
Duke University

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Michael Platt, Advisor

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David Fitzpatrick, Chair

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Scott Huettel

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William Wetsel

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Neurobiology in the Graduate School of Duke University 2009
Abstract

(Neuroscience (0317))

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Negotiating the complex decisions that we encounter daily requires coordinated neuronal activity. The enormous variety of decisions we make, the intrinsic complexity of the situations we encounter, and the extraordinary flexibility of our behaviors suggest the existence of intricate neural mechanisms for negotiating contexts and making choices. Further evidence for this prediction comes from the behavioral alterations observed in illness and after injury. Both clinical and scientific evidence suggest that decision signals are carried by electrical neuronal activity and influenced by neuromodulatory chemicals. This dissertation addresses the function of two putative contributors to decision-making: neuronal activity in posterior cingulate cortex and modulatory effects of serotonin. I found that posterior cingulate neurons respond phasically to salient events (informative cues; intentional saccades; and reward delivery) across multiple contexts. In addition, these neurons signal heuristically guided choices across contexts in a gambling task. These observations suggest that posterior cingulate neurons contribute to the detection and integration of salient information necessary to transform event detection to expressed decisions. I also found that lowering levels of the neuromodulator serotonin increased the probability of making risky decisions in both monkeys and mice, suggesting that this neurotransmitter contributes to preference formation across species. These results suggest that posterior cingulate cortex and serotonin each contribute to decision formation. In addition, the unique serotonergic projections to posterior cingulate cortex, as well as the frequent
implication of altered serotonergic and posterior cingulate function in psychiatric disorders, suggest that the confluence of cingulate and serotonergic activity may offer key insights into normal and pathological mechanisms of decision making.
To
Aunt Ona
Aunt Clarice
Auntie Anna
Aunt Helen
and
to my grandmothers:
Dorothy, Genevieve and Kitty
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List of Abbreviations and Symbols

Abbreviations

5-HT  serotonin

5HTT  serotonin transporter

ACC  anterior cingulate cortex

AD  Alzheimer’s disease

ADHD  attention-deficit and hyperactivity disorder

AIC  Aikake’s Information Criterion

ANOVA  Analysis of Variance

APOE  apolipoproteinE

BLA  basolateral amygdala

BR  the monkey Broome

C57BL/6J  C57BL/6J (wild-type)

CGp  posterior cingulate cortex

CNS  central nervous system

CV  coefficient of variance
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DRN</td>
<td>dorsal raphe nuclei</td>
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<tr>
<td>EV</td>
<td>expected value</td>
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<tr>
<td>FEF</td>
<td>frontal eye fields</td>
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<tr>
<td>GM</td>
<td>General Mills</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td>IT</td>
<td>inferior temporal region</td>
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<tr>
<td>LED</td>
<td>light-emitting diode</td>
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<tr>
<td>LIP</td>
<td>lateral intraparietal cortex</td>
</tr>
<tr>
<td>LNAA</td>
<td>large neutral amino acid</td>
</tr>
<tr>
<td>MT</td>
<td>area MT</td>
</tr>
<tr>
<td>NI</td>
<td>the monkey Niko</td>
</tr>
<tr>
<td>OCD</td>
<td>obsessive-compulsive disorder</td>
</tr>
<tr>
<td>OFC</td>
<td>orbitofrontal cortex</td>
</tr>
<tr>
<td>PCPA</td>
<td>para-chlorophenylalanine</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PSE</td>
<td>point of subjective equivalence</td>
</tr>
<tr>
<td>PTSD</td>
<td>post-traumatic stress disorder</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>PSTH</td>
<td>peri-stimulus time histogram</td>
</tr>
<tr>
<td>RPE</td>
<td>reward prediction error</td>
</tr>
<tr>
<td>RTD</td>
<td>rapid tryptophan depletion</td>
</tr>
<tr>
<td>SH</td>
<td>the monkey Sherry</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitors</td>
</tr>
<tr>
<td>TPH</td>
<td>tryptophan hydroxylase</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>WSLS</td>
<td>Win-Stay-Lose-Shift</td>
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<tr>
<td>WT</td>
<td>wild-type</td>
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Acknowledgements

It has been a privilege to work with my advisor, Michael Platt. His expansively creative research ideals and incomparable ability to spin a story continue to amaze and inspire me. My committee — David Fitzpatrick, Scott Huettel, Bill Wetsel and (formerly) Jill Stowe — have put up with some utterly dreadful presentations and have encouraged and challenged me. I thank them for their assistance on my voyage into the unknown. My studies of serotonergic function have been highly collaborative ventures, and I thank Cynthia Kuhn, Ramona Rodriguiz and Bill Wetsel for their assistance, encouragement, and contributions to my intellectual and professional development. The HPLC measurements described in these experiments were performed by Maria Bartolome (macaque plasma tryptophan), Masato Fukui and Dipendra Aryal (mouse brain serotonin). Ramona Rodriguiz, O’Dhaniel Mullette-Gillman and John Pearson have mentored and advised me, and I have appreciated their insight, encouragement and example. Melissa Furlong, Nandish Shah, James Zhang, Betty Jiang, and Sarah Boltuck helped me develop and implement the mouse experiments described in this thesis. It was a pleasure to work with them and I wish them well. Several colleagues volunteered helpful comments on portions of this thesis — Rebecca Ebitz, Jeff Klein, Victoria Long, O’Dhaniel Mullette-Gillman, John Pearson and Stephen Shepherd— and Bill Wetsel and Stephen Shepherd offered strategic advice that helped me formulate and write this work. Justin Long helped me make the figures used in the Discussion. As a Duke MSTP student, I owe partic-
ular homage to Sal Pizzo, Pat Burks, and Marjorie Miller for their commitment to education, to excellence, and to us as students. My medical deans, Mark Sebastian and Phil Goodman, have advised, encouraged and supported me. Bob Drucker has often hinted that, if all else fails, I could definitely get into a pediatrics residency. I really don’t know how to thank him, but it’s been good to know that there is a child-sized parachute nearby. Finally, I would like to thank my MSTP colleagues for their camaraderie and commiseration, my mother for teaching me how to write, the many friends who fed and encouraged me during the writing of this document, the wee free Nac MacFeegles, and my family (including Squiggles, Peanut and Wasabi) for their love and general awesomeness.
Every day, humans encounter myriads of seemingly trivial decisions. Surprisingly, even though our behavior depends on highly adapted neurochemical processes, we often find simple decisions difficult. Choosing the morning’s breakfast food and selecting the day’s professional attire may challenge us within the first hour after awakening. The more complex decisions that we consider — how to invest money, where to live, what career to pursue — reveal even more competing factors that influence our choices.

Even those decisions that seem to require only discrimination between better and worse options, or between reward and punishment, depend on multi-factorial evaluative processes. Valuation depends not only on quantifiable properties like size, weight, nutritional content, and dollar value, but also on the decision-maker’s current physiological and emotional state, past experience, socially acquired information, and idiosyncratic biases. Thus, although today’s calculated breakfast preference should depend rationally on the relative prices of the two options, experientially on yesterday’s choice, and energetically on the nutritional content of the two items, our decision may ultimately depend on the marketing strategies deployed by Kellog’s
and General Mills (GM). Similarly, investment choices that ought to follow rational calculations based on value and probability often reflect seemingly irrational and highly inconsistent biases toward (or against) perceived risk.

Decisions become even more challenging if, as in many psychiatric disorders, the neural systems that support decision-making are abnormal. Where we debate Eggo's versus cereal, a depressed patient may face anhedonic disinterest in breakfast; a schizophrenic patient may choose to avoid food for fear of poison; and an acutely manic patient may be so overwhelmed with grandiose plans that breakfast is irrelevant. Thus, counter-adaptive changes to the neuromodulatory systems that mediate decision making result in severe functional impairments.

Similarly, other neurological insults such as stroke and trauma cause distinctive behavioral alternations. The associations between pathological lesions and particular behavioral abnormalities are important clues to understanding the anatomical and chemical contributions to decision processes. Even with the help of such hints as the astonishing transformation of Phineas Gage and the forgetful geniality of H.M., however, comprehensively mapping neural decision processes has been challenging. Evaluative decisions appear to depend on recursive processing in the regions known as “association cortex”, which link sensory inputs to motor outputs. Unlike primary and secondary sensorimotor cortices, which were functionally characterized based on the observed movements and described sensations resulting from local stimulation, association cortices are not easily linked to distinct inputs and outputs. Thus, assigning particular decision-related functions to these brain structures has required careful experimental design. In this Introduction, I will discuss our current understanding of decisions from both behavioral and biological perspectives, with a particular focus on probabilistic components of decisions, before considering two particularly intriguing contributors to decision-making, posterior cingulate cortex and the neuromodulator serotonin. These ruminations will conclude in a summary of the experimental
rationale behind the specific aims of this thesis.

1.1 Decisions

1.1.1 Theories of rational choice

Over the last several centuries, economists and decision researchers have debated whether decisions depend upon rational calculation or heuristic estimations. Idealistic economic conceptions of human rationality are often traced back to Bernoulli’s famous solution for the St. Petersburg paradox (Bernoulli, 1738/1954). When asked to explain why people would only pay small fees to play a simple gamble with infinite expected return, Bernoulli suggested that such decisions depend on a subjective analysis of the gamble’s expected value and the participant’s current state. This rational decision framework, one of the earliest expected utility theories, admits that decisions are subjective and context-dependent but suggests that they depend on extensive (specifically, logarithmic) internal calculations of value.

Enlightenment economists and philosophers continued to develop the idea that decisions depended on personal, subjective utility through the 19th century. Adam Smith argued repeatedly, and across domains, that economic interactions depended on self-interested valuation of available options: whether choosing an occupation or acting cooperatively, “It is his [the individual’s] own advantage... which he has in view” (Smith, 1776/1976)\textsuperscript{1}. Notably, although Bernoulli’s aim was to develop “rules... whereby anyone could estimate his prospects from any risky undertaking in light of one’s specific financial circumstances” (Bernstein, 1996) and Smith’s interests were primarily economic, neither entirely excluded the possibility that decision-making agents might use other factors in calculating subjective utilities. The English philosopher Jeremy Bentham defined utility based on personal pleasure (Bernstein, 1996), but the focus of individual utility shifted completely toward monetary value.

\textsuperscript{1} See, for example, I.ii and IV.ii
when John Stuart Mill introduced an imaginary rational agent whose primary concern was the “[desire] to possess wealth” (Mill, 1844/2000). Although Mill’s “Economic Man” was admittedly “speculative”, the conceptual simplicity and relative tractability of analyzing an exclusively economic decision has made this fictitious figure useful to behavioral scientists and economic thinkers.

The mathematician John von Neumann and the economist Oskar Morgenstern rigorously axiomatized the definition of a rational agent, now dubbed “*homo economicus*” (Neumann & Morgenstern, 1944; Neumann, 1928/1959). Like Bernoulli, Smith and Mill, von Neumann and Morgenstern assumed that individuals attempt to maximize an evaluative, subjectively determined utility. Their expected utility theory strongly resembled Bernoulli’s — in both theories, for example, an agent choosing between two equal-valued options should be indifferent — and although the implications of the two theories are not identical, both definitions have influenced modern research. Together, these descriptions of utility form the basis of current economic utility theories.

*Risk in rational choice theories*

Both Bernoulli’s expected utility and the von Neumann/Morgenstern formulation estimate utility based on potential outcomes; importantly, both also depend on known outcome probabilities, known colloquially as uncertainty or expressed economically as risk. Notably, risk specifically implies known probabilities; uncertainty due to unknown probabilities is defined as ambiguity (Ellsberg, 1961; Knight, 1921). Thus, any outcome with a known probability attached to it is economically risky, while outcomes with unknown probabilities are ambiguous. Risk can be defined as the simple probability of reward (Bernoulli, 1738/1954), reformulated as a decision weight (Kahneman & Tversky, 1979), or calculated mathematically as the coefficient of variance (CV) of reward (E. Weber, Shafir, & Blais, 2004).
Empirical challenges to expected utility theories

Risk and ambiguity induce strong behavioral biases in people and animals that are difficult to explain on the basis of rational probability estimations or utility calculations. Committed gamblers will insist on buying car insurance beyond the legally required minimum (Kahneman & Tversky, 1979; Lee, McGreevy, & Barraclough, 2005). Thus, even though people are risk-seeking in one context (e.g., gambling), they avoid risk in other contexts (e.g., insuring a car), suggesting that they may not have consistent, rational reactions toward risk. The observation that betting at horse races shifts away from the most favored (and most likely to win) horses near the end of the day, even though the probability of a win increases toward the last race, provides additional evidence that responses to probabilistic bets do not depend on rationally calculated expected utilities (McGlothlin, 1956).

Several famous examples of irrational responses to uncertainty, such as the Allais and Ellsberg paradoxes (Allais, 1953; Ellsberg, 1961), have challenged behavioral economists to develop more comprehensive models to describe decision making (Lopes, 1995). In both paradoxes, people choosing between probabilistic outcomes with positive or zero magnitudes demonstrate unexpected preference reversals that are inexplicable under an expected utility framework. In addition, the observation that humans are more averse to ambiguity than to risk — even when the difference does not change rational expected value calculations (Ellsberg, 1961) — challenges the assumptions of expected utility frameworks.

Prospect Theory

Similarly, although expected utility theories often assume that attitudes toward risk should be consistent across contexts, choices made between positive, risky rewards and positive, guaranteed alternatives differ strongly from choices between risky and guaranteed options when reward values are negative: people are more likely to choose
a guaranteed option than a risky gamble when outcomes are positive, but switch
to choosing the gamble when outcomes are negative (Kahneman & Tversky, 1979).
This striking behavioral pattern led Daniel Kahneman and Amos Tversky to propose
that we evaluate risk in a non-linear fashion, and that we differentially value gains
and losses. Using these assumptions, they developed a modified form of expected
utility theory, known as Prospect Theory, that better accommodates many of the
empirical behaviors that violate expected utility theories (Camerer, 2000; Kahneman
& Tversky, 1979; Tversky & Kahneman, 1992).

The utility of expected utility

While expected utility theory and its derivatives have provided useful tools for an-
alyzing decisions across a variety of contexts, the tendency to analyze utility solely
in terms of monetary value — or even in terms of estimated value — continues
to challenge behavioral and biological decision researchers. Humans and animals
consistently distinguish simple, non-probabilistic magnitudes, and animals’ behav-
ior under simple matching law tasks indicates that they also discriminate reward
probabilities (R. J. Herrnstein Richard J., 1961; Hinson & Staddon, 1983; Lau &
Glimcher, 2005; A. N. McCoy, Crowley, Haghghian, Dean, & Platt, 2003; Wolford,
Miller, & Gazzaniga, 2000). Thus, we might suppose that organisms could achieve
economic rationality by integrating reward magnitude and probability to maximize
utility. On the other hand, we suspect intuitively and know empirically that humans,
like most other species, frequently make economically irrational decisions that violate
the predictions of expected utility theories (Allais, 1953; Ellsberg, 1961; Kahneman
& Tversky, 1979).

Empirical evidence confirms that our choices are influenced by emotion, culture,
and choice complexity (Dijksterhuis, Bos, Nordgren, & Baaren, 2006; Hsee & Rot-
tenstreicht, 2004; McClure et al., 2004; Rottenstreich & Hsee, 2001; E. U. Weber
& Hsee, 1998). Even the matching behavior that confirms sensitivity to probabilistic reinforcement results in submaximal choice patterns (R. Herrnstein & Heyman, 1979). Just as paradoxical responses to risk prompted the development of mathematical models such as expected utility, these observations of behavior that cannot be explained by utility theory have led both to the refinement of utility theory (e.g., Prospect Theory) and to the development of alternative behavioral explanations (Kahneman & Tversky, 1979; Simon, 1955; Gigerenzer, Czeslinski, & Martignon, 1999/2002).

1.1.2 Heuristic approaches

To better explain the limitations and irrationalities found by empirical studies of behavior, Herbert Simon challenged *homo economicus* and the framework of utility theory, and proposed that models of rational choice should consider the limitations of the organism as well as its environment. Simon agreed with Bernoulli’s assumption that decisions depended on both the agent and the environment, but argued that rationality was bounded by man’s limited knowledge and computational ability (Simon, 1955). In light of these observations, Simon suggested that human decision processes might simplify the problem of maximizing over a large set of alternatives. Rather than waiting to choose the optimal solution, we might choose the first satisfactory option. This alternative to utility theory’s optimization, which is known as satisficing, balances our satisfaction (or utility) against computational overload.

*Satisficing and aspiration levels*

Satisficing can also be explained as a simple rule-of-thumb, or heuristic, in which available options are compared to a threshold. When the value, worth, or utility of an option under consideration surpasses the desired threshold, or aspiration level, then it is deemed satisfactory. This decision rule replaces absolute maximization
with a much easier decision rule: instead of assigning and remembering utilities for all possible options (incidentally, a process which may not be environmentally or energetically feasible), a decision maker needs only to compare each option to the aspiration level.

Evidence for this strategy can be found in multiple situations and across species.\(^2\) In Chapter 2, I show that monkeys choosing between two visual cues are much more likely to choose an option if its associated juice reward exceeds 153 ul; although there is some variability in the monkeys’ choices (an observation that may result from their need to explore alternatives in a frequently changing environment), this observation suggests that the monkeys may be categorizing reward values as larger or smaller than a 153 ul threshold. Similarly, in Chapter 3, I show that the same monkeys again appear to apportion choices around a threshold (203 ul). While the threshold varies with context, the behavioral pattern appears similar in both studies; furthermore, previous analyses indicate that an aspiration level can — and probably should — vary with context (Selten, 1998).

**Win-Stay-Lose-Shift**

Several game theoretic analyses have suggested that a heuristic strategy built on comparisons to an aspiration level, Win-Stay-Lose-Shift (WSLS) (Thorndike, 1911), is the optimal solution for repeated prisoner’s dilemma games (Imhof, Fudenberg, & Nowak, 2007; Nowak & Sigmund, 1993). In a repeated interaction context where multiple choices are available simultaneously, this decision rule leads agents to repeat decisions that led to supra-threshold rewards (if win, then stay) but to switch away from an option after receiving a sub-threshold payout (if lose, then switch) (Bar-

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\(^2\) For a related example, in which stickleback fish cooperation and defection can be described as a tit-for-tat strategy, see (Milinski, 1987). If cooperation parallels a win, and defection matches a loss, then much of the literature on repeated-interaction cooperative behavior and tit-for-tat strategies could be reframed in WSLS terms (cf (Wedekind & Milinski, 1996)).
In a repeated prisoner’s dilemma context, where there are two possible choices (cooperate or defect) and two possible outcomes (win or lose), using an WSLS strategy allows an agent to both exploit available options and to quickly change decisions in response to experienced consequences.

Intriguingly, this strategy may also be successful for more complicated social interactions. In a language immersion setting, where students learn by interacting with other students, the decision to use (and thus learn) a particular language can depend on the number of other people using that language (Matsen & Nowak, 2004). This number represents an aspiration level; if enough students use the language, then the aspiration level is surpassed, and a new student will join the conversation. On the other hand, if the number of students using the language is below the aspiration level, then a learner will switch to an alternative language. The proposed aspiration level — two to three students — is comparable to the number of concordant individuals needed to begin an informational cascade (Banerjee, 1992; Bikhchandani, Hirshleifer, & Welch, 1998), suggesting that switch/stay decisions based on a simple comparative decision rule may contribute to a wide variety of social phenomena.

The utility of heuristics

Although the WSLS heuristic supports successful, approximately optimal decisions across multiple contexts, using only a single heuristic for all decision environments eventually leads to counterproductive behavior. In changing environments, heuristic decision makers adapt by modifying favorite heuristic strategies (e.g., changing an aspiration level) or by choosing alternative heuristic decision rules (Gigerenzer & Todd, 1999; Tversky & Kahneman, 1974). Such flexible decision making allows agents to simultaneously approximate optimization while maximizing speed and minimizing computational effort (Payne, Bettman, & Johnson, 1988). These observations, when combined with experimental evidence for Prospect Theory, probability matching, and
local maximization, suggest that humans and other organisms use a wide variety of strategies to negotiate probabilistic environments.

1.1.3 Summary

Although utility theories provide useful mathematical summaries of the interplay between objective valuation and self-interested consideration, their rigorous axioms limit their explanatory power. Increasingly nuanced versions of utility theories have been adapted to empirical observations (Kahneman & Tversky, 1979; Tversky & Kahneman, 1992), but experimental evidence continues to challenge such models. Alternative heuristic, or rule-of-thumb, summaries of decisions have evolved to summarize and rationalize our variegated choice strategies.

Importantly, the observation that decision making depends on prioritizing environmental information for internal processing is explicit in the heuristic and bounded rationality perspectives of decision making. Since humans have only limited processing capacity and time, simplification and prioritization maximize personal satisfaction despite the excessive computational demands of the environment (Gigerenzer et al., 1999/2002; Gigerenzer, 2000; Simon, 1955). Similarly, neuronal decision processes need to filter, process and prioritize relevant information — in other words, to manipulate our internal representation of the external environment into a tractable and useful form. Such manipulations can be quantified in terms of information theory and entropy, or as energy-efficient processing (Montague, 2007; Shannon, 1948). Alternatively, they may qualitatively link vivid environmental features to internal representations of salience (Hall, 1994; Posner, Snyder, & Davidson, 1980). In the next section, I will summarize the salience view of informational and attentional prioritization before describing a current framework for understanding internal representations of salience.
1.2 Salience

Even before we approach a decision, when we open our eyes and survey our environment, we need to make sense of our surroundings. The visual sensory information that reaches our brain contains relevant and irrelevant information. Whether our gaze falls on a fruiting orchard, a lion-filled savannah or a modern art gallery, our ability to negotiate the risks and rewards — be they food, death, or art critics — depends critically on our ability to extract relevant information from our environment.\(^3\)

The visual system, in particular visual orienting, is a particularly useful background system for testing stimulus detection and perception. Since extensive research has thoroughly characterized the basic anatomy and function of visual sensation and eye movement, many perceptual and decision-related studies depend on visual stimuli and visuosaccadic responses to investigate internal decision processes. In this section, I will first define salience before describing criteria for characterizing a region as a salience map representing an abstract visual salience. Next, I will use the example of the frontal eye fields to demonstrate that neural mapping of salience is a realistic proposal.

1.2.1 Defining salience

The word \textit{salience} is often used to describe the ephemeral quality that makes environmental features stand out. Salience is defined literally by this attentional prominence (Simpson & Weiner, 1989), and in colloquial use, we generally assume that anything that attracts our attention must be salient. The latter observation reveals an important distinction in our understanding of salience and attentional modulation: while some stimuli stand out because of intrinsic physical features, others

\(^3\) Some of the most entrancing examples of information come from social information processing in non-human primates. Although baboons pay more attention to perineal swellings and hippopotami than to art critics, their complex social interactions offer a particularly captivating illustration of the power and relevance of contextual information (Cheney & Seyfarth, 2007; Sapolsky, 2005).
Physical features such as visual contrast, bright color, and sudden appearance contribute to the attention-grabbing power of salience (James, 1892). Such *intrinsic* features might evoke rapid, strong responses from our visual system as a result of evolutionary selection processes that encouraged sensitivity to environmental information relevant for survival. In attentional terms, these features can be described as contributing to “bottom-up” regulation of attention, that is, activating neuronal responses at early stages of neuronal processing, without extensive post-sensory modulation.

Alternatively, if we have acquired associations with the stimulus that make it important, it may catch our attention because of those associations. The underlying *acquired* characteristics, which are not intrinsic but reflect experience, valuation or conditioning, can endow a stimulus with informational, emotional or motivational relevance. Recognition of such non-physical features involves modulation of sensory regions by higher-level cortices, a contribution known as “top-down” regulation.

Both intrinsic and acquired characteristics contribute to our understanding of salience. Notably, the attention-grabbing qualities resulting from either type of feature do not necessarily indicate valence (e.g., reward versus punishment). Thus, the color of a bright red object sighted from afar could alert us to the possibility that important information is present, even though the object might not be immediately recognizable as a sweet, nutritious fruit or as a slimy, poisonous frog. If we are driving, however, we may quickly react to a red light by slowing down or we may barely notice the red litter along the median. In this thesis, I assume that both intrinsic and acquired characteristics contribute to the attention-grabbing qualities that we summarize as salient.
1.2.2 Integrating features: defining salience maps

Neurons in regions such as area MT (MT), inferior temporal region (IT) and amygdala respond to specific features of the external environment (motion, objects and emotion, respectively). While these representations highlight external locations with particularly strong feature-specific salience, they maintain separate detailed representations that can be further developed by identifying locations with multiple co-existing salient features. This possibility raises a new question. Is there a brain region that integrates the feature-specific information encoded by areas such as MT, IT and the amygdala to form a cohesive representation of external salience? If such a salience map exists — that is, if some region of the brain encodes attention-grabbing power across space and across multiple features — where is it? Before addressing this question, I will describe the characteristics that motivate classification of a region as a salience map. Then, since frontal eye fields (FEF) has been well-characterized as a probable salience map, I will describe evidence that the frontal eye fields function as a salience map for detecting relevant visual stimuli (K. G. Thompson & Bichot, 2005) and I will propose that posterior cingulate cortex functions as a salience map for mapping reward and motivational characteristics.

The core characteristic of a salience map is that it provides a spatial representation of attentional relevance based on integrated feature-specific maps. Thus, instead of responding to a particular feature, and binding spatial location to a specific feature, neurons that contribute to a salience map should respond more generally to noteworthy stimuli. I suggest that five characteristics of neuronal activity support the description of a salience map.4

1. Neurons respond to stimuli across a variety of contexts. The anatomic and functional convergence of local feature maps causes sensitivity to the integrated

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4 For complementary descriptions, see (Fecteau & Munoz, 2006) and (K. G. Thompson & Bichot, 2005).
strength of attention-grabbing or motivationally relevant features. This criterion also suggests that neuronal activity may reflect both intrinsic visual features (or “bottom-up” features) and learned relevance (or “top-down” features such as those resulting from conditioned associations between a cue and reward information).

2. **Neuronal responses are not feature-selective.** Neurons may have poor sensitivity to unitary attention-grabbing visual features such as color, contrast, orientation or motion, although their responses may be correlated with features when they are task-relevant.

3. **Neuronal activity reflects sensorimotor integration.** Neuronal activity modifies perceptual processing and motor preparation in similar ways.

4. **Neurons have spatial receptive fields.** They respond more strongly to stimuli presented at a defined spatial locus than at other loci. Importantly, this is a comparative definition that does not preclude stimulus-sensitivity outside of the neuronal receptive field.

5. **Stimulus responses are not exclusively spatial.** Neurons respond to salient stimuli independent of eye movements, and may even respond to stimuli outside of the neuronal receptive field. One implication of this response pattern is that neurons may respond to stimuli that attract covert attention, but are not foveated.

Taken together, these criteria describe responses that permit prioritization of spatial location by a somewhat abstract measure of salience that depends on integration of feature-selective responses. Although there may be a universal salience map in the brain, previous studies suggest that multiple salience maps may exist which integrate over different (but potentially overlapping) feature sets and perform different (but potentially overlapping) functional roles. For example, neurons in the frontal eye fields appear to integrate both distinctive visual features and task-relevant visual
characteristics to localize task-relevant cues within visual space. In contrast, the amygdala — despite its strong association with fear responses (Amaral, 2003) — may eventually be described as a salience map due to its responses to task-relevant non-social stimuli (Ousdal et al., 2008). Since there is extensive experimental evidence supporting the characterization of FEF as a salience map, I will use it as an example to illustrate the functional characteristics of a salience map. In later sections, I will also provide evidence for classifying posterior cingulate cortex as a salience map.

1.2.3 Salience map example: frontal eye fields

Neurons in the macaque frontal eye fields demonstrate all five of the features that comprise a salience map; in fact, an extensive body of literature documents FEF responses to salient information across contexts, across visual features, and across space. This section will summarize the evidence that FEF functions as a salience map, using the criteria described above (p. 13).

**FEF neurons are sensitive to stimulus relevance.** FEF neuronal activity discriminates between informationally relevant and irrelevant stimuli presented in the neuronal receptive field. When monkeys perform an oddball task, in which saccades to one unique stimulus (the oddball) within a set of stimuli results in reward, FEF neurons respond more strongly if an oddball is presented in the neuronal receptive field than to an identically situated, motivationally irrelevant stimulus (Schall, Hanes, Thompson, & King, 1995; Schall & Thompson, 1999). In addition, task-relevant learning contributes to this effect (Bichot & Schall, 1999), indicating that both contextual visual distinctiveness and learned contextual salience are integrated in FEF.

Finally, in experiments that parallel the random-dot motion experiments described above, FEF neuronal activity approximates behavioral discrimination (K. G.
Thompson, Bichot, & Sato, 2005). When monkeys could distinguish between targets and distractors based on color differences, neuronal responses varied according to the relevance of the stimulus placed in the neuronal receptive field. However, FEF neurons did not distinguish between targets that were behaviorally indistinguishable due to similar coloring.

**FEF neurons are not specific for feature detection.** Importantly, FEF neuronal activity discriminates targets from distractors based on features other than color similarity. When all targets presented include randomly moving dots and the oddball is identified by unique motion coherence (i.e., in the opposite direction to all the other targets), FEF neurons differentiate the presence of oddball versus distractor in the neuronal receptive field (Sato, Murthy, Thompson, & Schall, 2001). Thus, FEF perceptual discrimination is maintained whether salient, distinguishing information is conveyed by color or by motion coherence. Further evidence for this assertion comes from the observation that FEF neurons respond to informationally salient, task-relevant oddballs as well as to task-irrelevant, visually-distinctive distractors (Bichot, Chenchal Rao, & Schall, 2001).

**FEF neurons contribute to visuomotor activity.** Stimulating FEF neurons, even in the absence of task demands, elicits directional saccades (Bruce, Goldberg, Bushnell, & Stanton, 1985). Thus, activity in frontal eye fields can be presumed to contribute to visuomotor indications of internal decision processes.

**FEF neurons have spatial receptive fields.** Early mapping studies demonstrated that FEF neurons respond to the appearance of small spots of light and that these responses are spatially selective, with large quadrant- or hemifield-sized receptive fields (Mohler, Goldberg, & Wurtz, 1973; Bruce & Goldberg, 1985).
**FEF neurons respond to covertly attended stimuli, independent of saccade.** In a “no-go” version of the oddball tasks described above — in which monkeys did not saccade to the oddball — Thompson and colleagues found that FEF neurons discriminate oddballs from other cues (K. G. Thompson, Bichot, & Schall, 1997). Notably, the timing and responsiveness of neuronal activity did not indicate the presence or absence of saccade. Furthermore, in experiments that dissociated the oddball’s location from the direction of the saccade, FEF neuronal activity reflects the location of the oddball rather than the direction of the saccade (Murthy, Thompson, & Schall, 2001). Thus, while FEF neurons appear to contribute to motor planning and may bind salient information to spatial locations, their activity appears to encode the locations of salient stimuli.

These studies confirm that FEF neurons are likely to integrate salient information and represent the probability of motivationally relevant information or stimuli across space. Thus, FEF activity promotes directing attention to potentially informative or motivating stimuli, and thereby supports stimulus evaluation and action selection.

### 1.2.4 Summarizing salience and salience maps

The example of FEF neurons demonstrates that at least one part of the brain responds to a generalized, or integrated, abstraction of attention-grabbing features in visual space. As described above, microstimulation experiments suggest that FEF’s representation of visual space contributes to visual orienting and attentional allocation. Importantly, however, the observation that FEF neurons do not discriminate large from small rewards (Ding & Hikosaka, 2006; Leon & Shadlen, 1999) suggests that these neurons provide primarily spatial information. This possibility raises yet another question: is there a brain region that integrates reward/motivational information with spatial information, or that can serve as a salience map for assessing reward? Before providing evidence that a strategically located region of the
brain, the posterior cingulate cortex, may fulfill this function, I will suggest a simple characterization of decision processes in which CGp links perceived, prioritized and remembered motivational information to outcome evaluation and action selection.

1.3 Neural contributions to decisions

As behavioral researchers and economists try to describe and understand decision-making strategies, neuroscientists have focused on the neural mechanisms that contribute to decisions. The difficulty of describing human decisions (e.g., as economically rational, heuristic, or adaptive across contexts) hints at the challenges that hinder description of neural decision processes. This section will present a simple framework for understanding the neural processes that contribute to decision making.

1.3.1 The process of decision: From perception to outcome

The process of decision making requires detection and characterization of relevant stimuli within the external sensory environment. Evaluative processes characterize relevant stimuli and predict the value of possible responses. During this process, working memory is activated to recall previously experienced action-outcome evaluations. The outcomes of actions that have been selected and completed are evaluated and stored in long-term memory. At each of these stages, efficient, effective decisions require that relevant information be prioritized. The process of decision making is not, of course, as simple as this description might suggest (but cf (Rangel, Camerer, & Montague, 2008)). The regions that subserve these stages, even when they are anatomically distinct, modulate each other and interact. Furthermore, the recursive nature of memory storage and outcome recall introduces additional layers of feedback loops.

To make consideration of these non-linear processes tractable, I will first describe three components of decisions — detection, evaluative action selection and outcome
evaluation — with examples of brain regions that contribute to each component. Although memory is critical for many relevant learning processes, this thesis does not focus on the contributions of memory to decisions, and I will not review these contributions in any detail. Then, after proposing that the regions that subserve action selection and outcome evaluation are linked by posterior cingulate cortex and modulated by serotonergic projections, I will focus on two specific contributors to decision making, posterior cingulate cortex and serotonin.

1.3.2 Detection

Simple stimulus detection is generally tested by asking a subject to respond as soon a relevant stimulus becomes evident. For example, to determine the minimal signal the retina can detect, a subject sitting in a darkened room is asked whether he (or she) observed a flash of light after opening a shutter (Hecht, Shlaer, & Pirenne, 1942). This simple procedure identifies the signal strength necessary to cross the subject’s perceptual threshold (Posner et al., 1980). These experiments comprise the simplest perceptual judgments, in which we report whether or not a stimulus is present.

The ability to discriminate the directionality of moving dots is representative of discriminatory, perceptual evaluations that range in complexity from detecting a flash of light or tactile vibration to culturally-influenced assessments of the riskiness of a financial purchase (Hecht et al., 1942; Hernandez, Zainos, & Romo, 2000; E. U. Weber & Hsee, 1998). Results obtained by recording from MT neurons in behaving monkeys illustrate the probability that neuronal activity subserves discrimination of the external characteristics that shape our behavioral responses (Romo, Hernandez, Zainos, & Salinas, 1998; Romo, Hernandez, Zainos, Brody, & Lemus, 2000; Salzman, Murasugi, Britten, & Newsome, 1992); and with the supposition that regions whose activity discriminates features are likely to contribute to contingent behavioral responses.
Stimulus detection example: discriminating random dot coherence

In these experiments, complex perceptual decisions were tested by requiring monkeys to report the direction of moving dots. In an experiment designed by Newsome and colleagues (Newsome, Britten, & Movshon, 1989), monkeys shown an array of randomly moving dots make a saccade to indicate whether the majority of the dots are moving to the right or to the left. If a monkey correctly discriminates the direction of movement, then it is rewarded with a squirt of fruit juice. Behaviorally, these experiments establish that about 6% of the dots need to be moving in the same direction for the monkeys to report a directional signal with reasonable accuracy (i.e. 82% correct).

In addition to testing behavioral sensitivity, Newsome et. al. recorded from neurons in MT (Newsome et al., 1989). They found that neuronal activity, like behavior, was sensitive to directionality: when the dots were moving almost entirely randomly, MT neuronal activity was similar whether the dots were slightly more likely to move toward the left or the right. Even before the dot coherence approached and surpassed the coherence threshold for behavioral discrimination, MT neuronal activity began to differentiate dot directionality. When a discriminable percentage of the dots moved in a neuron’s preferred direction while within its receptive field, that neuron fired more strongly than if the dots moved in the opposite direction. To confirm that MT neurons not only reflect perceptual stimulus differences, but contribute to the translation from external stimuli through perception to behavioral responses, the authors performed another experiment. Instead of recording from MT neurons, they applied small electrical currents to microcolumns of MT neurons with congruent direction-selectivity (Salzman et al., 1992). This microstimulation biased the monkeys’ responses in the preferred direction of the stimulated microcolumn, suggesting that MT activity contributes to perceptual evaluation or action selection.
involved in indicating the perceived direction of motion.

Other evidence for neuronal feature discrimination

While MT neurons respond selectively to motion, other regions of the brain respond to other qualities. For example, IT neurons respond selectively to particular shapes, such as faces or hands (Gross, 2008). New data suggests that the amygdala, which was once thought to detect only threatening or fear-filled stimulus (Amaral, 2003), detects a broader range of emotional features (Pessoa & Ungerleider, 2004) and discriminates social signals such as the emotional valence of relevant faces (Pessoa, Kastner, & Ungerleider, 2002).

The contributions of both IT and amygdalar activity to behavior are demonstrated by the functional impairments resulting from lesions in these two brain regions. Lesioning the entire temporal lobe, which eliminates both the amygdala and the inferior temporal region region, causes a unique constellation of signs eponymously named Klüver-Bucy syndrome: affected animals fail to recognize objects, learn poorly and show decreased emotional reactivity, diminished fear responses and increased sexual drive. The emotional hyporeactivity and loss of fearful behavior can be replicated with focal amygdala lesions (Aggleton & Passingham, 1981). Consistent with IT responses to visual objects and its proposed role in object recognition, the impairments in visual function and learning result from IT lesions (Gross, 1994).

1.3.3 Action evaluation and selection

In perceptual discrimination experiments, actions acquire attentional priority via association with reward. If a monkey correctly discriminates the direction of maximal coherence of field of randomly moving dots, he receives a reward. If the monkey is instead offered a choice between two simultaneously available options, he has to evaluate the relative desirability, or in economic terms, calculate the expected utility,
of the two options in order to choose to his best advantage. Since monkeys generally choose the largest, sweetest or highest-probability option present, it is reasonable to assume that these choices reveal the motivational value of options.

Thus, just as monkeys’ responses to flashes of light or to randomly moving dots disclose perceptual thresholds, monkeys’ choices between rewarding options reveal their preferences, or the relative motivational value placed on each option. This behavioral metric allows comparison of neuronal responses to decision variables such as reward magnitude and probability. Importantly, action evaluation is a fundamentally memory-dependent processes; thus, although I am not detailing memory’s contributions to decisions, I note that the monkey must remember the previous outcomes to evaluate the worth of potential actions. Three brain regions, lateral intraparietal cortex (LIP), prefrontal cortex (PFC) and anterior cingulate cortex (ACC), have been implicated in evaluation and action selection; they are particularly worth noting as examples of neural contributions to action selection. Each of these regions functions slightly differently in action selection: LIP and PFC, respectively, appear to prioritize value-based and historical or rule-based contributions to decisions, while ACC selects and maintains decision strategies. In this section, I will summarize the evidence for each of these contributions before discussing outcome evaluation regions.

**Lateral intraparietal cortex**

Neurons in LIP, a visuomotor region that appears to link reward evaluation to visuosaccadic responses, signal both the magnitude and probability of available rewards (M. Platt & Glimcher, 1999). To demonstrate LIP’s sensitivity to these decision-theoretic variables, Platt and Glimcher showed monkeys two conditioned visual cues (one red and one green) before indicating the rewarded saccade with a color-matched central cue. To compare responses to reward magnitude and probability, Platt and Glimcher varied the juice payout and reward probability separately.
Unsurprisingly, LIP neurons encoded only movement directionality once the rewarded saccade was signaled; but before the required saccade direction was revealed, activity scaled with the magnitude and probability associated with the cue in the neuronal receptive field. When monkeys were allowed to choose freely between the same two visual cues, both behavioral preferences and neuronal activity reflected the economic expected value, or motivational salience, of available options. Thus, LIP activity reflected the magnitude and probability of reward, and predicted behavioral responses. A pioneering study that used choices between juice rewards and socially relevant images to determine the motivational value of visual social information demonstrated that LIP activity reflected a more abstract measure of desirability (Klein, Deaner, & Platt, 2008).

Prefrontal cortex

A dramatic clinical correlation between PFC damage and erratic behaviors (Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994; Ratiu, Talos, Haker, Lieberman, & Everett, 2004) has encouraged scientists to think of PFC as a region involved in cognitive control. More recent studies have suggested a role for PFC in learning choice-outcome associations and implementing resulting behavioral “rules” (Balleine & Dickinson, 1998; Lee, Rushworth, Walton, Watanabe, & Sakagami, 2007; Miller & Cohen, 2001). Evidence for this function comes from neurophysiological recordings in macaques that demonstrate reward predictive activity, or expectation signals, before reward delivery and outcome reporting after reward (Hikosaka & Watanabe, 2000; M. Watanabe, 1996). Recent studies confirm that in a matching pennies game, PFC neurons encode past choices and rewards as well as the historically influenced decision value that contributes to future decisions (Barraclough et al., 2004).
Anterior cingulate cortex

Although anterior cingulate cortex (ACC) has often been described as a neural conflict manager, recent studies have revealed a probable role for ACC in learning and updating strategic decisions. Monkeys with focal ACC lesions fail to learn from submaximal responses: they fail to update behavior by reversing options and maintaining newly optimal choice patterns following submaximal or erroneous experiences (Kennerley, Walton, Behrens, Buckley, & Rushworth, 2006; Rudebeck et al., 2008). Where the regions described above as reward-sensitive or evaluative often encode values and preferences that are primarily determined by reward magnitude and probability, ACC neurons appear to represent the motivational value of an action (Matsumoto, Matsumoto, Abe, & Tanaka, 2007; Rushworth, Buckley, Behrens, Walton, & Bannerman, 2007). Contributions to learning from failures represent important components of both utilitarian and heuristic decision strategies (cf (B. Y. Hayden, Pearson, & Platt, 2009)).

1.3.4 Outcome monitoring (and prediction)

Regions such as ACC, PFC and LIP that evaluate options and select actions depend on memory for the maintenance of reward information across time, and on memory retrieval to predict that information. Not surprisingly, then, regions that respond differentially to outcomes also often also predict outcomes or respond differentially to the relative expected values of options. These retrospective responses to remembered value contribute prospectively to decision-making. Examples of these processes have been found in neurons in the orbitofrontal cortex, dorsal raphe nuclei, ventral tegmental area and posterior cingulate cortex, which are associated with evaluative and predictive reward responses that contribute to action selection and decision.
Orbitofrontal cortex

When monkeys choose between options with punishment and reward outcomes, orbitofrontal cortex (OFC) neuronal firing rates reflect the relative values of available rewards (Roesch & Olson, 2004). If, as this observation suggests, OFC signals reward outcomes, then neurons in this region should reflect the relative magnitudes of available rewards. In two more recent studies, Padoa-Schioppa and Assad quantified monkeys’ preferences for juice rewards to develop a “menu” of options with relative values indicated by the monkeys’ choices (Padoa-Schioppa & Assad, 2006, 2008). The interpretive framework that these authors propose is somewhat complicated, but the both studies suggest that, consistent with previous studies showing correlations between reward preferences and OFC activity (Hikosaka & Watanabe, 2004), neurons in OFC can encode relevant decision variables such as the juice chosen, the strength of preference, and the relative value of the reward chosen. Macaque lesion studies that demonstrate failure to update decision values with experienced outcomes following OFC lesions provide further evidence for the hypothesis that OFC contributes to outcome evaluation (Rudebeck et al., 2008; Schoenbaum, Chiba, & Gallagher, 2000; Wallis, 2006).

Dorsal raphe nucleus

Neurons that produce the neurotransmitter serotonin may also contribute to reward sensitivity. Serotonergic dysfunction is associated with anhedonic and risk-seeking maladaptations (Deakin, 2003; Dolan, Anderson, & Deakin, 2001), and serotonin is thought to convey information about negative consequences (Zhang, Lu, & Bargmann, 2005), but little is known about the outcome sensitivity of the dorsal raphe nuclei (DRN) cells that produce serotonin. A recent study showed that DRN neuronal activity predicted future reward magnitudes and responded differentially to rewards delivered (Nakamura, Matsumoto, & Hikosaka, 2008), suggesting
that these neurons (and by extension, serotonin, if the neurons recorded are in fact serotonergic) may contribute to evaluative processes. While there are few studies that investigate the contributions of serotonergic DRN neurons to decision, there is extensive circumstantial and clinical evidence that serotonin modulates affective and evaluative components of decision. These observations, especially in light of the extensive anatomical spread of neuromodulatory efferents, raise important questions about the nature of neuromodulatory contributions to decisions. Serotonergic contributions are particularly intriguing given the clinical evidence for serotonergic involvement in risky decision making. In Section 1.5, I will discuss this evidence and outline my rationale for investigating serotonergic contributions to decisions.

Dopaminergic neurons

The observation that dopaminergic neurons in the ventral tegmental area of the midbrain respond to reward probability and to the absence of predicted reward has led to the hypothesis that these neurons encode a learning signal, often called reward prediction error (RPE). As monkeys learn a cue-reward association, dopaminergic responses to reward transfer to the cue. These neurons then respond with phasic increases to unexpected rewards and their activity is suppressed after omission of expected rewards (Fiorillo, Tobler, & Schultz, 2003; Schultz, Tremblay, & Hollerman, 1998); thus, their activity appears to indicate unexpected positive and negative changes in predicted reward. Signaling the difference between expected and received reward is important for learning and for updating information about experienced rewards. In addition to the evidence provided by the neurophysiology studies just referenced, neuroimaging studies have implicated ventral tegmental area (VTA) neurons in reward monitoring and updating, and functional studies confirm that dopamine modulates responses to reward and contributes to addiction (Blum et al., 2000; Dreber et al., 2009; Fiorillo et al., 2003; Kuhnen & Knutson, 2005; Wise, 2002).
Posterior cingulate cortex

Tonic CGp neuronal activity discriminates large from small rewards and tracks the risk level associated with a risky gamble (A. N. McCoy et al., 2003; A. McCoy & Platt, 2005b). CGp is thus sensitive to both reward magnitude and risk when contextually relevant. These results may reflect discrimination that contributes to representation of the decision value, i.e. the motivational salience of an option, or more generally responsiveness to contextually relevant features. Notably, CGp neurons also respond when expected reward is omitted, and maintain a representation of received reward between trials (A. N. McCoy et al., 2003; B. Y. Hayden, Nair, McCoy, & Platt, 2008). CGp microstimulation biases choice away from repetition of a preferred risky gamble (B. Y. Hayden et al., 2008), suggesting that CGp contributes to the evaluation of and response to economic or heuristic calculations of utilities. Accumulating evidence suggests that, consistent with its anatomical connections to regions that bias action selection and strategic decisions, posterior cingulate cortex links outcome evaluation to strategic action selection. Intriguingly, CGp’s tendency to respond to informationally relevant cues across contexts raises the possibility that CGp may function as a salience map. I will discuss the possible functions of posterior cingulate cortex in more detail in Section 1.4.

1.3.5 Summary

Although the neuronal contributions to decision making are complex and continue to challenge characterization, accumulated evidence implicates neurons in both detection and differentiation of visual stimuli, and in reward differentiation and outcome evaluation. Regions such as MT and IT contribute to detecting relevant stimulus features, such as motion and shapes, that are necessary for simple discriminatory decision making and in more complex, naturalistic decision contexts. Neuronal activity in LIP, PFC and ACC participate in action evaluation and selection, while
neurons in OFC, DRN, VTA and CGp reflect reward outcomes (Fiorillo et al., 2003; Kennerley et al., 2006; Klein et al., 2008; M. Platt & Glimcher, 1999; Wallis, Anderson, & Miller, 2001; Wallis & Miller, 2003a; Walton, Croxson, Behrens, Kennerley, & Rushworth, 2007; Schoenbaum et al., 2000; Schultz, 2001).

In previous sections of this thesis, I have hinted that CGp might tie together the regions that contribute to outcome evaluation and action selection. It is difficult to isolate entirely distinct functions for each of the regions described above, partly since neurons in each of the regions described represent some version of outcome or decision value. However, the differences in their functional contributions to decisions suggest complementary contributions to a multi-factorial decision process. Thus, LIP contributions to action selection depend on valuative signals that are often correlated with utility theories’ expected value calculations (M. Platt & Glimcher, 1999); but ACC updates outcome evaluation for strategy selection (Kennerley et al., 2006). Thus, these two regions might represent alternative modes of evaluation that contribute complementary (though potentially competitive and interactive) calculations to action selection.

Both these brain regions — like most of the regions described above — are anatomically linked to CGp. ACC and LIP send and receive projections to and from CGp (Parvizi, Van Hoesen, Buckwalter, & Damasio, 2006). Thus, like PFC and OFC, which both receive projections from CGp (Pandya, Van Hoesen, & Mesulam, 1981; Vogt & Pandya, 1987), neuronal activity in these regions is likely influenced by CGp’s putative outcome-evaluative representations. In addition, the projections from ACC and LIP to CGp are likely to refine CGp reward estimations and update the value of actions. Summarily, current evidence suggests that the action selection processes in LIP, ACC and PFC — which prioritize outcome valuation, strategy application, and history-based rule application, respectively (Kennerley et al., 2006; Klein et al., 2008; M. Platt & Glimcher, 1999; Wallis et al., 2001; Wallis & Miller,
utilize outcome-evaluative signals from CGp, which are maintained over time and interact with memory. In return, CGp receives signals from LIP and ACC that may update spatial and action-predictive prioritization. The VTA neurons described above respond strongly to environmental changes, particularly in reward contingencies, and are thus likely to signal unexpected events that might contribute to strategic or heuristic shifts via connections with ACC and PFC (Fiorillo et al., 2003; Schultz, 2001). Finally, CGp’s projections to the OFC may contribute to the formation of stimulus-outcome associations, whether by responses to unexpected events or by maintaining representations of reward outcomes (Morecraft, Geula, & Mesulam, 1992 Sep 15, 1993; Pandya et al., 1981; Rolls, 2000; Schoenbaum et al., 2000). Of note, the DRN neurons referenced above project extensively throughout frontal and decision-related regions. These putatively serotonergic neurons project to PFC, ACC, OFC and CGp and are likely to modulate activity in each of these regions (Baumgarten & Gothert, 1997).

Although these proposed functions are necessarily speculative, they are consistent with evidence implicating each of these regions in decision making; and suggest that decisions are, consistent with the complexity and variety of behavioral explanations for decisions (Sections 1.1.1, 1.1.2), dependent on multiple modes of evaluation, processing, and strategic action selection. In particular, this summary model sites CGp at a decision-making nexus where it appears to contribute uniquely to recognition and processing of salient motivational and informational events. I will discuss CGp contributions to decisions in more detail in the next section. Finally, I will emphasize the importance of neuromodulatory processes for decision making by discussing the evidence that implicates serotonin in decisions.
1.4 Posterior cingulate cortex

Although it is difficult to simplify neural decision processes into independent stages, most of the regions described above can be identified as contributing to action selection or to outcome evaluation. Posterior cingulate cortex appears to link these two processes, and may function as a salience map for prioritizing attentional allocation based on reward-based or decision-relevant features. In this section, I will describe the evidence for CGp contributions to decisions, from the clinical, anatomical, and functional points of view. I will first describe clinical observations that implicate CGp dysfunction in abnormal decision tendencies. I will then summarize the anatomical clues to CGp function before discussing the functional evidence that links CGp activity to outcome evaluation, attention and spatial awareness. Finally, I will comment on the possibility that CGp may act as a salience map, prioritizing visuospatial attention based on integrated information about such acquired features as reward and decision value.

1.4.1 CGp dysfunction in disease

Hints that posterior cingulate cortex contributes to decision making and attentional processes have come from the disrupted functionality observed in disease or after cortical damage. The abnormal decision patterns observed in schizophrenia and borderline personality disorder have been associated with CGp dysfunction (Ha et al., 2004; Hazlett et al., 2005; Haznedar et al., 2004). Memory failures in Alzheimer’s disease (AD) have been linked with the timing and magnitude of decreases in CGp energy metabolism (Minoshima et al., 1997; Valla, Berndt, & Gonzalez-Lima, 2001; Valla et al., 2002); and the normal functional connectivity between CGp and hippocampus disappears in AD (Sorg et al., 2007). Importantly, the apolipoproteinE (APOE) genotype that predicts worse prognosis is correlated
with great CGp abnormalities (Drzezga et al., 2005). Post-mortem histopathological studies also confirm striking posterior cingulate cortex atrophy in AD (B. Jones et al., 2006). Consistent with these observations, which link CGp dysfunction to suboptimal memory and spatial attention, removing cingulate cortex causes neglect and amnesia (Valla et al., 2001; Watson, Heilman, Cauthen, & King, 1973). In addition, CGp hypoactivity is associated with both the diagnosis of schizophrenia and with decreased ability to focus attention on a relevant clue to the exclusion of distractors (Laurens, Kiehl, Ngan, & Liddle, 2005; Laurens, Kiehl, & Liddle, 2005). Although the physiological bases of these diseases are complex and multifactorial (and generally poorly understood), the observed symptoms suggest associations between CGp hypofunction and altered attention and memory — both associations that are consistent with the proposal that CGp contributes to directing attention, outcome evaluation and spatial orienting.

1.4.2 CGp anatomy

The observation that posterior cingulate cortex’s anatomical connections include both evaluative regions and pre-motor cortices (A. McCoy & Platt, 2005a) also supports the hypothesis that CGp activity might mediate the transition from outcome evaluation to action selection. CGp has extensive reciprocal connections with the visuomotor regions LIP and FEF (Beckmann, Johansen-Berg, & Rushworth, 2009; Klein et al., 2008; Morecraft et al., 1993; Pandya et al., 1981; M. Platt & Glimcher, 1999; Parvizi et al., 2006). CGp is also closely interconnected with ACC, a region thought to contribute to selecting decision strategies and learning from errors (Kennerley et al., 2006; Parvizi et al., 2006). Posterior cingulate neurons also project to PFC, which appears to update and apply decision rules (Pandya et al., 1981; Vogt & Pandya, 1987; Wallis et al., 2001; Wallis & Miller, 2003a); and to OFC, which is thought to encode and update evaluative decision variables (Barraclough et
1.4.3 Functional characteristics of CGp activity

Studies of CGp activity during visual and decision tasks further support the suspicion, introduced by anatomical and clinical observations, that CGp contributes to decisions by linking visuomotor, evaluative and attentional information. In the following sections, I will summarize evidence that CGp does fulfill this role. First, CGp neurons respond following visual cues and visual saccades, and these responses are often spatially selective. In addition, although attention to tasks and differentiation of task-relevant information appear to operate on different time scales, CGp neurons monitor and differentiate task-relevant reward information. Finally, CGp evaluative responses reflect more abstract judgments than juice reward magnitude, as they discriminate between auditory cues as well as degrees of morality.

CGp reports visual cues and saccades

Although initial explanations of CGp function labeled the region as limbic or paralimbic, electrophysiological studies quickly established that the region’s functionality involved sensorimotor processes and spatial memory. Single-neuron recordings in cats revealed phasic responses after visual saccades and tonic modulation by orbital position (C. Olson & Musil, 1992), and analogous experiments in macaques confirmed these findings (C. Olson, Musil, & Goldberg, 1996). Although neurons in these early studies responded only infrequently to abrupt-onset visual cues, they did respond both to low-frequency flashes of light and to large visual targets; CGp neurons also
responded to contextual changes such as dimming or brightening ambient light (C. Olson & Musil, 1992). More recent studies have confirmed and extended these findings, both by demonstrating that these neurons respond phasically to small visual cues and by clarifying the spatial encoding schemes used in CGp (Dean, Crowley, & Platt, 2004; Dean & Platt, 2006; B. Hayden, Smith, & Platt, 2009).

**CGp neurons are spatially selective**

The studies that initially characterized CGp visual responses confirmed that CGp neurons are often spatially selective (Vogt et al., 1992): in cats performing visual fixation and visual saccadic tasks, CGp neuronal activity varied with the direction of gaze and, after saccade, of saccade (C. Olson & Musil, 1992). Although spatial selectivity across this population of neurons was not clearly linked to contraversive or ipsiversive saccades, later studies using macaques indicated that CGp neurons tended to have broad contralateral tuning: neurons responded more strongly following contraversive than ipsiversive saccades, and responded more strongly to contralateral target onset than to ipsilateral targets (Dean et al., 2004; C. Olson et al., 1996). Further experiments revealed that while posterior cingulate neurons can encode in either retinocentric (eye-centered) or non-retinocentric (head-centered) coordinate systems, they were more likely to use non-retinocentric coding. Similarly, while CGp neurons can encode spatial location as either egocentric or allocentric (relative to surroundings), allocentric encoding is more frequent (Dean & Platt, 2006). Thus, while CGp’s spatial sensitivity involves multiple coordinate systems, it is biased toward a contextual mapping consistent with a role in attentional allocation.

**CGp responses operate on phasic and tonic time-scales**

The observation that CGp neuronal activity has both phasic and tonic components (C. Olson & Musil, 1992) suggests that CGp responses contribute to both
the immediate recognition and the memory of relevant events. A recent analysis of CGp activity in monkeys performing an evaluative decision task demonstrated that neuronal responses to reward were maintained through a 1-second interval following reward, and into the next trial (B. Y. Hayden et al., 2008).

While this observation suggests that tonic CGp activity contributes to the memory necessary for evaluating the value of potential actions, other studies link tonic modulations to attention rather than to decision. For example, both neuroimaging and neurophysiological experiments show that CGp activity decreases when attention is allocated to task performance or to monitoring visual cues, and that phasic responses to visual stimuli are independent of tonic modulations (Fox et al., 2005; B. Hayden et al., 2009). It is difficult to clearly link these tonic modulations with CGp contributions to decisions, but worth noting that these observations support a role for CGp in attention and, potentially, memory.

**CGp neurons monitor task-relevant reward information**

In a single-target saccade task for liquid reward, CGp neuronal activity reflects reward sizes across blocks, suggesting that CGp neurons may encode saccade value based on experienced reward information (i.e., an acquired feature of visual cues) (A. N. McCoy et al., 2003). Furthermore, CGp neurons differentiate levels of risk in a gambling task where monkeys choose between guaranteed reward and a variable reward gamble (A. McCoy & Platt, 2005b). Consistent with the supposition that CGp contributes to reward evaluation and action selection, CGp activity measured in each of these studies reflected task-relevant information that predicted behavior: in the first study, reward magnitude, and in the second study, risk.

In addition, the temporal development of CGp responses in these tasks — i.e., responses consistently follow events rather than preceding them — suggests that CGp monitoring of task-relevant information could contribute to memory-guided de-
cisions. As previously noted, CGp neurons maintain a representation of experienced reward between trials; and microstimulation of CGp following reward delivery biases choices away from the generally preferred risky option (B. Y. Hayden et al., 2008).

*CGp responds ubiquitously across contexts*

Imaging studies provide extensive evidence that CGp activity contributes to attentional, evaluative or visuospatial processes across a variety of contexts. These studies measure activity over longer time windows than most related neurophysiological studies and are thus likely to describe tonic CGp function (B. Y. Hayden et al., 2008). Several studies confirm that CGp differentiates rewards from non-rewards and losses in financial decisions (Knutson, Fong, Bennett, Adams, & Hommer, 2003; Kuhnen & Knutson, 2005). In addition, CGp activity correlates with moral judgments and predict punishment magnitude in socially oriented studies (Buckholtz et al., 2008; Robertson et al., 2007), and differentiates emotional, threatening and neutral words (Maddock, Garrett, & Buonocore, 2003; Maddock & Buonocore, 1997). Although such social information seems distant from economic reward evaluation, these studies may indicate a broader, multi-contextual role for evaluative functionality in posterior cingulate cortex.

**1.4.4 Summary**

The studies summarized above support the hypothesis that CGp links evaluative processes that depend on experienced reward to visuomotor decision output. CGp neuronal responses following both reward and saccade reflect subjective valuation of experienced rewards, and thus could contribute to outcome monitoring and evaluation. Furthermore, CGp maintains such evaluative representations over time, and CGp microstimulation affects future decisions. The combination of observed value-representation, visuospatial sensitivity and attention observed in CGp in consistent
with a role for CGp in decision making.

*Does CGp function as a salience map?*

It should also be evident that CGp fulfills many of the criteria for definition as a salience map (Section 1.2.2, p. 13). CGp neurons respond to visual stimuli across a variety of contexts and, in addition, respond to auditory stimuli, changes in the external environment and internally motivated sensory signals such as pain (Morrison, Peelen, & Downing, 2006). Furthermore, these neurons respond differentially based on decision-related information such as reward and risk; and the observation that CGp neurons respond differentially to equal-valued risky and safe options confirms that neuronal activity is not tied to reward magnitude independent of other contributions to task-relevant decision variables (A. McCoy & Platt, 2005b). CGp neuronal activity reflects both perceptual processing, specifically decision variables, and motor preparation. CGp neurons have broad, often contralateral spatial receptive fields.

*Remaining questions*

However, several questions remain unanswered. While CGp neurons respond to visual stimuli across multiple contexts, it is not clear whether these responses are exclusively spatial, i.e. whether evaluative information in decision or saccade tasks is inextricably linked to spatial and saccadic maps. In addition, while CGp responses to risk may be task-relevant, the correlation between risk levels and monkeys’ subjective expected utility (as revealed by their choice allocation) introduces the possibility that CGp neurons respond to risk as a unitary feature.

This thesis describes two sets of experiments designed to address these questions. First, in Chapter 2, I will describe results that demonstrate that CGp responses generalize across and beyond visual contexts. Specifically, I show that CGp neurons respond phasically to visual cues, after saccade, and following reward delivery.
across multiple contexts; and, furthermore, that CGp neurons also respond to reward delivery independent of visual cues. Second, to confirm that CGp neurons track task-relevant decision variables in the presence of risk (Chapter 3), I will describe evidence that as monkeys’ preferences shift across contexts in a gambling task, CGp neurons track the revealed preferences rather than risk, and thus reflect an integrated measure of reward and motivational information.

1.5 Serotonin

Several of the brain regions described above that contribute to decision making (Section 1.3) receive extensive innervation from serotonergic neurons in the DRN. Regions that contribute to action selection, such as the PFC and ACC, and regions that differentiate reward outcomes, such as the OFC and CGp, all receive significant input from serotonergic neurons (Baumgarten & Gothert, 1997; Varnas, Halldin, & Hall, 2004). This observation suggests that serotonin is likely to modulate decision making. Serotonin, like CGp, has been implicated in decision making by evidence from psychiatric disorders with profound behavioral deficits. Although the evidence provided by the clinical observations described below is associative and does not establish causal mechanisms, it suggests that serotonin contributes to decisions and, more specifically, to decisions involving risk. In this section, I will summarize the evidence for a link between serotonergic function and decision making that led me to suppose that lowering serotonin might cause an increased tendency to make economically risky choices.

1.5.1 Serotonin is implicated in psychiatric disorders

One of the disorders most strongly linked with serotonergic dysfunction is depression, a disorder in which negative emotional states are associated with altered perceptions of the world (Deakin, 2003; Jans, Riedel, Markus, & Blokland, 2007). De-
pression is associated with increased impulsive behavior as well as increased risk of suicide (Apter et al., 1990; d’Acremont & Linden, 2007). Genetic studies have shown that alternative forms of the serotonin transporter gene modulate risk for depression following stressful events, and are linked to differential probabilities of suicidal behavior (Caspi et al., 2003; Nielsen et al., 1998); in addition, serotonin transporter polymorphisms modulate the risk of post-traumatic stress disorder (PTSD) (Koenen et al., 2009). The increased risk of suicide associated with depression may depend on physiological changes in serotonergic function (Brown & Linnoila, 1990; Nielsen et al., 1994, 1998; Nordstrom et al., 1994). Serotonergic deficits have also been associated with addictive behaviors, attention-deficit and hyperactivity disorder (ADHD) and impulsive personality disorders as well as impulsive/compulsive syndromes such as Tourette’s and obsessive-compulsive disorder (OCD) (Deakin, 2003; Fletcher, 1995; Grados, 2009; Harrison, Everitt, & Robbins, 1999; Higley et al., 1996; Mossner, Muller-Vahl, Doring, & Stuhrmann, 2007; Wong et al., 2008).

Since serotonin contributes to both neurogenesis and formation of brain structure, developmental deficits of serotonergic function may contribute to the pathology of psychiatric disorders (Sodhi & Sanders-Bush, 2004). Both autism and Down’s syndrome are thought to reflect disrupted serotonergic development (Whitaker-Azmitia, 2001; Chugani et al., 1999; DeLong, 1999; Pardo & Eberhart, 2007).

Drugs that modulate serotonergic activity ameliorate symptoms of many of the conditions just mentioned. For example, selective serotonin reuptake inhibitors (SSRI) have been used to treat autism as well as depression, and some of the antipsychotics used to treat schizophrenia act at serotonergic receptors (e.g. risperidone, clozapine) (Roth, Hanizavareh, & Blum, 2004; Veenstra-VanderWeele, Anderson, & Cook, 2000). This observation further supports the supposition that serotonergic dysfunction contributes to altered behavioral patterns observed in psychiatric conditions. Thus, both observational clinical evidence and successful treatment strategies.
implicate serotonergic mechanisms in disorders associated with increased impulsivity or abnormal responses to risk, such as depression, schizophrenia, impulsive personality disorders, PTSD and autism (Akhondzadeh, 2001; Cook, 1990; Corchs, Nutt, Hood, & Bernik, n.d.; Deakin, 2003; DeLong, 1999; Pardo & Eberhart, 2007; Svenningsson et al., 2006; Marek, Carpenter, McDougle, & Price, 2003).

1.5.2 Serotonin is implicated in risky decision making

Notably, genetic studies also suggest that serotonin may contribute to impulsive and risk-seeking behaviors (Chau, Roth, & Green, 2004; Gainetdinov et al., 1999; Higley et al., 1996; Kreek, Nielsen, Butelman, & LaForge, 2005; Nordstrom et al., 1994). The observation that regions with extensive serotonergic innervation, including frontal cortical regions such as ACC (Kennerley et al., 2006), OFC (Padoa-Schioppa & Assad, 2008), CGp, and prefrontal cortex (Barraclough et al., 2004), contribute to strategic decision making supports the general supposition that serotonin contributes to decisions. In fact, low serotonin is associated with pathological gambling as well as excessive impulsivity (Dolan et al., 2001; Nordin & Sjodin, 2006); and serotonin receptor variants predict willingness to take risk in financial decisions (Kuhnen & Chiao, 2009).

Behavioral research also indicates that serotonin contributes to impulsivity, or unwillingness to wait for reward (Winstanley, Dalley, Theobald, & Robbins, 2004). Subjects are more likely to choose a smaller reward delivered sooner than a larger, delayed reward following dietary manipulations that lower brain serotonin levels than under normoserotonergic conditions (Schweighofer et al., 2008). Although impulsivity may not represent the same psychological construct as economic risk (J. Evenden, 1999a), behavioral studies suggest that temporal impatience and economic risk-attitudes are closely related (B. Y. Hayden & Platt, 2007; M. L. Platt & Huettel, 2008).
1.5.3 Summary and experimental direction

Thus, disorders associated with serotonergic dysfunction present with evidence of impaired decision-making, particularly with respect to risk. This observation suggests that serotonin contributes to outcome evaluation and action selection, though it does not specify a particular mechanistic contribution. The trend for dysfunctional serotonin to be associated with increased risk-taking behaviors, especially given recent evidence for a correlation between low serotonin and pathological gambling (Nordin & Sjodin, 2006), supports the specific hypothesis that low serotonin levels may cause increased risk-taking behaviors.

I tested this hypothesis in two species of animals. In Chapter 4, I demonstrate that lowering brain serotonin in monkeys choosing between guaranteed and risky reward options increases the likelihood that the monkeys will choose the risky option. In Chapter 5, I describe an analogous experiment in mice, with complementary results.

1.6 Experimental rationale and specific aims

The overarching goal of my dissertation was to investigate the neural processes that contribute to decision making. Since risk is an essential component of decision making, the correlation between posterior cingulate activity and risk preference (A. McCoy & Platt, 2005b), and between dysfunctional serotonergic systems and pathologically excessive risk-taking (Deakin, 2003; Nordin & Sjodin, 2006; Akhondzadeh, 2001) suggested that both CGp and serotonin may be particularly relevant components of the decision-making process. The first two aims of this dissertation focus on the role of CGp in decision-making. Since I suspected that CGp functions as a salience map, I first asked whether CGp neurons detect contextually relevant events across tasks, before determining that CGp activity reflects heuristically-guided pref-
erences across risky lotteries. The third and fourth aims ask whether serotonin modulates revealed preferences for a risky option in two species, mice and monkeys.

Aim 1: To determine whether CGp neuronal activity reports salient events across contexts, both inclusive and independent of visuospatial and visuomotor relevance

The hypothesis that posterior cingulate cortex monitors salient information — or, as posited above, functions as a salience map — predicts that neuronal activity in CGp should phasically report the occurrence of informationally relevant stimuli; that while CGp neurons might encode the spatial directionality of target-directed saccades motivated by reward, they should report the appearance of relevant stimuli independent of spatial location and independent of saccade; and, furthermore, that CGp neurons should report the occurrence of uncued, non-visual events such as unexpected reward. To test these hypotheses, I recorded from CGp neurons in awake monkeys performing four tasks:

1. To confirm that CGp neurons respond to informative stimuli, including rewards, in a decision task, I recorded from individual CGp neurons while monkeys performed a two-target visual choice task in which reward size was contingent on target color but not on location.

2. To confirm that CGp neurons respond to informative operant stimuli when no decision is available, I recorded from CGp neurons in monkeys performing a single-target operant task in which reward size was contingent on target color.

3. To confirm that CGp responses to visual stimuli are independent of visuomotor planning, I recorded from CGp neurons in monkeys performing a single-target Pavlovian task in which reward size was cued by target color.

4. To confirm that CGp neurons respond to stimuli independent of task relevance, I recorded from CGp neurons in monkeys receiving uncued rewards.
I found that that CGp neurons responded following motivated saccades, and that this post-saccadic activity was modulated by spatial location; that CGp neurons responded to informationally relevant visual cues across all three tasks, largely independent of spatial information; and that CGp neurons responded to reward delivery whether cued or not. These results are consistent with the hypothesis that phasic CGp activity reflects the occurrence of relevant events, and that CGp might function as a salience map.

**Aim 2:** To determine whether CGp neuronal responses to risk reflect encoding of contextually relevant information, i.e. contextual preferences and choice probability.

If posterior cingulate cortex activity reflects salient information across contexts, and contributes to the development of preferences, then tonic CGp activity should reflect and predict preferences across contexts. Thus, CGp neurons that are sensitive to risk as a distinctive feature of frequently chosen options (A. McCoy & Platt, 2005b) should reflect preference for a more rewarding safe option even in the presence of a risky option. I tested this hypothesis by recording from CGp neurons in two contexts differentiated by the reward sizes available to monkeys performing a gambling task. In one context, when mean reward values for a safe option and a risky option were equal, the monkeys preferred the risky option; but when the safe option offered a higher average reward than the risky option, monkeys preferred the safe option. CGp neurons reflected the choice preference within each context, and predicted reward-history-dependent preferences across both contexts. These results confirm that CGp monitors contextually integrated reward information and clarify the function of CGp in decisions made under risk.

**Aim 3:** To assess the contribution of brain serotonin to economically risky decisions in monkeys.
Extensive clinical and circumstantial evidence associates low serotonin (5-HT) with increased risk-taking behaviors. If serotonin plays a causal role in the development or expression of risk preferences, then lowering brain 5-HT should increase the probability of risky choices. I tested this hypothesis in three monkeys choosing between a risky option and a safe option (gambling task, as described in Aim 2). When the monkeys underwent rapid tryptophan depletion (RTD), a dietary manipulation that decreased brain serotonin levels, they became more likely to choose the risky option. Comparing the monkeys' behavior across a range of lotteries confirmed that the monkeys valued the risky option more under low-serotonin conditions: they were more willing to give up juice available from the safe option to choose the risky gamble. These results confirm a causal relationship between low serotonin and increased tendencies to choose economically risky options.

**Aim 4:** To assess the contribution of brain serotonin to economically risky decisions in mice.

Since serotonergic mechanisms are broadly conserved across species, lowering brain serotonin should have the same effect in mice as in monkeys. I first established basic mouse behavior in a task analogous to the gambling task used in monkeys. Mice, like many other species, strongly prefer a safe option to a double-or-nothing gamble. I then used para-chlorophenylalanine (PCPA), an irreversible inhibitor of the rate-limiting enzyme in serotonin synthesis, tryptophan hydroxylase (TPH), to lower brain serotonin levels in a subset of mice and compared their behaviors to that of sham-treated mice. PCPA-treated mice, like monkeys, are more likely to choose the risky gamble than normo-serotonergic mice. This observation confirms that serotonin plays an important role in modulating behavioral responses to environmental risk.
Neurons in posterior cingulate cortex respond phasically to informative events

Neurons in posterior cingulate cortex (CGp) signal reward expectations, reward uncertainty and level of task engagement via tonic modulations in activity but respond phasically to the onset of visual targets, after saccades to those targets, and following rewards delivered for gaze shifts. The presence of both tonic and phasic domains of response suggests the hypothesis that CGp neurons dynamically signal the instantaneous allocation of neural resources to the current information stream. In this view, salient events that provide new information should trigger CGp neurons to respond phasically while state-dependent vigilance levels should modulate tonic activity. To test the impact of event salience on phasic responses, we recorded from 91 CGp neurons while two monkeys (*Macaca mulatta*) performed three tasks for fluid rewards as well as while they received random uncued fluid rewards. Individual CGp neurons responded to the onset of visual targets associated with rewards, whether or not they triggered overt orienting; operantly-conditioned saccades rewarded with fluid; and reward delivery. Notably, CGp neurons were as likely to respond phasically to
unpredicted, surprising rewards outside of any task as to fully predicted rewards delivered for task performance. These results support the hypothesis that CGp dynamically signals changes in the ongoing stream of information linking the external environment to internal state.

2.1 Introduction

The term salience is often used to describe stimuli that stand out from the ongoing stream of sensory inputs, engage attention, and trigger enhanced processing in the brain (James, 1892; Muller & Rabbitt, 1989; Posner et al., 1980). Although typically associated with sensory processing, the concept of salience can in principle be extended to other types of events, such as motor behavior and reward outcomes, that violate expectations or alter the relationship between the internal and external environment. Salience broadly writ not only contributes to enhanced sensory processing (Treisman & Gelade, 1980; Posner et al., 1980), but also learning (Mackintosh, 1975; Pearce & Hall, 1980) and motor adaptation (Abrams & Jonides, 1988; Rosenbaum, 1980). Although the neural mechanisms underlying the attentional modulation of sensory processing have been the subject of intense scrutiny, the processes that mediate the extraction of information from other types of salient events, such as motor behavior and reward, remain poorly understood.

Based on evidence from anatomy, brain imaging, and neurophysiology, we hypothesized that posterior cingulate cortex (CGp) contributes to the processing of informational salience, independent of its sensory provenance. CGp is extensively interconnected with brain areas involved in attention, such as parietal cortex, as well as brain areas involved in motivation and emotion, such as anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) (A. McCoy & Platt, 2005a; Pandya et al., 1981; Parvizi et al., 2006). Furthermore, brain imaging studies demonstrate activation in CGp in response to salient visual events (B. Hayden et al., 2009; C.
Olson et al., 1996), overt orienting (Berman et al., 1999), emotional events (Greene, Nystrom, Engell, Darley, & Cohen, 2004; Maddock, 1999; Morrison et al., 2006), recalling information from autobiographical memory (Cabeza et al., 2003; Maddock, Garrett, & Buonocore, 2001), and reward itself (Kable & Glimcher, 2007; Knutson et al., 2003; Kuhnen & Knutson, 2005; Nieuwenhuis et al., 2005; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001; Small et al., 2003).

Moreover, neurophysiological studies have shown that CGp neurons respond phasically to infrequent or unpredictable visual stimuli and overt orienting (Dean et al., 2004; C. Olson & Musil, 1992) as well as task-relevant reward delivery and omission (A. N. McCoy et al., 2003). Furthermore, CGp neurons respond with tonic modulations in activity to signal reward expectations (A. N. McCoy et al., 2003; Kuhnen & Knutson, 2005; B. Y. Hayden et al., 2008), memory for reward outcomes (B. Y. Hayden et al., 2008), choice (A. McCoy & Platt, 2005b; B. Y. Hayden et al., 2008), and reward uncertainty (A. McCoy & Platt, 2005b). Together, these observations are consistent with the hypothesis that posterior cingulate cortex contributes to the detection and transmission of salient information independent of sensory modality (e.g., visual vs. gustatory), overt orienting, or active decision making.

To test this hypothesis, we studied the activity of 91 CGp neurons in four task contexts in two male macaque monkeys. In three contexts, monkeys received juice reward for either choosing a target for overt orienting based on its color-reward associations, shifting gaze to a single target associated with reward, or fixating while a peripheral stimulus was illuminated followed by reward. In the fourth context, monkeys received uncued reward. In each context, we measured phasic responses by comparing activity following events such as visual cue onset, saccade initiation, and reward delivery to the immediately preceding pre-event baseline. Neurons responded to informative visual stimulus onset in all three contexts, whether or not overt orienting to target was required; following saccade initiation whether a choice
was or was not required; and to both cued and surprising reward. Furthermore, CGp
neurons responded as often to uncued, surprising reward as to cued reward. These
results suggest that CGp dynamically signals salient information that contributes
to the on-line assessment of the sensory environment, monitoring of movement, and
evaluation of reward.

2.2 Results

To confirm that monkeys learned the reward associated with red and green cues, we
examined choices between large and small rewards in a color-based target choice task
(Figure 2.1). In this task, a large reward was associated with one color and a small
reward with the other in a block of trials. Notably, the location of the colored cues
was not predictable (Lau & Glimcher, 2005, 2008; Sugrue, Corrado, & Newsome,
2004). Monkeys chose the cue associated with the large reward more often than the
small reward across the range of potential reward options (Figure 2.4a). Monkeys
behavior was well fit with a logistic function with a steep slope indicating excellent
reward-color association (beta=0.04, p<0.01).

Sensitivity to color-cued reward information was also evident in single-target
operant trials in which monkeys shifted gaze to a single target location that was
differentially rewarded based on target color (Figure 2.2). Consistent with a pre-
vious report (A. N. McCoy et al., 2003), the slope of the line relating peak eye
velocity to saccade amplitude (the main sequence) decreased as reward magnitude
increased (Figure 2.4b: linear regression R = 0.045, p<0.01). In addition, reac-
tion time increased as reward magnitude increased (Figure 2.4c: linear regression
R=0.062, p<0.01). On such delayed saccade trials, increased response latency and
decreased velocity may reflect a speed-accuracy trade-off motivated by the desire for
larger rewards (Dean & Platt, 2006).

Monkeys also performed Pavlovian fixation trials in which they received reward
after maintaining central fixation while a red or green peripheral cue was illuminated; again, color predicted reward size within a block of trials (Figure 2.3). Reward magnitude did not influence the probability of error in any of the tasks (choice, error probability in large reward vs small reward trials, n.s.; operant, n.s.; Pavlovian, n.s.); since the monkeys average error rates on these tasks are low (choice: BR 6.4% error, NI 16.5%; operant: BR 8.6%, NI 14.9%; Pavlovian: BR 5.9%, NI 16.3%), this observation suggests that the monkeys were strongly motivated to collect as much reward as possible within an experimental session. Next, we assessed whether neurons in CGp responded to the occurrence of salient events while monkeys performed each of these tasks as well as following uncued rewards. We recorded from a total of 91 CGp neurons across all tasks. A subset of these neurons were studied in each task: colored target choice, 38 neurons; single target operant task, 30 neurons; Pavlovian fixation task, 48 neurons; uncued reward delivery, 68 neurons. Each task had a different set of salient events. The choice task included illumination of two targets, choice via saccade, and reward delivery. The operant orienting task included illumination of a single target in a predictable location, gaze shift, and reward delivery. The Pavlovian fixation task included fixation onset, peripheral target onset, and reward delivery. Finally, the uncued reward task included only the unpredictable delivery of reward. To probe neuronal responses to these events, we compared neuronal activity in the two 200ms epochs following each event to a 200ms epoch (baseline) immediately preceding the relevant event.

Figure 2.5 shows peri-stimulus time histograms (PSTHs) for two example neurons recorded during the color choice task; one neuron reported target onset (a), and the other responded after saccades (b) and reward delivery (c). Across the subpopulation of 38 neurons, CGp neurons responded to all three salient events with either increased or decreased firing rates, as described previously (McCoy et al., 2003, (A. N. McCoy et al., 2003)). Across epochs, more cells responded by significantly increasing activity
after saccade (Table 2.1), while more cells tended to decrease activity after reward (Table 2.1). Since a sizeable fraction of CGp neurons shows some degree of spatial selectivity (Dean et al., 2004; C. R. Olson, Musil, & Goldberg, 1993; C. Olson et al., 1996; C. Olson & Musil, 1992), we also assessed whether phasic responses were modulated by chosen target location. To test this possibility, we used a factorial ANOVA to identify significant interactions between neuronal responses to events and chosen target location. (ANOVA, firing rate vs epoch and chosen target location). Neurons were most likely to distinguish spatial location following saccade onset (Table 2.1); only a small fraction of the population signaled target location following cue onset (2 neurons) or reward delivery (4 neurons), consistent with previous results (A. N. McCoy et al., 2003). To examine the aggregate response, we sorted the cells by whether responses to each event were positive or negative (cue, saccade, reward; cells with significant or nonsignificant event responses) and calculated the mean firing rate per epoch for each group (Figure 2.5c,d). Both populations responded significantly after all events (though activity in the increasing population did not differ from baseline in the second cue epoch).

These observations confirmed that CGp neurons report salient events, such as visual cue onset and reward delivery, that contribute to reward associations and the expression of choice. We next asked whether neuronal activity was modified by events in an operant context where cues were informationally relevant but choice was not required and monkeys earned reward by shifting gaze to a single target (Figure 2.2). We found neurons that reported cue, saccade and reward on these trials. The activity of the first example neuron shown in Figure 2.6a,b increased following target onset and saccade onset, while that of the second neuron (c,d) decreased following saccade onset and reward. Many neurons responded to events with positive and negative changes in firing frequency (Table 2.1). Again, neurons responded more strongly following saccade than following cue or reward, both individually (Table 2.1) and
across the population (Figure 2.6c,d).

Having confirmed that CGp neurons report salient events associated with overt orienting for rewards, we next asked whether CGp neurons also respond phasically to visual stimuli and rewards in a Pavlovian context, where conditioned color cues predict reward in the absence of gaze shifts. As in the choice and operant tasks, neurons responded to both cue onset and reward delivery (Figure 2.7a,b; example neurons). In the subpopulation of 48 neurons, more neurons responded to reward delivery than to cue onset (Table 2.1). Figure 2.7c,d shows the neuronal population activity sorted by positive or negative responses. These data make plain that CGp neurons respond phasically to informationally relevant information independent of active choice or overt orienting for reward (contra (C. Olson et al., 1996) but consistent with (C. R. Olson et al., 1993)).

The presence of phasic responses to task events across all three contexts suggest the possibility that CGp neurons respond to any surprising or motivationally informative events, including uncued reward delivery in the absence of visual stimulation. To test this idea, we studied the neuronal activity of 68 CGp neurons while monkeys received uncued rewards while sitting quietly in the experimental chamber. Consistent with data from the three active tasks, CGp neurons responded to reward delivery with increasing (Figure 2.8a) or decreasing (Figure 2.8b) phasic responses. Across the subpopulation, more neurons responded with decreasing firing rate than increasing firing rate (Table 2.1). To summarize population activity, we sorted the neurons into groups that responded to reward with increased (Figure 2.8c) or decreased (Figure 2.8d) phasic response and plotted the mean activity during the three epochs analyzed surrounding reward. We found that both groups of neurons responded significantly to reward in both epochs.

Since neurons responded both to rewards associated with task performance and after delivery of uncued rewards, we asked whether the probability or magnitude
of responses to reward varied across contexts. We speculated that phasic neuronal responses to reward might vary with the informational demands of each task context. To test this idea, we first compared the proportions of neurons that responded to reward in either post-reward epoch (Choice task, 44.5% responded; Operant, 53.3%; Pavlovian, 39.6%; Reward, 32.3%) across the four contexts using pairwise t-tests to look for significant differences between each pair of contexts (i.e. Choice vs Operant, Choice vs Pavlovian, etc; one-way ANOVA including all four groups n.s., $F=1.4$, all post-hoc t-tests n.s. corrected for multiple comparisons, Tukey HSD). None of these comparisons revealed significant differences across these four contexts.

2.3 Discussion

Our results demonstrate that CGp neurons respond phasically to informative events in a variety of contexts, independent of sensory stimulation, active choice, or overt orienting. Consistent with previous reports (Dean et al., 2004; C. Olson et al., 1996), the spatial sensitivity of these phasic responses was strongest immediately following saccade initiation. Most notably, CGp neurons responded to uncued rewards outside of task context as well as to rewards delivered to motivate behavior (contra (C. Olson et al., 1996) but see (C. R. Olson et al., 1993)). We cautiously interpret this observation as suggesting that phasic CGp responses may reflect the occurrence of informationally-significant events independent of their sensory or motor provenance. If so, CGp activity may signal the dynamic allocation of neural processing to information in the ongoing processing stream that links the internal and external environments.

Some learning theories have emphasized the importance of the vividness, or salience, of informative events for drawing attention and inducing learning (Hall, 1994). Strongly predictive conditioned stimuli provide information relevant to behavior: in a decision context, known reward associations contribute to choice forma-
tion; in an operant context, conditioned stimuli prompt behavioral responses; and in Pavlovian contexts, conditioned stimuli predict future events. Previously published data showed that responses of CGp neurons to conditioned stimuli approximately parallel behavioral indices of learning (Gabriel & Orona, 1982; A. McCoy & Platt, 2005b), suggesting that these neurons may bind motivational information to external events (Freeman, Cuppernell, Flannery, & Gabriel, 1996). Our observation that CGp neurons respond to informative events in a variety of contexts (decision, operant and Pavlovian), support the hypothesis that these neurons monitor the environment for behaviorally important information.

In classic learning models, stimulus-response relationships that are fully predicted induce no new learning (Kamin, 1969; Rescorla & Wagner, 1972). Thus, unpredictable stimuli contribute more to new learning than do predictable stimuli (Dickinson, 1980; Mackintosh, 1975; Pearce & Hall, 1980). This fundamental observation is summarized as associative strength in classical conditioning or attentional theories of learning, belief uncertainty in Bayesian updating models, and as a reward prediction error in temporal difference learning algorithms (Courville, Daw, & Touretzky, 2006; Pearce & Hall, 1980; Rescorla & Wagner, 1972; Sutton & Barto, 1981; Sutton, 1988). Rare and unexpected events, such as the surprising rewards used in this study, modulate both the allocation of attention and learning rate (Shannon, 1948; Pearce & Hall, 1980; Posner et al., 1980; Einhäuser, Mundhenk, Baldi, Koch, & Itti, 2007; Itti & Baldi, 2008).

Such novel or changing stimuli activate neurons in the basolateral amygdala (BLA), which may bind new task-relevant information to appropriate stimuli (Holland & Gallagher, 2004; Pickens et al., 2003). Unlike CGp neurons, BLA neurons do not maintain representations of stimulus-association encoding after learning (Gottfried, O’Doherty, & Dolan, 2002) and do not obviously encode reward valuation. They do, however, reflect stimulus intensity and novelty (Gottfried et al., 2002;
J. P. O'Doherty, Deichmann, Critchley, & Dolan, 2002), consistent with a role in the recognition and encoding of new information. Similarly, the phasic responses of CGp neurons to unexpected reward and reward omission (A. N. McCoy et al., 2003) suggest sensitivity to the informational content of events. In this view, CGp may serve as a general-purpose learning device (Gabriel, Sparenborg, & Kubota, 1989) that serves to allocate neural resources to the ongoing stream of information about external stimulus events, rewards and punishers, decisions, and motor behavior. Future functional studies using lesion or microstimulation techniques will be needed to fully evaluate this hypothesis.

2.4 Materials and methods

2.4.1 Surgical and behavioral procedures

All procedures were approved by the Duke University Institutional Animal Care and Use Committee and were designed and conducted in compliance with the Public Health Services Guide for the Care and Use of Animals. Using standard surgical techniques, a head restraint prosthesis, scleral search coil and small stainless steel recording chamber were surgically implanted. The chamber was located over posterior cingulate cortex, at the intersection of the midsagittal and interaural planes, and was kept sterile with regular antibiotic washes and sealed with sterile caps (cf. (Dean et al., 2004)). The animals received analgesics and antibiotics after all surgeries.

Monkeys indicated their choices and performed tasks with gaze shifts. Eye position was sampled via a scleral search coil at 500 Hz (Riverbend Instruments) and recorded by computer (Gramalkn Experiment Control System, Ryklin Software). Visual stimuli were LEDs presented on a large stimulus display panel (LEDtronics); the LEDs were positioned one degree apart and fill 50 degrees of the visual field on the horizontal axis and 40 degrees on the vertical axis. A computer-driven solenoid was used to deliver liquid rewards whose magnitude was linearly correlated with the
open time of the solenoid.

2.4.2 Behavioral paradigms

We recorded from 91 single neurons in the posterior cingulate cortex of two male rhesus macaques while they either performed one of three tasks (saccade color choice trials, saccade single target trials and continuous fixation trials) or received reward delivered at random, unpredictable times (reward only trials). In each task, red and green peripheral target LEDs on an LED board (A. N. McCoy et al., 2003) were used to indicate high or low reward delivery, and the color-reward associations were reversed every 25-50 trials. Fluid reward delivery on successful trials was controlled by solenoid open time as described previously (A. N. McCoy et al., 2003; A. McCoy & Platt, 2005b; B. Y. Hayden & Platt, 2007), but within each task, failure to complete a trial through peripheral target fixation was not rewarded (error trial).

2.4.3 Task contexts

Choice trials. Two target LEDs were placed diametrically around a central fixation point. In each trial, one LED was red and the other green, but the color-location association was varied randomly. One color was associated with a large reward on every trial and the other with a small reward. The color-reward associations varied at the end of every block of 25-50 trials. To start a trial, the monkey was required to foveate a centrally located LED. Then the two peripheral targets were illuminated and after a brief delay, the central fixation LED was turned off, cuing the monkey to fixate on either of the two peripheral targets. After fixation, juice reward was delivered via a computer-driven solenoid.

Operant trials. Each trial again began with foveation of a central illuminated LED. A randomly selected red or green LED was illuminated in only a single target location following central fixation. When the central LED turned off, the monkey fixated on
the target LED to receive a fluid reward. The LED colors again indicated high or low reward size within a block of 25-50 trials, and color-reward associations were varied at the end of every block; target location did not vary within a session.

Pavlovian (conditioning) trials. As in the saccade single target trials, monkeys fixated on a central illuminated LED to begin the trial and trigger illumination of a single red or green peripheral LED in the neuronal receptive field. However, the central and target LEDs were turned off simultaneously, and the monkey was not required to saccade to the target LED. Target LED color cued high or low reward within each block and this association changed at the end of every block, but target location remained constant within each session.

Uncued reward trials. The monkeys received unpredictable, irregular juice rewards while sitting in a dimly lit room (backlighting at 12V for monkey BR and 5V for monkey NI) in the absence of any task or pertinent visual stimuli. All rewards were the same size (solenoid open time 150 ms). Microelectrode recording techniques. Single electrodes (Frederick Haer) were lowered by microdrive (Kopf) until the waveform of a single neuron was isolated. Individual action potentials were identified in hardware by time and amplitude criteria (BAK electronics). Neurons were selected on the basis of the quality of isolation and visual or saccade-related modulation. We used a hand-held digital ultrasound device (Sonosite 180) placed against the recording chamber to confirm that recordings were made in areas 23 and 31 in the cingulate gyrus and ventral bank of the cingulate sulcus, anterior to the intersection of the marginal and horizontal rami. Analysis Custom Matlab functions were used to process and analyze neuronal activity and eye movements. Neuronal spike rates (Hz) were calculated during three 200ms epochs surrounding each event (cue illumination, saccade initiation, reward delivery). The baseline firing rate for each event was defined as the
mean activity in the 200ms epoch preceding each event, and two 200ms post-event epochs were defined immediately following each event (0-200ms, 200-400ms). Thus, we analyzed neuronal activity in nine 200ms epochs for the choice and operant task (cue, saccade, reward), six 200ms epochs for the Pavlovian task (cue, reward), and three for the uncued reward task (reward). We used one-way ANOVAs and t-tests to compare the firing rate in each post-event epoch to the baseline measured before the relevant event (significance at p<0.05).
2.5 Figures and Tables
Figure 2.1: Choice task: monkeys chose between two distinctly colored visual targets. Within each block of 25-50 trials, each color (red, green) cued a single reward size but appeared randomly at the two symmetrically placed target locations. Top panel, trial events; bottom panel, sample reward schedule.
Figure 2.2: Operant (saccade) task: monkeys saccade to a single target location to receive reward. Monkeys fixated the central cue to begin each trial. After a single peripheral colored target was illuminated (one target location per session), the monkeys maintained fixation until the central cue dimmed. They received a reward (cued by color) after looking at the peripheral target.
**Figure 2.3:** Pavlovian (fixation) task: monkeys receive reward after continuously fixating a central cue during peripheral target presentation. Monkeys fixated a central cue to begin each trial, and maintained fixation while a peripheral colored target was illuminated. Once the peripheral target dimmed, monkeys received a reward, with magnitude cued by color, and the trial ended.
Figure 2.4: Monkeys distinguish large from small rewards. A. Choice behavior. Monkeys choose the large reward more often across all pairs of reward options, confirming that they distinguished large and small rewards. B, C. Monkeys responses distinguish large from small rewards in the operant task. The slope of the line relating peak eye velocity to saccade amplitude (the main sequence) was inversely correlated to reward magnitude (B). Additionally, the monkeys response latency increased as reward magnitude increased (C), confirming that monkeys were sensitive to reward size on single-target trials.
Figure 2.5: CGp neurons respond to informationally relevant events in the choice task. An example neuron that responds to target onset by sharply increasing firing rate is shown in A. The peri-stimulus time histogram (PSTH) is aligned to target onset; arrows indicate target onset, saccade initiation and reward delivery. The light grey bar highlights the 200ms baseline epoch preceding target onset, and the two dark grey bars show the two 200ms epochs analyzed following target onset. Similarly, B and C show a neuron that responded after saccades (B, aligned to saccade initiation) and rewards (C, aligned to reward delivery); light grey bars indicate baseline epochs before relevant events, and dark grey bars enclose post-movement and post-reward epochs. Post-event epochs with firing that differs significantly from the relevant pre-event epoch are indicated with an asterisk (p<0.05). D. Population activity of cells with increasing activity during either of the two epochs following each event; E, cells with decreasing activity. Light bars indicate baseline activity during the 200ms epoch preceding each event; dark bars show activity during the two 200ms epochs following each event. Asterisks indicate a significant difference between the relevant epoch and the relevant baseline (p<0.05). Error bars are standard error of the mean.
Neurons respond to relevant events in the operant task. A, B. PSTH showing the activity of a single neuron whose activity increased immediately following target onset (A, aligned to stimulus onset) and eye movement (B, aligned to saccade); panels C and D illustrate the firing of a single neuron whose activity decreased following both movement (C, aligned to saccade) and reward (D, aligned to reward). Arrows indicate salient events; light grey bars include the baseline epoch preceding an event, while dark grey bars indicate the two epochs analyzed following an event; asterisks indicate that activity in a post-event epoch differed significantly from the baseline activity preceding the relevant event (p<0.05). Neuronal activity sorted by response direction (E, increasing from baseline; F, decreasing). Light bars indicate baseline activity during the 200ms epoch preceding each event; dark bars show activity during the two 200ms epochs following each event. Asterisks indicate a significant difference between the relevant epoch and the relevant baseline (p<0.05). Error bars, S.E.M.
**Figure 2.7:** CGp neurons respond to visual stimuli and reward in the Pavlovian task. The single neuron shown in A increases firing following target onset and reward (PSTH aligned to target onset), while the neuron shown in B decreases activity following reward (aligned to reward). Light grey bar, 200ms baseline epoch; dark bars, post-event epochs. Asterisks indicate a significant difference between firing rate in an epoch and that of the immediately preceding baseline epoch. CGp population activity calculated after sorting individual neurons by response direction (C, increasing from baseline; D, decreasing). Light bars indicate baseline activity during the 200ms epoch preceding each event; dark bars show activity during the two 200ms epochs following each event. Asterisks indicate a significant difference between the relevant epoch and the relevant baseline (p<0.05). Error bars, S.E.M.
Figure 2.8: CGp neurons report uncued rewards. Individual posterior cingulate neurons respond to reward delivery. A. PSTH for a single CGp neuron whose activity increased significantly following reward delivery. B. PSTH for a neuron whose activity decreased after reward. Light grey bar, 200ms baseline epoch; dark bars, post-event epochs. Asterisks indicate significantly different neuronal activity relative to the baseline epoch. C, D: Posterior cingulate population responses to reward calculated from groups of neurons sorted by increasing (C) or decreasing (D) activity following reward. Light grey bars indicate mean baseline frequency; dark grey, activity during the early (0-200ms) and late (200-400ms) epochs after reward. Asterisks indicate a significant change relative to baseline. Error bars are S.E.M.
Table 2.1: Neuronal responses to events across contexts. We compared neuronal activity during two 200-ms epochs (early: 0-200ms; late: 200-400ms) after each event (cue, saccade, reward) to a 200-ms baseline immediately preceding the relevant event (i.e. -200-0ms). In each of the four task contexts, neurons responded to events by both increasing and decreasing activity relative to baseline. Numbers in parentheses indicate total number of neurons in each task; boxes left blank reflect inapplicable analyses, e.g. the absence of saccade in the Pavlovian task.

<table>
<thead>
<tr>
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<th>Choice (38)</th>
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<th>Pavlovian (48)</th>
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<td>Early reward</td>
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In economics, decision making is often modeled as a policy of maximizing subjective expected utility. By contrast, applying a heuristic, or rule of thumb, can minimize the need for explicit utility calculations, thus reducing cognitive load. One such heuristic, the WSLS strategy, renders decisions based on a simple comparison between rewards received and a threshold. Previous studies have implicated dorsolateral prefrontal cortex and anterior cingulate cortex, brain regions involved in behavioral control, in the application of strategic heuristics to action selection. However, the role of heuristic coding in the neural transition from outcome valuation to action selection remains obscure. To examine these influences, we recorded from neurons in CGp, a brain area implicated in reward evaluation and risky decision making. Monkeys repeatedly chose between a probabilistic reward and a guaranteed option in two reward contexts. Although preferences varied with context, monkeys’ local choice patterns followed a relative-outcome-based decision rule analogous to the classic WSLS strategy. Consistent with contextual effects on preferences in this task, neuronal activity in CGp reflected the history of both rewards and choices; impor-
tantly, neuronal representation of choice bias also depended on context. Consistent with heuristic encoding of rewards, neuronal activity differentiated above-threshold “wins” from below-threshold “losses”. Finally, these signals predicted the likelihood of staying with the same choice or exploring the alternative on the next trial, concordant with the use of a WSLS strategy. These results support the hypothesis that CGp contributes to a heuristic-based evaluation of rewards and strategic decision making.

3.1 Introduction

Normative decision making models often assume that choosers compute the subjective expected utility of available options in a Bayesian fashion using complete knowledge of the environment (Bernoulli, 1738/1954; Neumann & Morgenstern, 1944). In this framework, decisions between alternatives are rendered by comparing their relative subjective utilities, which are monotonic functions of their objective values. Alternatively, the cognitive demands of such decisions can be simplified by the use of strategic rules of thumb, or heuristics (Simon, 1955; Gigerenzer, 2008). One such strategy, Win-Stay-Lose-Shift (WSLS), compares experienced rewards to a subjective threshold, or aspiration level, to choose between exploitation, or repeating choices, and exploration, or sampling alternatives. The uncertainty inherent in adaptive decision making encourages strategies that select exploration or exploitation based on integration of previous experience (Daw, O’Doherty, Dayan, Seymour, & Dolan, 2006) or critical re-evaluation of options’ estimated subjective values (Bayer & Glimcher, 2005; J. O’Doherty, Critchley, Deichmann, & Dolan, 2003; P. N. Tobler, O’doherty, Dolan, & Schultz, 2006). Alternatively, when choice patterns depend more strongly on recent history than on long-term integration, such strategies use comparisons between recent reward and a normative reward threshold (Lee, Conroy, McGreevy, & Barraclough, 2004; Soltani, Lee, & Wang, 2006) as the relevant
decision variables. Although recent research has implicated dorsolateral prefrontal cortex (DLPFC), ACC, and LIP in experience-based decision making (M. Platt & Glimcher, 1999; Barraclough et al., 2004; Kennerley et al., 2006), the role of strategic heuristics in the transformation of outcome valuation to action selection, both within and across reward contexts, remains unclear.

One brain area that may contribute to heuristically-guided reward outcome evaluation and decision formation is the posterior cingulate cortex (CGp). Anatomically, CGp is connected with brain areas involved in cognitive control, including ACC; action selection, such as the supplementary eye fields and LIP; and reward and motivation, such as the amygdala and caudate (Pandya et al., 1981; Morecraft et al., 1993; A. McCoy & Platt, 2005a). Moreover, neuronal activity in CGp reflects reward magnitude (B. Y. Hayden et al., 2008) and omission (A. N. McCoy et al., 2003), tracks reward uncertainty (A. McCoy & Platt, 2005b), and predicts future choices in a simple two-alternative task (B. Y. Hayden et al., 2008). Finally, microstimulation in CGp promotes switching away from a risky gamble toward a safe alternative (B. Y. Hayden et al., 2008).

We tested whether CGp activity reflected the use of a heuristic model of outcome evaluation by recording the activity of neurons in this area while monkeys chose between a safe option and a risky gamble in two contexts. Across contexts, we found that choices reliably followed a simple heuristic analogous to WSLS. Moreover, CGp neurons encoded both choices and outcomes in a simplified scheme compatible with this heuristic; their activity reflected choice bias within contexts but was rescaled across contexts ACCording to the WSLS heuristic. Furthermore, across contexts CGp neurons predicted future choices. Our results demonstrate that CGp activity reflects reward categorization and predicts decision probabilities in a manner compatible with a simple heuristic encoding, thus implicating this area in outcome evaluation and decision making guided by such strategies.
3.2 Materials and methods

3.2.1 Surgical and behavioral procedures.

All procedures were approved by the Duke University Institutional Animal Care and Use Committee and were designed and conducted in compliance with the Public Health Service’s Guide for the Care and Use of Animals. Using standard surgical techniques, a head restraint prosthesis, scleral search coil and small stainless steel recording chamber were surgically implanted. The chamber was located over posterior cingulate cortex, at the intersection of the midsagittal and interaural planes, and was kept sterile with regular antibiotic washes and sealed with sterile caps (cf. (Dean et al., 2004). The animals received analgesics and antibiotics after all surgeries.

3.2.2 Behavioral tasks

Monkeys indicated their choices with gaze shifts. Eye position was sampled via a scleral search coil at 500 Hz (Riverbend Instruments) or an infrared camera at 60 Hz (Arrington) and recorded by computer (Gramalkn Experiment Control System, Ryklin Software). Visual stimuli were yellow circles (1°; ±10° fixation accuracy required) presented on a computer monitor positioned 50cm in front of the monkey. A computer-driven solenoid was used to deliver liquid rewards.

During gambling trials, two targets were illuminated diametrically around a central fixation circle (Figure 3.1a). One target was associated with a “safe” reward outcome of a constant amount of juice on every trial, while choosing the other, “risky,” target resulted in either a larger or smaller juice reward, each with probability 0.5. The locations of the safe and risky targets were varied every 25-50 trials. The monkeys were free to select either target. In an initial set of behavioral experiments, the sizes of the large and small rewards offered for the risky option, as well as the size of the reward offered for the safe option, were varied independently.
For electrophysiological recordings, we then selected one reward context in which monkeys consistently preferred the risky option ("Equal Values" Condition: safe, 143 uL juice; risky, 43 or 243 uL), and one context in which monkeys preferred the safe option ("Biased Values" Condition: safe, 213 uL; risky, 43 or 243 uL). In each recording session, we randomly varied both the reward context and the locations of the safe and risky targets every 25-50 trials. Only recording sessions with a minimum of four blocks — fully counterbalanced, with two for each reward context and two for each location assignment — were included in analysis.

3.2.3 Microelectrode recording techniques.

Single electrodes (Frederick Haer) were lowered by microdrive (Kopf) until the waveform of a single neuron was isolated. Individual action potentials were identified in hardware by time and amplitude criteria (BAK electronics). Neurons were selected on the basis of the quality of isolation and visual or saccade-related modulation. We used a hand-held digital ultrasound device (Sonosite 180) placed against the recording chamber to confirm that recordings were made in areas 23 and 31 in the cingulate gyrus and ventral bank of the cingulate sulcus, anterior to the intersection of the marginal and horizontal rami.

3.2.4 Analysis

Data were analyzed off-line using custom Matlab (The MathWorks, Inc.) scripts to compute saccade direction, amplitude, latency, and peak velocity. Custom Matlab and R scripts were used to complete additional data processing. Statistics were computed using Matlab or Statistica. For behavioral data, we calculated the mean frequency of risky choice per day for each set of available rewards and used multiple regression and ANOVAs to quantify the relationship between available reward and the probability of choosing the risky target. To further explore the local influences
on decisions, we fit each monkey’s behavior using a model (Lau & Glimcher, 2005) that incorporates experienced reinforcement, previous choices, and contextual bias:

\[ \log(p_R/p_S) = \sum_{j=1:n} (\alpha_j(r^j_R - r^j_S)) + \gamma \]

where \( p_R \) = probability of a risky choice and \( p_S \) probability of a safe choice. The right-hand side of the equation comprises three pieces: a weighted sum over past trials of reward outcomes (\( r^j_R = \) reward received from a risky choice on trial \( j \), \( r^j_S = \) reward received from a safe choice on trial \( j \)); a weighted sum over previous choices (\( c^j_R = 1 \) for a risky choice, 0 otherwise; \( c^j_S = 1 \) for a safe choice, 0 otherwise); and an overall bias toward or away from the risky option (\( \gamma \)). We used Akaike’s Information Criterion (AIC) to compare the explanatory power of models including a moving window of one to ten trial histories prior to the current trial, and retained the window size that produced the best fit (lowest AIC value).

We measured firing rates for each trial during three 400-ms intervals, each aligned to a specific event during the trial: (i) 0-400ms following illumination of the eccentric targets (“post-target”); (ii) 0-400ms following saccade onset (“post-choice”); (iii) 50-450ms following reward onset (“post-reward”; excluding a 50ms window immediately following reward onset to remove the solenoid artifact). We used ANOVAs to determine the relationships between neuronal firing rates and contextual choice allocation as well as between neuronal activity and outcomes with respect to context. Logistic regression was used to quantify the relationship between neuronal firing rate and probability of repeating a choice, independent of reward.

### 3.3 Results

#### 3.3.1 Monkeys use a threshold-comparative decision heuristic

Previous studies from our lab showed that monkeys are risk-seeking when offered a choice between constant rewards and risky gambles with equivalent mean expected
values (A. McCoy & Platt, 2005b) but adapt to delayed reward availability in the same task by more frequently choosing the safe option (B. Y. Hayden & Platt, 2007). Thus, monkeys’ choices in this task are context-dependent. We therefore predicted that increasing the value of the safe reward relative to the expected value of the risky option would bias monkeys’ choices toward the safe option. To test this hypothesis, we modified the payoffs of the gambling task (Figure 3.1a) by varying both the risk (reward coefficient of variance) of the gamble and the magnitude of the safe reward (Figure 3.1b). For both monkeys, there was a significant inverse correlation between the ratio of the expected values of the two options (EVsafe/EVrisky) and the likelihood of choosing the risky option (Linear regression, mean frequency of risky choice per session and EV ratio vs EV ratio: monkey NI, R=0.59, p<0.01; monkey BR, R=0.68, p<0.01).

Given this behavior, we reasoned that a model that reflected both choice history and reward experiences would accurately describe the monkeys’ decisions. To quantify the effects of past reinforcement, preceding choices, and contextual bias on decisions, we used a model in which choice probabilities depend on each of these factors (Lau & Glimcher, 2005). Consistent with previous studies (A. McCoy & Platt, 2005b; B. Y. Hayden et al., 2008), we found that monkeys’ choices were best explained by their experiences on the most recent two trials (AIC1,2 <AIC3-10). Although choices were influenced by both previous choice and reward history, recent rewards influenced decisions more strongly than did prior choices (Table 3.1).

We therefore explored the relationship between reward outcomes and ensuing decisions. The probability of repeating a choice was correlated with reward size (Figure 3.1c); monkeys were most likely to repeat choices after receiving large rewards but sampled more frequently following small rewards, a pattern consistent with the application of the “win-stay/lose-switch” heuristic. In this strategy, rewards are classified as “wins” or “losses” relative to an internal threshold, or aspiration level (Si-
mon, 1955). To test this idea formally, we estimated a normative reward threshold based on the reward size at which monkeys were equally likely to switch or stay with the preceding choice (Figure 3.1d; 203 uL juice reward). We then categorized rewards greater than the 203 uL threshold as “wins” and designated rewards less than this threshold as “losses”. Consistent with a WSLS heuristic, the monkeys repeated choices significantly more often following wins (82.8 ± 2.8% repeats) than after losses (39.1 ± 3.8% repeats; ANOVA, mean daily frequency of repeating a choice after a win or loss vs previous outcome (win/loss), F=81.9, p<0.01).

Based on these findings, we identified two reward contexts for electrophysiological recordings: in the first, the mean expected values of the two options were equal (Equal value condition), but in the second, the safe option offered a larger reward than the expected value of the risky option (Biased value condition). To confirm that the monkeys’ behavior reflected the use of a WSLS strategy in these two contexts, we determined the probability of repeating a choice following above-threshold rewards, or wins, and below-threshold rewards, or losses (Figure 3.1e). As predicted by this strategy, the monkeys almost always repeated choices after wins (EV: 90.0% ±1.5 (SEM) repeats, BV: 91.1% ±1.6) but were more likely to sample the alternative option after losses (EV: 57.8%±4.2 repeats, BV: 51.5%±2.8; main effects ANOVA, mean probability of repeating per session and outcome category vs reward context and win/loss outcomes: reward context n.s., win/loss F=168.46, p<0.01).

3.3.2 Neuronal activity reflects contextual choice bias

Previous studies have shown that the activity of CGp neurons varies with decision variables such as the subjective value of a target (A. McCoy & Platt, 2005b) or the likelihood of choosing a particular option in the future (B. Y. Hayden & Platt, 2007). Based on these observations, we hypothesized that neuronal activity in CGp would also reflect context-dependent decisions arising from simple heuristics like WSLS. To
test this idea, we proposed that neuronal activity reflected the monkeys’ empirical choice biases in each context. In addition, we hypothesized that CGp activity would reflect a heuristically-defined categorization of reward outcomes as wins and losses. We then reasoned that if CGp mediated heuristic decision making, neuronal activity would encode both reward outcomes and switch/stay decisions. Thus, neuronal activity should differentiate the possible outcome/decision combinations. Furthermore, we predicted that if CGp contributed to the development of heuristic decisions, neuronal activity would predict ongoing and future stay or switch decisions.

To test these predictions, we first assessed the relationship between neuronal activity and the frequency of choosing risky and safe options across contexts. We identified neurons whose activity reflected context-dependent choice frequencies by a factorial ANOVA comparing firing rates across choice (risky vs safe) and context (equal value vs biased value). Within the population of 38 neurons, 26 cells (68%) had activity that was significantly modulated by choice allocation during at least one epoch (post-target, post-choice, post-reward; Table 3.2). Figure 3.2 (A,B) shows the activity of one such neuron following choice. Firing rate increased following choices of the risky option in the Equal value context and following choices of the safe option in the Biased value context (ANOVA: choice n.s., context n.s., interaction term F=10.97, p<0.01), confirming that the activity of these neurons reflects choice bias. Across the population (Figure 3.2 C, D, E), neuronal activity was also significantly modulated by choice bias during the 400 ms following target onset (ANOVA, choice n.s. (p=0.051), context n.s., interaction F=49.07, p<0.01), following choice (choice F=4.67, p=0.03, context n.s., interaction F=32.34, p<0.01) and following reward delivery (choice n.s., context n.s., interaction F=15.93, p<0.01).

If, as this result suggests, population activity in CGp mediates observed decision behavior, firing rates should predict the reward ratio at which monkeys were indifferent to the two options (Padoa-Schioppa & Assad, 2008). Thus, if neuronal firing
rates in the post-choice epoch are directly proportional to the frequency of selecting a given option, it should be possible to estimate behavioral indifference points from firing rates (cf Figure 3.11b: Subject NI, EVsafe/EVrisky = 1.2; Subject BR, EVsafe/EVrisky = 1.3). Consistent with our hypothesis, we found that post-decision firing rates did predict the monkeys’ indifference point (EVsafe/EVrisky = 1.2).

Notably, although neuronal firing reflected relative choice bias – an indicator of both subjective expected utility and heuristic preference – within each reward context, the neuronal representation of the risky option rescaled across contexts. In all epochs, population activity was higher when the monkeys chose the risky option in the equal values context than when they chose the risky option in the biased values context. Thus, the representation of choice bias in CGp is relative and heuristic rather than absolute and invariant (but see (Padoa-Schioppa & Assad, 2008)).

3.3.3 CGp activity reflects heuristically defined outcomes

Next, since the monkeys’ behavior was well described by a WSLS strategy, we predicted that neuronal activity would reflect the recent history of rewards categorized as wins or losses by this heuristic. To test this idea, we analyzed the relationship between neuronal firing and wins and losses across contexts. In this analysis, the activity of the example neuron shown previously was significantly modulated by the previous reward outcome across contexts (Figure 3.3 3a,b; ANOVA, firing rate vs previous win/loss and context: win/loss F(1,133)=4.78, p=0.03, context n.s., interaction term n.s.). The activity of 26 neurons (68%) was significantly influenced by prior wins and losses as defined by the heuristic threshold (Table 3.3). Population activity (Figure 3.3c,d,e) also reflected previous wins and losses before (win/loss on the previous trial F=21.00, p<0.01, context n.s., interaction n.s. (p=0.07)) and after choice (win/loss on the previous trial F=12.49, p<0.01, context n.s., interaction n.s.), and after reward delivery (win/loss on the current trial F=50.39, p<0.01,
context $F=8.06$, $p<0.01$, interaction n.s.). Crucially, this pattern of response to wins and losses was independent of context, suggesting that CGp activity reflects strategically-relevant variables derived from the underlying task parameters.

### 3.3.4 CGp activity distinguishes outcome/decision combinations

We next reasoned that if CGp neurons mediate the translation of outcomes into heuristically-guided decisions, neuronal activity should distinguish the four possible outcome/choice situations (win-stay, loss-stay, win-switch, loss-switch). We therefore sorted trials into four categories based on preceding outcome and ensuing choice and used a one-way ANOVA to identify cells whose activity differed across these four categories. This analysis showed that the activity of 17 (50%) of the neurons studied, including the example neuron shown previously (Figure 3.4a; ANOVA, firing rate vs categories, $F=8.0$, $p<0.01$), varied significantly across these four situations (post-target epoch, 8 cells significant; post-choice epoch, 8 cells, post-reward epoch, 8 cells). Population activity also differentiated the outcome/choice combinations (Figure 3.4b,c,d, ANOVA, firing rate vs categories: post-target, $F=13.2$, $p<0.01$; post-saccade, $F=5.3$, $p<0.01$; post-reward, $F=14.6$, $p<0.01$). Notably, the response patterns of the population changed between the early and post-reward epochs. Neuronal activity following target onset (Figure 3.4b) and saccade (Figure 3.4c) differentiated between relative wins and losses as well as between stay/switch decisions (main effects ANOVA, firing rate vs previous trial’s win/loss outcome and current trial’s switch/stay decisions: post-target effect of outcome, $F=5.3$, $p<0.05$, effect of decision, $F=19.4$, $p<0.01$; post-saccade effect of outcome, $F=5.0$, $p<0.05$, effect of decision, $F=4.0$, $p<0.05$), implying an integration of outcome and decision information in these decision-related epochs. In contrast, neuronal activity immediately following reward delivery (Figure 3.4d) differentiated outcomes but not future decisions (main effects ANOVA, reward epoch firing rate vs the current trial’s outcome
and the next trial’s decision: effect of outcome, $F=33.7$, $p<0.01$; effect of decision n.s.), suggesting that CGp neurons encode outcomes but not decisions immediately following reward. Thus, CGp activity appears to shift from outcome encoding in the reward epoch to integrated outcome/decision encoding in the decision epochs. This temporal progression suggests that CGp neurons assess outcomes immediately following reward and translate these outcomes into decision biases, raising the possibility that neuronal activity during the decision epochs though not during the reward epoch – should predict decisions independent of reward outcomes.

3.3.5 **CGp activity predicts the probability of repeating a choice**

We therefore asked whether neuronal activity predicted the probability of repeating choices across contexts and across epochs. The post-sACCadic firing rate of the example neuron shown previously predicted the likelihood of repeating a choice (same example neuron, Figure 3.5a; data fit with cumulative normal function, switch/stay choice on the current trial vs firing rate: Wald stat 8.1, $p<0.01$). Since both neuronal activity and future choices depended on reward outcome, we repeated this analysis with previous reward included as an independent regression term to determine whether firing rate predicted future choices independent of reward outcome. Even after controlling for the effects of reward outcome on choice (Wald stat 7.4, $p<0.01$), the firing rate of the example neuron continued to significantly predict decisions (Wald stat 5.3, $p=0.02$). Likewise, 22 of the neurons studied (58%) significantly predicted decisions during at least one of the epochs analyzed (Table 3.4); of these, 15 (68%) predicted the probability of repeating a choice during the decision epochs even when previous outcome was included as a factor in the analysis. Only 4 neurons (18%) predicted choice on the next trial independent of outcome in the reward epochs, suggesting that neuronal encoding shifts from reflecting outcome during the reward epoch to predicting decision during the decision epochs.
Similarly, we used logistic regression to assess whether the probability of repeating a choice depended on population firing rate following target onset, choice and reward delivery; again, since rewards influence later choices, we also included the most recent outcome as a factor in this regression. CGp activity predicted the probability of repeating a choice during the two decision epochs (Figure 3.5b,c,d; ANCOVA, switch or stay on this trial vs firing rate and previous win/loss: post-target firing rate Wald stat 20.3, p<0.01 and previous outcome Wald stat 1052.0, p<0.01; post-sACCadic firing rate Wald stat 4.2, p=0.03 and previous reward Wald stat 1058.9, p<0.01) but not after reward (ANCOVA, switch or stay on the next trial vs firing rate and current trial’s win/loss: effect of post-reward firing rate Wald stat 0.96, n.s., and reward Wald stat 771.8, p<0.01). Thus, CGp neuronal activity shifts from reflecting outcome information immediately after reward to predicting decisions prior to choice. These data support the hypothesis that CGp neurons contribute to the expression of heuristically-guided behaviors by linking reward outcomes to decisions.

3.4 Discussion

Neuronal activity in CGp, as well as other brain areas (Leon & Shadlen, 1999; M. Platt & Glimcher, 1999; Tremblay & Schultz, 1999; A. N. McCoy et al., 2003; Wallis & Miller, 2003b; Cromwell, Hassani, & Schultz, 2005; Matsumoto & Hikosaka, 2008; Padoa-Schioppa & Assad, 2008), varies with rewards received and subjective biases for a particular option (A. McCoy & Platt, 2005b; Kable & Glimcher, 2007; B. Y. Hayden et al., 2008) in a particular context. Such observations are consistent with the hypothesis, motivated by economic theory, that CGp encodes the subjective utility of options under consideration or already chosen (A. McCoy & Platt, 2005b; Kable & Glimcher, 2007). By contrast, applying a heuristic, or rule of thumb, can minimize the need for explicit utility calculations, thus reducing cognitive load. We found that the choices of monkeys across two contexts were consistent with a simple
win-stay-lose-shift heuristic, which renders decisions based on a simple comparison between rewards received and a threshold (Simon, 1955). Monkeys more frequently repeated a choice following receipt of supra-threshold rewards (wins) but tended to explore the alternative option after subthreshold rewards (losses).

Categorizing reward magnitude relative to a threshold aspiration level ameliorates the difficulty of explicit analytic decisions required for classical utility maximization. In addition, this heuristic simplification allows accommodation across a large range of rewards by rescaling neuronal value representations within a finite range of firing rates. Macaques, like humans, may benefit from simplified computations that reduce cognitive load during decision making (Simon, 1955). Heuristic use explains behaviors such as loss aversion in humans (Camerer, 2000) and capuchins (Chen, Lakshminarayanan, & Santos, 2006) that would be considered irrational under classical utility assumptions. In such cases, decision-making agents simplify reward assessment by comparing gains and losses to a contextually determined reference point, or status quo (Kahneman & Tversky, 1979). Similarly, in iterative Prisoner’s Dilemma games, most humans use the aptly named WSLS heuristic to choose between cooperative behavior and defections (Wedekind & Milinski, 1996). Although similar threshold-comparative rules may apply across contexts, heuristic selection is sensitive to small environmental changes. Monkeys playing an inspection game against a computer component whose algorithm depended only on the monkeys’ previous choices used a WSLS strategy (Barraclough et al., 2004). However, when the computer’s strategy incorporated both reward and choice information, effectively neutralizing the benefit of a WSLS strategy, the monkeys optimized their decision strategy by abandoning WSLS in favor of random, unpredictable choice allocation. The fact that monkeys relied on simple biases except when confronted with a savvy opponent suggests that cognitive load reduction may be a fundamental goal of decision making—at least in monkeys. Such heuristics may also prove important for understanding the neuronal
circuits mediating decisions. Previous studies indicated that neuronal activity in posterior cingulate cortex varied with contextual biases (A. McCoy & Platt, 2005b) and reward outcomes (A. McCoy & Platt, 2005b; B. Y. Hayden et al., 2008), and predicted future decisions (B. Y. Hayden et al., 2008). Importantly, our observation that posterior cingulate’s representation of the risky option rescales across contexts indicates that CGp represents relative, heuristic biases rather than monotonically encoding absolute subjective values. Our results also demonstrate that CGp signals heuristically defined win/loss outcomes. In addition, neuronal activity distinguished WSLS outcome/decision combinations and predicted future WSLS decisions. These results suggest that CGp activity not only reflects variables important for updating strategic decision calculations but also contributes to the outputs of internal heuristic analyses.

The temporal evolution of CGp activity further supports the hypothesis that CGp contributes to heuristic decision making by translating outcomes into decisions. The transition from outcome-only to integrated outcome/decision encoding suggests that CGp contributes to future strategic decisions. This hypothesis is supported by the observation that microstimulation in CGp concurrent with reward delivery increases monkeys’ tendency to explore a less frequency chosen option within a single context (Hayden et al., 2008, (B. Y. Hayden et al., 2008)). Our observations suggest that this effect is due to posterior cingulate’s contribution to action selection via heuristic encoding of reward values: by categorizing rewards in a simple, context-dependent menu, CGp condenses rich contextual information and transforms outcome valuation for action selection via a computationally parsimonious decision rule.

Several other cortical areas have been implicated in outcome assessment, decision formation or decision expression. Several of these regions, including DLPFC, LIP and ACC, are reciprocally connected with CGp (Pandya et al., 1981; Vogt & Pandya, 1987; Morecraft et al., 1993). Neurons in DLPFC, like those in CGp, maintain out-
come information across trials and signal changes in behavior (Barraclough et al., 2004). The application of prior reward information to repeated decisions appears to depend on ACC activity: although monkeys with ACC lesions initially respond to changes in reward contingencies, they fail to sustain these preferences (Kennerley et al., 2006). This observation, in combination with the observation that ACC activity reflects the value of actions rather than objects (Matsumoto et al., 2007), suggests that ACC supports heuristic use across repeated decisions. These observations strongly suggest that ACC and CGp work together to evaluate reward outcomes and form decision biases using simple heuristics such as WSLS.
3.5 Figures and Tables

**Figure 3.1:** Monkeys use a win-stay/lose-shift heuristic to make decisions across contexts in a simple gambling task.
A. On each trial, a monkey initially fixated (1-2 deg) a central yellow LED (200-800 ms). Two peripheral, diametrically opposed, centrally equidistant yellow LEDs were then illuminated (200-800 ms). The fixation LED was then extinguished, cuing a free choice via gaze shift to either target (1-2 deg) within 350 ms. Correct trials were rewarded with juice and a 300 ms noise. Example Reward Schedule: the risk and mean reward sizes for each target were varied orthogonally. In experiments used to assess reward sensitivity, the range of reward differences for the risky target was 0-303uL across blocks of trials, and the expected value difference between the two options varied from 0 to 153 uL across blocks. B. The likelihood of a risky choice decreases inversely to the relative value of the safe reward. We varied the magnitude of the safe reward and variance of the risky rewards independently to establish preferences across a range of options. Based on these observations, we selected a subset of options with equal expected values at which the monkeys more often choose the risky option (Equal value condition; blue circle) and a subset biased toward the safe choice at which the monkeys more frequently chose the safe option (Biased value condition; red circle). Lines shown are the best-fit lines for each monkey (NI grey dashed; BR continuous black lines). Points represent the mean probability of the risky choice across sessions, and points and lines are staggered for visibility; whiskers represent 1 S.E.M. C. Choice allocation at a subset of target values results in two reward contexts with opposite choice biases. In the Equal value condition, the average expected values of the two options are equal and monkeys choose the risky option more frequently. In the Biased value condition, the safe reward size is larger than the mean risky reward and monkeys more often select the safe option. D. The probability of repeating a previous choice depends linearly on the previous reward received; monkeys repeat choices more frequently following large rewards but switch to the alternative after small rewards. The monkeys become indifferent between switch and stay decisions at 203 uL juice reward. E. Monkeys’ choices reflect
a win-stay-lose-shift strategy. Following supra-threshold rewards, or wins, monkeys repeated approximately 90% of choices. In contrast, monkeys were roughly indifferent between switching and staying following sub-threshold rewards, or losses.
Figure 3.2: A,B: Peri-stimulus time histograms for a single CGp neuron aligned to saccade onset. Firing rates were largest in each context when monkeys selected their preferred option (A: Equal values; B: Biased values). The grey block indicates the 400 ms window following saccade initiation. C-E: Population responses also signal contextual choice bias. When the two options have equal expected values and the risky choice is chosen more often, posterior cingulate activity is higher when monkeys select a risky option (Equal value context; C, 0-400ms after target onset, post-hoc Fisher’s LSD, p<0.05; D, 0-400 ms after saccade initiation, p<0.01; E, 50-450 ms after reward onset, p=0.06). In contrast, posterior cingulate activity is relatively increased following a safe choice when available average rewards are unequal (Biased value context) and the safe choice is preferred (after target, p<0.01; after saccade, p<0.01; after reward, p<0.01).
Figure 3.3: CGp neurons signal heuristically-assessed outcomes across contexts. A, B: Peri-stimulus time histograms for a single CGp neuron aligned to saccade onset demonstrate that the firing rates of the neuron were largest in each context on trials following receipt of wins (A: Equal values; B: Biased values). The grey block indicates the 400 ms window analyzed following saccade initiation. C, D, E: Population firing rates following wins exceed those following losses in both the equal and biased value contexts (C, 0-400ms after target onset, ANOVA firing rate vs most recent previous reward p<0.01; D, 0-400 ms after saccade initiation, p<0.01; E, 50-450 ms after reward delivery, p<0.01).
Figure 3.4: CGp neurons signal outcome/decision combinations in the decision epochs. In the simple WSLS behavior described in this paper, the two possible outcomes and two possible decisions produce four possible outcome/decision situations (lose/switch, win/switch, lose/stay, win/stay). A. The example neuron shown previously differentiated these four outcome/decision situations after choice and responded most strongly to wins and on trials on which the monkey chose to return to the previously chosen option. B, C, D: During the two epochs surrounding choice indication (B, C) and the post-reward epoch (D), CGp population activity distinguished the four outcome/decision combinations. Notably, CGp activity distinguished both wins from losses and switch decisions from stay decisions during the decision epochs, but only differentiated wins from losses in the reward epoch.
Figure 3.5: CGp neurons signal the probability of repeating choices across contexts. A. The activity of the example neuron shown in this plot (the same neuron shown in Fig 2, 0-400ms following saccade initiation) significantly predicted the probability that, on the current trial, the monkey repeated the preceding choice; increased firing rate predicted higher probabilities of staying with an option rather than switching to the alternative. B, C, D: The CGp population response predicts the probability of repeating a choice during the decision epochs. After both target onset (B) and saccade (C), CGp firing rate predicts the probability of repeating a choice on the current trial (binomial regression, decision to repeat the previous choice vs firing rate). Similarly, CGp activity following reward delivery (C) predicts the probability of repeating a choice on the next trial (binomial regression, decision to repeat the current trial’s choice on the next trial vs firing rate).
Table 3.1: Monkeys' decisions in the gambling task are motivated by integration of short reward and choice histories. We fit each monkeys' choices with a model that included previous rewards, previous choices, and contextual bias, and used Akaike's Information Criteria to identify the history length (k; number of previous trials) with the greatest explanatory power. To adjust for the effect of reward magnitude on choice, we multiplied each reward coefficient by the mean expected value of the risky choice and report the adjusted values here.

<table>
<thead>
<tr>
<th>Monkey BR:</th>
<th>Equal Value Condition (BR, NI, k=2)</th>
<th>Biased Value Condition (BR, k=2; NI, k=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Previous trial</td>
<td>Penultimate trial</td>
</tr>
<tr>
<td>Reward coefficient</td>
<td>0.7076</td>
<td>0.4508</td>
</tr>
<tr>
<td>Choice coefficient</td>
<td>-0.1964</td>
<td>0.2790</td>
</tr>
<tr>
<td>Bias</td>
<td>1.0707</td>
<td></td>
</tr>
<tr>
<td>Monkey NI:</td>
<td>Previous trial</td>
<td>Penultimate trial</td>
</tr>
<tr>
<td>Reward coefficient</td>
<td>1.3286</td>
<td>0.7551</td>
</tr>
<tr>
<td>Choice coefficient</td>
<td>-1.2108</td>
<td>0.0643</td>
</tr>
<tr>
<td>Bias</td>
<td>0.9193</td>
<td></td>
</tr>
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</table>
Table 3.2: CGp neuronal activity is modulated by contextual biases. We used a factorial ANOVA to compare the effect of choice and context on neuronal activity during the 400ms epochs following target appearance, saccade initiation and reward delivery. A significant interaction term ($p < 0.05$) indicated that a cell’s activity was significantly correlated with contextual bias across contexts. Consistent with previous studies, we found that neuronal activity was either positively or negatively correlated with choice bias.

<table>
<thead>
<tr>
<th></th>
<th>Risky choice</th>
<th>Context</th>
<th>Interaction (with bias)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of positively correlated cells</td>
<td>Post-target</td>
<td>Post-saccade</td>
<td>Post-reward</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
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<td>10</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Number of negatively correlated cells</td>
<td>Post-target</td>
<td>Post-saccade</td>
<td>Post-reward</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>10</td>
</tr>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 3.3: CGp neurons track previous wins and losses across contexts. To assess the contribution of heuristically categorized outcomes to posterior cingulate activity, we assessed the relationship between neuronal firing and previous outcomes (ANOVA, firing rate vs win/loss outcome). Neurons whose activity depended significantly on previous outcomes ($p<0.05$) were categorized as positively (increasing activity following wins relative to losses) or negatively (higher firing rate for losses than wins) correlated with outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Post-target</th>
<th>Post-saccade</th>
<th>Post-reward</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of positively correlated cells</td>
<td>5</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Number of negatively correlated cells</td>
<td>4</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 3.4: CGp neuronal activity predicts the probability of repeating a choice across contexts. To analyze the contribution of neuronal activity to switch/stay decisions, we compared the probability of repeating a choice to neuronal firing rate for each neuron (binomial regression, probability of repeat vs firing rate). Neurons whose activity significantly predicted the probability of repeating a decision (p<0.05) were further categorized by the relative strength of their responses on switch or stay trials.

<table>
<thead>
<tr>
<th></th>
<th>Post-target</th>
<th>Post-saccade</th>
<th>Post-reward</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stay &gt;switch</td>
<td>5</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Stay &lt;switch</td>
<td>4</td>
<td>3</td>
<td>10</td>
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</tbody>
</table>
Serotonin shapes risky decision making in monkeys

Some people love taking risks, while others avoid gambles at all costs. The neural mechanisms underlying individual variation in preference for risky or certain outcomes, however, remain poorly understood. Although behavioral pathologies associated with compulsive gambling, addiction, and other psychiatric disorders implicate deficient serotonin signalling in pathological decision making, there is little experimental evidence demonstrating a link between serotonin and risky decision making, in part due to the lack of a good animal model. We used dietary RTD to acutely lower brain serotonin in three macaques performing a simple gambling task for fluid rewards. To confirm the efficacy of RTD experiments, we measured total plasma tryptophan using high performance liquid chromatography (HPLC) with electrochemical detection. Reducing brain serotonin synthesis decreased preference for the safe option in a gambling task. Moreover, lowering brain serotonin function significantly decreased the premium required for monkeys to switch their preference to the risky option, suggesting that diminished serotonin signalling enhances the relative subjective value of the risky option. These results implicate serotonin in risk-sensitive decision making and, further, suggest pharmacological therapies for treating patho-
logical risk preferences in disorders such as problem gambling and addiction.

4.1 Introduction

Making adaptive decisions requires evaluation of the costs and benefits of available options as well as their uncertainty or risk. In economics, risk is typically defined as known probabilistic variation in the distribution of outcomes (Sharpe, 1964; E. Weber et al., 2004). Economists and psychologists have long known that risk so defined strongly influences decisions made by humans and nonhuman animals (Bernoulli, 1738/1954; Neumann & Morgenstern, 1944; Kahneman & Tversky, 1979). Typically, individuals prefer safe bets to risky gambles. However, these preferences can reverse depending on financial status (Guiso & Paiella, 2007), physiological state (Caraco, 1981), and contextual features of the task such as the delay between rewards (Rachlin, 2000; B. Y. Hayden & Platt, 2007), reward options available (Rottenstreich & Hsee, 2001; Bateson, 2002; Dickhaut et al., 2003), and whether the choice is between probabilistic losses or gains (Kahneman & Tversky, 1979). Importantly, the same individual can be risk-seeking in one context and risk-averse in another, like the compulsive gambler who insures his car (Friedman & Savage, 1948).

The neural correlates of probabilistic reinforcement and risk-sensitive decision making have been probed in a number of recent neurophysiological and neuroimaging studies. For example, activation of prefrontal and parietal cortex is correlated with risk (Knutson & Cooper, 2005; Huettel, Stowe, Gordon, Warner, & Platt, 2006), and neurons in posterior cingulate cortex have been shown to signal the subjective utility of risky options (A. McCoy & Platt, 2005b). Building on these correlative findings, brief disruption of neural processing within the right dorsolateral prefrontal cortex by transcranial magnetic stimulation increases risk-taking behavior in a simple gambling task (Knoch et al., 2006), thus directly implicating this structure in the
Individual differences in risk preference may result from neuromodulatory influences on these brain areas. Indeed, the impaired decision making found in multiple psychiatric disorders, including addiction, attention-deficit hyperactivity disorder, pathological gambling, schizophrenia, depression, and personality disorders, is sometimes characterized as increased preference for risk (Kapur & Remington, 1996; J. Evenden, 1999a; B. J. Jones & Blackburn, 2002; Lyne, Kelly, & O’Connor, 2004; Sodhi & Sanders-Bush, 2004; Nordin & Sjodin, 2006), and neuromodulators such as dopamine and serotonin have been implicated in these disorders. In particular, impaired function of the serotonergic neuromodulatory system is found in several risk-seeking behavioral pathologies (J. Evenden, 1999b; B. J. Jones & Blackburn, 2002; Marek et al., 2003; Chau et al., 2004; Clarke et al., 2005; Svenningsson et al., 2006; Jans et al., 2007) and genetic studies have implicated serotonergic dysfunction in impulsive and risk-taking behavior (Nordstrom et al., 1994; Higley et al., 1996; Gainetdinov et al., 1999; Chau et al., 2004; Kreek et al., 2005).

These observations invite the hypothesis that serotonin signaling contributes to decision making by systematically altering perceptions of risk and reward or the translation of those perceptions into decision and action. Previous studies have tested the functional relationship between altered serotonin function and impulsivity (J. Evenden, 1999a; Winstanley, Dalley, et al., 2004), temporal discounting (Schweighofer et al., 2008), and reward cue processing (R. Rogers et al., 2003). While these studies reflect multiple facets of risk (J. Evenden, 1999a; M. L. Platt & Huettel, 2008), the functional relationship between serotonin and preferences for economically defined risk, or uncertainty, has not yet been assessed. We therefore designed our experiment to measure behavioral responses to risk by parameterizing economic uncertainty and value. Specifically, we probed the role of serotonin in the processes mediating choices between risky and certain options by systematically
altering serotonin levels in three adult male rhesus macaques (Macaca mulatta) performing a gambling task (Figure 1a). We used rapid tryptophan depletion (RTD), a well-characterized dietary manipulation that rapidly, reliably, and reversibly lowers brain serotonin (Moja, Cipolla, Castoldi, & Tofanetti, 1989; Reilly, McTavish, & Young, 1997; Klaassen, Riedel, Deutz, Someren, & Praag, 1999), to bypass the complex, often poorly understood, interactions between serotonergic antagonists and neurophysiological systems and induce a global deficit in serotonin synthesis. Since RTD significantly lowers brain serotonin by dramatically reducing plasma, and therefore brain, tryptophan (Young, Ervin, Pihl, & Finn, 1989; Reilly et al., 1997), plasma tryptophan serves as an index for brain serotonin levels after RTD (Tagliamonte, Biggio, Vargiu, & Gessa, 1973; Young et al., 1989; Reilly et al., 1997). We modified this paradigm for use in awake, behaving monkeys and confirmed tryptophan depletion within each subject by measuring total plasma tryptophan (Figure 1b).

The task we used was designed specifically to probe decision making in the presence of economic risk (A. McCoy & Platt, 2005b). In this task, modeled on a classic foraging task (Kacelnik & Bateson, 1996), animals were offered a choice between two options. The safe option offered a guaranteed juice reward while the risky option offered either a larger or smaller volume, selected randomly. This task, which we dubbed a “gambling task”, allowed us to quantify both risk preference, defined by preferences when the two options had equal expected value, and the shift in the amount the monkeys will pay for the risky option, defined in economics as the “safety premium” (Asch & Quandt, 1990). Overall, we found that lowering brain serotonin decreased the monkeys’ likelihood of choosing the safe option and increased their valuation of the risky option. Our results demonstrate for the first time that serotonin signaling functionally contributes to decision making under economic risk.
4.2 Methods

4.2.1 Surgical and training procedures

All procedures were approved by the Duke University Institutional Animal Care and Use Committee and were designed and conducted in compliance with the Public Health Service’s Guide for the Care and Use of Animals. Surgical and training procedures were performed as described in detail elsewhere (A. N. McCoy et al., 2003). The monkeys’ eye position was monitored with either a scleral search coil (A. N. McCoy et al., 2003) or using a small infrared camera (Arrington Eyetracker) placed in the monkey’s peripheral visual field and focused on the pupil. This camera sampled horizontal and vertical eye position at 60Hz and transmitted this information to the Gramalkn Experiment Control System (ryklinsoftware.com). Since the 60Hz Eyetracker transmitted data at a slower rate than the 500Hz scleral search coil system used by the other two subjects (monkeys BRand NI), the temporal parameters within each task were modified to allow sufficient time for the system to recognize saccade initiation and completion. However, we held the inter-trial interval constant at 1 second.

4.2.2 Behavioral paradigms

Visual stimulus presentation and eye position measurement were performed as described previously (A. McCoy & Platt, 2005b). On each visual gambling trial, a monkey initially fixated (± 1-2°) a central yellow light-emitting diode (LED) (200-800 ms). Two peripheral yellow LEDs were then illuminated diametrically opposite each other, equidistant from the fixation LED (200-800 ms). The fixation LED was extinguished, cuing the monkey to initiate a gaze shift to either target (± 1-2°) within 350 ms. Correct trials were rewarded with juice and a 300 ms noise; no reward was given if the monkeys failed to complete the trial. Juice rewards were controlled by
a computer-driven solenoid that, when open, permitted juice flow through a tube to the monkey’s mouth. Within the range of reward values used, solenoid open time linearly predicts the volume of juice delivered (A. McCoy & Platt, 2005b). Within each block of 25-50 trials, one target was associated with a “safe” reward outcome of a constant amount of juice on every trial, while the other “risky” target was randomly rewarded with less or more than the certain amount of juice with probability 0.5. The locations of the safe and risky targets were varied every block. The sizes of the large and small reward offered for the risky option, as well as the size of the reward offered for the safe option, were varied orthogonally (Figure 4.1). The range of reward differences for the risky target was 20-250ms (23-253uL) across blocks of trials, and the expected value difference between the two options varied from 0 to 80 ms (0-83uL) across blocks. When there was no payoff difference between the two options, risk (the coefficient of variation in the size of the large and small reward offered by the risky option) was varied as previously described (A. McCoy & Platt, 2005b). Similarly, for two monkeys, when risk was held constant, the payoff difference between the average reward sizes of the safe and risky options was varied.

4.2.3 Rapid Tryptophan Depletion

For depletion (RTD) and control experiments, the monkey was fed a low-protein diet (80% fruit and vegetables, 20% normal monkey biscuits) for 24 hours. The monkey was then fed a mix (Supplementary Info., Appendix A) prepared no more than 48 hours previously. Since previous research had already demonstrated that maximal tryptophan depletion is reached within 3-6 hours following administration of the depletion mix (Carpenter et al., 1998), we performed behavioral testing 3-6 hours after administration of the RTD mix. A subset of depletions were performed by feeding the monkey a portion of the depletion mix on the evening preceding the experiment, as a replacement for the monkey’s normal low-protein meal, and the
remainder of the mix 3-6 hours before behavioral testing was performed, to mitigate satiation. Within one hour after either baseline or RTD behavioral testing, a 1-3 mL venous blood sample was drawn under approved anesthetic criteria for analysis of total plasma tryptophan levels. Immediately following the blood draw, the monkey was given his normal daily supply of biscuits and fruit to quickly re-establish normal tryptophan levels (Carpenter et al., 1998).

Plasma total tryptophan was measured by HPLC followed by electrochemical detection using a modification of literature methods (Krstulovic et al., 1984). Samples were injected directly onto a C18 reverse-phase ODC column and eluted with a mobile phase comprised of 0.5 M citric acid, 0.05 M Na2HPO4, 0.1 mM EDTA and 8% acetonitrile. Samples were detected with a BAS LC-4B amperometric detector with a dual 3 mM carbon electrode at a potential of .85V vs. an Ag/AgCl reference electrode. Samples were quantitated in comparison to external standards.

Total tryptophan has been shown to relate predictably to free plasma tryptophan: if anything, this measure underestimates the degree of depletion for free plasma tryptophan that is available for transport into the central nervous system (CNS) (Moja et al., 1989). The degree of depletion observed in the monkeys was equivalent to that reported in humans and nonhuman primates following this procedure (Moja et al., 1988; Young et al., 1989; Klaassen et al., 1999). We additionally confirmed successful depletion in a subset of experiments by measuring plasma tyrosine and comparing the ratio of plasma tryptophan to plasma tyrosine (Supplementary Info., Appendix A).

4.2.4 Experimental schedule

Each monkey performed the gambling task under both depletion (RTD) and control conditions. The monkeys were well-trained before data collection commenced. To minimize the effects of order, experience or time, the order of experiments was pseu-
dorandomized, with no more than four consecutive repetitions of the same condition permitted (e.g. Figure 4.1).

4.2.5 Analysis

Behavioral data were analyzed off-line; custom Matlab and R scripts were used for data processing, including sorting reward and risk variables (Matlab script, T. Hanson, Duke University) and computing saccade direction, amplitude, latency, and peak velocity. Monkeys performed from 102–443 trials per day (mean=256 trials: monkey BR, mean=278 trials; monkey SH, mean=195 trials; monkey NI, mean=432 trials; blocks in which the monkey failed to sample both targets were excluded from analysis.). Thus, although performing studies in primates precludes study of a large number of subjects, each subject performed a large number of trials across repeated experiments (Total trials: Monkey BR, 3573; Monkey NI, 2466; Monkey SH, 1964). Such repeated experiments in a small number of subjects can provide a reliable population estimate of behavior.

To analyze the behavioral changes induced by RTD, we calculated the mean frequency of choosing the safe choice for each monkey, experimental session, and experimental context (e.g. reward, depletion) and determined the effect of RTD on the probability of choosing the safe target using Analysis of Variance (ANOVA); we also calculated the temporal window over which monkeys integrated the history of rewards (Supplementary Methods, Appendix A). In addition, response latency and peak saccade velocity provide metrics useful for testing the possibility that lowering serotonin levels affects motor control or attention (Baumgarten & Grozdanovic, 1995; Gobbi, Murphy, Lesch, & Blier, 2001; K. Watanabe, Lauwereyns, & Hikosaka, 2003 Oct). Notably, since saccade velocity increases linearly with saccade amplitude, eye velocity is commonly normalized with saccade amplitude, a measure known as the “main sequence” (Bahill, Clark, & Stark, 1975). The sensitivity of these markers
necessitates their use on a trial-by-trial basis rather than as daily means. Thus, we used all choices to analyze these aspects of behavior and, to confirm that behavioral changes reflected serotonin depletion rather than changes in attention or motor performance, additionally included response latency and peak eye velocity relative to saccade magnitude (the “main sequence”) as co-factors in logistic regression analyses (A. McCoy & Platt, 2005b). Statistics were computed using Statistica (StatSoft).

4.3 Results

We first asked whether low serotonin levels influenced monkeys’ preferences as revealed by a simple risky decision making task in which monkeys prefer the risky option when choices with equal expected value are presented rapidly (every 1-3 sec) (A. McCoy & Platt, 2005b; B. Y. Hayden & Platt, 2007). Based on prior results, we held the inter-trial interval constant at 1 second, a range in which monkeys were previously risk-seeking, and assessed preferences in monkeys in two different contexts designed to elicit different choice likelihoods: one in which the two options had the same value and another in which the safe option had a higher value than the risky option. In the first condition, monkeys chose between safe and risky options with equal expected value and were thus able to choose either option without forfeiting long-term average reward intake. In the second context, the monkeys forfeited reward by choosing the risky option. Thus, orthogonalizing risk and payoff differences permitted quantification of the effects of RTD on risk preferences and reward valuation in two different contexts. At baseline, the combination of these two contexts resulted in an apparent overall preference for the safe option, though monkeys clearly chose the risky option more frequently when both options were matched for expected value.

We confirmed successful serotonin depletion in monkeys by measuring levels of plasma tryptophan, a reliable marker for brain serotonin (Tagliamonte et al., 1973;
Young et al., 1989; Reilly et al., 1997), in each macaque immediately following each session. RTD effectively depleted plasma tryptophan in each of the three monkeys and across all 3 monkeys considered together (Figure 4.2). In contrast, plasma tryptophan levels did not decrease when monkeys were fed a balanced amino acid mix that also contained tryptophan (Supplementary Figure A.1).

Overall, monkeys chose the safe option significantly less often following RTD (Figure 4.3a). Since these experiments involved multiple monkeys and reward contexts, we used multiple linear regression to investigate the influence of subject identity and relative reward value on revealed preferences (Multiple regression, mean daily frequency of safe choice vs depletion condition, subject, reward context, order of experiments, consumption time, mix volume: Depletion condition regression coefficient=0.19, p=0.03; Subject n.s.; Reward context regression coefficient=0.78, p<0.01; order, coefficient=-0.22, p<0.01; consumption time n.s.; mix volume n.s.). Importantly, the effect of RTD on choice frequency was significant independent of the order of experiments, timing of mix consumption (morning vs evening, as described in Methods), or liquid volume consumed with the mix (n.s.). Although baseline preference for the safe option varied across individuals, each monkey chose the safe target less frequently following RTD (Figure 4.3b). Furthermore, the monkeys chose the safe option less frequently following RTD (Figure 4.3c) whether they initially preferred the risky option (Equal expected value (EV) context) or the safe option (Unequal EV context). Moreover, following consumption of the balanced amino acid mix, monkeys’ preferences were similar to baseline preferences (Supplementary Figure A.2), indicating that the behavioral changes observed following RTD were due to the physiological effects of tryptophan depletion rather than consumption of the amino acid drink.

Since monkeys’ preferences were significantly modulated by decreasing serotonin, we asked whether preferences varied continuously with plasma tryptophan, and by
extension, serotonin levels in the CNS. We used the mean probability of choosing the safe option in each session to calculate the overall frequency of safe choices and found a weak but significant linear correlation between the frequency of safe choices and measured plasma tryptophan \((y = 0.39 + 0.0015x; y\) is the probability of the safe choice, \(x\) is plasma tryptophan; \(r = 0.40, p = 0.03\)). The effect of plasma tryptophan levels on preference for the safe option survived inclusion of individual and reward context in the model (tryptophan level regression coefficient=0.20, \(p = 0.01\); subject regression coefficient=0.22, \(p < 0.01\), all subjects in the same direction; reward EVcontext regression coefficient=0.77, \(p < 0.00001\), both contexts in the same direction). Although this observation suggests graded modulation of neural systems mediating reward processing and decision making, the strong influence of inter-individual variation and decision context prompts us to tender this conclusion with caution.

One interpretation of these results is that the subjective value of the risky option increased (or the subjective value of the safe option decreased) following serotonin depletion. Analysis of the monkeys’ safety premium \((\text{Asch} \ & \text{Quandt}, 1990)\), or point of subjective equivalence (PSE) between the risky and safe options, confirmed this supposition. To calculate the safety premium for each experimental condition, we used regression to find the optimal fit describing the relationship between the probability of choosing the safe option and the difference between the two options’ expected value (Figure 4.4). We found strong linear correlations between choice likelihood \((y)\) and the difference in EV \((x = (EV_{safe} - EV_{risky}))\) between safe and risky options:

**Baseline**, \(y = 0.32 + 0.006x \ (r = 0.93, p < 0.001)\)

**RTD**, \(y = 0.26 + 0.005x \ (r = 0.91, p = 0.01)\)

Notably, both relationships have similar slope and differ primarily in their intercept. Based on these analyses, we calculated that the solenoid open time at which monkeys
were indifferent to the two options (choice likelihood=0.5) increased by 60% when tryptophan depleted (baseline, 30ms solenoid open time; RTD, 48 ms), a payoff difference about double that which monkeys reliably discriminate when rewards are delivered in the absence of risk (A. N. McCoy et al., 2003). We repeated this analysis for each monkey (Supplementary Info, Appendix A) and found that each subject’s safety premium increased following tryptophan depletion (Monkey SH, 60% increase: baseline PSE=20ms, RTD PSE=32ms; Monkey NI, 39% increase: baseline PSE=38ms, RTD PSE=53ms; Monkey BR, 100% increase: baseline PSE=26ms, RTD PSE=52ms).

In addition to confirming that the monkeys value the safe option less (or the risky option more) when tryptophan-depleted, this analysis suggests that RTD does not negatively affect monkeys’ ability to discriminate relative reward sizes. To confirm that low serotonin did not impair monkeys’ reward discrimination ability, we examined behavior in a simple reward discrimination task (A. N. McCoy et al., 2003). We found no significant differences in performance in the baseline and depleted conditions (Supplementary Figure A.4), suggesting that RTD does not affect reward discrimination.

Serotonin might contribute to valuation of risky and safe options via opponency with dopamine, a hypothesis that posits that serotonergic activity conveys a negative reward signal by inhibiting dopamine neurons (Daw, Kakade, & Dayan, 2002). Although our experiment was not designed to specifically test this hypothesis with negative rewards or aversive outcomes, we suspected that the rewards associated with the safe or risky option might represent relative gains and losses compared to the reference point of the mean expected reward. Previous observations show that, consistent with this idea, monkeys were more likely to sample the safe option after receiving a smaller than average reward (loss) than after a larger reward (gain) (A. McCoy & Platt, 2005b). If serotonergic responses do report undesirable outcomes,
the decreased serotonin function resulting from RTD should preferentially diminish
the effects of losses, thus inducing the monkeys to choose the risky option more
frequently after receiving a small reward. To test this prediction, we analyzed the
effects of RTD on the likelihood of a safe choice on trials immediately following a
loss, a win, or a safe reward outcome (Figure 4.5). After RTD, the monkeys were
significantly less likely to choose the safe option on trials following such “losses” while
their preferences did not change significantly following “wins.” Although these results
do not definitively confirm the serotonin-dopamine hypothesis, reframing wins and
losses relative to the large reward suggests that RTD more strongly affects choices
following relative losses (safe reward and small risky reward; Baseline 61.5%±3.5%;
RTD 50.3%±3.1%; ANOVA; logistic regression, choice vs depletion, Wald stat 54.7,
p<0.000001) than following relative gains (large risky reward).

Alternatively, since serotonin also contributes to memory and impulsive behavior
(Riedel, Sobczak, & Schmitt, 2003; Winstanley, Dalley, et al., 2004), these effects
might reflect a shift in the temporal window over which the monkeys integrated the
history of prior rewards. To test this possibility, we determined the dependence of
monkeys’ choices on rewards received over the preceding ten trials (Supplementary
Methods, Appedix A). The monkeys’ choices were influenced by the preceding two
to three trials in both baseline (2.5±0.4 trials) and RTD (3.3±0.4) conditions (t-test
n.s.). Thus, at least in this well-learned task, RTD does not appear to strongly influ-
ence the monkeys’ memory or attention to the history of reward. Since low serotonin
levels can also influence motor control and attention (Baumgarten & Grozdanovic,
1995; Gobbi et al., 2001), we investigated whether RTD affected behavioral indices
of these processes. Although response latency appeared to decrease following RTD
(2963 baseline trials, mean latency 209.1±0.7 ms; 5070 RTD trials, mean latency
207.2±0.5 ms; one-way ANOVA F=5.7, p=0.17), these effects were very small and
largely driven by one monkey (Subject BR 1068 baseline trials, mean 198.3±0.9 ms,
2478 RTD trials, mean 198.5±0.9 ms, ANOVA F=0.02, n.s.; Subject NI 858 baseline trials, mean 223.5±1.2 ms, 1608 RTD trials, mean 217.4±1.0 ms, ANOVA F=14.23, p<0.01; Subject SH 1037 baseline trials, mean 208.4±1.1 ms, 984 RTD trials, mean 212.3±1.2 ms, ANOVA F=5.5, p=0.019. However, peak saccade velocity as a function of amplitude (the “main sequence”) increased significantly following RTD (2935 baseline trials with mean 42.0±0.3, main sequence slope=42.1; 5054 RTD trials with mean 48.5±0.3, main sequence slope=21.3, one-way ANOVA F=164.1, p<0.00001). This oculomotor effect included a significant subject effect (ANOVA; effect of RTD, F=66.2, p<0.00001; effect of subject, F=257.0, p<0.00001) although all three monkeys showed an increase in velocity as a function of amplitude (subject BR, 1067 baseline trials with mean 46.0±0.2, 2478 RTD trials with mean 52.6±0.5; subject NI, 858 baseline trials with mean 45.2±0.3, 1608 RTD trials with mean 49.2±0.2; subject SH, 1010 baseline trials with mean 35.0±0.87, 968 RTD trials with mean 36.7±0.90). Thus, movement velocity, a potential index of motivation, increased following RTD—perhaps reflecting greater valuation of the potential large rewards.

There was no overall effect of tryptophan depletion on the frequency of errors (incomplete trials; 3087 baseline trials, mean error frequency 4.0%±0.3%; 5288 RTD trials, mean error frequency 4.1%±0.2%; ANOVA F=6.9, p<0.01, subject effect F=87.2, p<0.00001), though one monkey failed to complete slightly, but significantly, more trials following serotonin depletion (subject NI, 879 baseline trials with 2.4%±0.5% errors, 1696 RTD trials±0.5%, ANOVA F=11.2, p<0.001; subject BR, 1076 baseline trials with 0.8%±0.2% errors, 2517 trials with 1.5%±0.2% errors, ANOVA F=2.9, p=0.09; subject SH, 1132 baseline trials with mean 8.4%±0.8% errors, 1075 RTD trials with 8.5%±0.8% errors, ANOVA F=0.004, n.s.). Furthermore, RTD did not affect the frequency with which monkeys switched targets, a measure of exploratory sampling behavior (ANOVA, daily mean sampling frequency vs depletion
condition, \( F=1.0 \), n.s., mean baseline sampling frequency±s.e.m. =25.5%±2.4%, mean RTD sampling frequency 29.1%±2.5%).

Thus, the only consistent effect of RTD on metrics of motor performance and attention was an increase in saccade velocity. Nonetheless, we further assessed the contribution of tryptophan depletion and choice context on preferences by including these metrics as factors in our analysis. To accurately reflect trial-by-trial variation in saccade metrics, we coded each choice as a binomial variable (safe, risky) and assessed factor effects across all correct trials using logistic regression. This analysis confirmed the main result that tryptophan depletion significantly decreased preference for the safe choice (Wald stat 38.7, \( p<0.00001 \)), even in the presence of significant effects of subject (Wald stat 34.1, \( p<0.00001 \)), relative reward context (Wald stat 689.6, \( p<0.00001 \)) and peak velocity as a function of amplitude (Wald stat 4.7, \( p=0.03 \)). Response latency was not correlated with preference (Wald stat 2.9, \( p=0.09 \)).

4.4 Discussion

Our results demonstrate for the first time that reducing brain serotonin synthesis decreases preference for a safe reward option in monkeys. Furthermore, the monkeys’ safety premium (the amount monkeys would “pay” to choose the risky option) increased with diminishing serotonin levels, confirming that decreased preference for the safe option reflects a decrease in its subjective value relative to the risky option. This shift in preferences appears to reflect choices following relative losses, an observation that supports serotonin’s putative role as indicator of negative rewards.

Lowering brain serotonin function also resulted in modest changes in attention and motor control. RTD increased saccade velocity as a function of amplitude (the “main sequence”) but did not influence error frequency or response latency. These results suggest that low serotonin is associated with faster behavioral responses, consistent with increased motivation, attention or temporal impulsivity (Scholes et
Importantly, the effects of reduced serotonergic activity on preferences were independent of changes in behavioral metrics of attention or motor control, confirming the main effect that lowering serotonin function systematically decreases preference for the safe option.

While this paper demonstrates behavioral modulation following successful tryptophan depletion in awake, behaving monkeys, several caveats are warranted. To ensure the monkeys’ comfort while maintaining consistent amino acid intake, we varied the amount of water used in the RTD mix. Consistent with previous experiments (A. McCoy & Platt, 2005b), we found that monkeys’ preferences did not depend on the volume of fluid intake. Similarly, the effectiveness of RTD did not depend on the slightly variable wait time between mix consumption and behavioral testing or on the order of experiments (although behavior did shift over time). The consistent behavioral effects of RTD despite variability in these experimental parameters suggest that changes in risk preferences revealed here were the result of changes in serotonergic function. The similarity between monkeys’ behavior at baseline and after consuming a balanced amino acid mix further supports this supposition, as it indicates that observed behavioral changes were due to low tryptophan rather than to consumption of an amino acid bolus in fluid. Furthermore, neither plasma tryptophan nor the plasma tryptophan/tyrosine ratio (an imperfect proxy for the plasma tryptophan/LNAA ratio but cf (Carpenter et al., 1998)) differed between baseline and balanced mix conditions.

Our data further supports the observation that context modulates the likelihood of choosing a safe option in the presence of uncertainty (Kahneman & Tversky, 1979; A. McCoy & Platt, 2005b; B. Y. Hayden & Platt, 2007). We confirmed that low serotonin diminishes the likelihood of safe choices independent of the initial safe choice likelihood, indicating that serotonin exerts a global influence on reward valuation and decision making independent of context. Since monkeys’ preferences shifted
consistently toward the risky choice across contexts, rather than toward indifference, the use of additional reward contexts also provided an additional confirmation that, in this task, serotonin depletion did not diminish the ability to learn to discriminate rewards.

It is worth noting that many of the tasks used to probe the influence of the serotonergic system on behavior address temporal impulsivity. Since calculations of both time and probability-weighted value contribute to time-sensitive decision making (Kacelnik & Bateson, 1996; J. Evenden, 1999a; B. Y. Hayden & Platt, 2007), as does the context within which choices are made (Kahneman & Tversky, 1979; Small et al., 2001; Huettel, Song, & McCarthy, 2005), impulsive behavior might reflect either unwillingness to wait or preference for risky options. By holding delays constant, we were able to focus on the contribution of serotonin signalling to the valuation of probabilistic rewards. Although the methods of this study do not directly address temporal impulsivity, the increased saccade velocity observed in our study is consistent with heightened temporal impulsivity following serotonin depletion as described in previous studies (Harrison, Everitt, & Robbins, 1997; Winstanley, Dalley, Theobald, & Robbins, 2003; Scholes et al., 2007).

The distinction between risk sensitivity and temporal impulsivity reflects the complexity of both the serotonergic system and the behavioral pathologies it influences. In fact, prior studies of the influence of serotonin on decision making have often yielded contradictory results (Jacobs & Azmitia, 1992; Hoyer, Hannon, & Martin, 2002; Varnas et al., 2004). serotonin signaling relies on over fifteen distinct, differentially localized receptor subtypes whose functions often conflict. Because individual components of the serotonin system may contribute conflicting signals in the decision process, this system is not truly the sum of its parts. Thus, results obtained using nonspecific agents (such as selective serotonin reuptake inhibitors, or SSRIs) must be interpreted with caution. For example, although SSRIs suppress
temporally impulsive behavior in pigeons and rats (Bizot, Le Bihan, Puech, Hamon, & Thiebot, 1999; J. L. Evenden, 1999; J. L. Evenden & Ryan, 1999; Wolff & Leander, 2002) and serotonin depletion increases temporal impulsivity (Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000), specific 5-HT2 receptor agonists slightly increase impulsivity (J. L. Evenden, 1998, 1999) while 5-HT1A receptor agonists shift preferences toward indifference (J. L. Evenden & Ryan, 1999).

Given the effects of global serotonin levels on risk sensitivity observed in this study, it is likely that multiple components of the serotonin system, rather than any single element, contribute to these behaviors. Thus, a single perturbation of the system, whether via environmentally induced serotonin depletion or genetic modifications of components of the serotonin system, modulates behavior via a cascade of signaling events (Canli & Lesch, 2007). These complicated polygenic and epigenetic interactions, which obscure the detailed mechanics of serotonergic contributions to behavior, contribute to the combination of genetic and contextual influences on psychiatric diseases (Bennett et al., 2002; Jans et al., 2007; Kreek et al., 2005; Ren-Patterson et al., 2006). Nonetheless, our results demonstrate that serotonin contributes to the neural processes that translate perceived rewards and risk into action.

Although humans, unlike the monkeys studied here, tend to be risk-averse for gains, monkeys and humans both show contextual modulation of risk preferences (Kahneman & Tversky, 1979; Rachlin, 2000; Bateson, 2002; A. McCoy & Platt, 2005b; B. Y. Hayden & Platt, 2007). Notably, humans become risk-seeking when gambling for small financial rewards, which may be comparable to the small juice rewards our monkeys receive (Markowitz, 1952). These behavioral observations suggest that our results may generalize to humans. Furthermore, the serotonergic system is strongly conserved across primate species (Bennett et al., 2002), suggesting that the modulatory mechanisms influencing risky decision-making may be common.
to monkeys and humans.

Our results suggest the possibility that low serotonin levels may underlie the willingness of problem gamblers to continue betting despite excessive losses (Rachlin, 2000). Based on these considerations, it seems surprising that low serotonin levels should persist at such high frequencies in the human population (Caspi et al., 2003). From an evolutionary point of view, however, low serotonin may not always be pathological and in some circumstances may in fact be beneficial. For example, developmentally diminished serotonin found in adolescent vervet monkeys may promote impulsive and risk-taking behaviors that improve social status in adulthood even as they introduce immediate risk or danger (Higley et al., 1996; Fairbanks et al., 2004). Similarly, high CNS serotonin turnover during the mating season may support increased aggression and mating behavior in rhesus macaques, increasing the probability of reproduction (Mehlman et al., 1997). The persistence of low serotonin function at high frequencies within the population may thus reflect evolutionary pressures favoring risky, but potentially advantageous behavior. Thus, although low serotonin levels are often associated with behavioral pathologies, willingness to take risks may be beneficial in some contexts. Nonetheless, our observation that low serotonin decreases valuation of safe rewards relative to risky options emphasizes the continued importance of investigating potential serotonergic therapies for behavioral pathologies like problem gambling, addiction and schizophrenia.
4.5 Figures
Figure 4.1: Top panel, task: after monkeys fixated a central stimulus, two peripheral targets were illuminated. The central stimulus was then dimmed to indicate the opportunity to saccade to either target. Middle panel, example reward schedule: in each block, one target was associated with a guaranteed reward while the other target offered a variable reward. The risk and mean reward sizes for each target were varied orthogonally. Bottom panel, example treatment order (B, Baseline; RTD, Rapid Tryptophan Depletion): the treatment for each session was pseudorandomized to minimize the effects of treatment order.
Figure 4.2: Ingestion of tryptophan-free amino acid mixture significantly lowers plasma tryptophan in three monkeys. Baseline levels (blue bars) were measured from macaques in their normal physiological state (monkey SH, mean ± s.e.m. = 8.53 ± 0.78 ug/ml (n=6 measurements), monkey BR 9.21 ± 0.35 ug/ml (n=4), monkey NI baseline mean ± s.e.m. 9.8 ± 0.60 ug/ml (n=2)) and post-RTD levels (red bars) were measured following a 24-hour low-protein diet and administration of the RTD mix (monkey SH 3.36 ± 1.00 ug/ml (n=5), monkey BR 3.20 ± 0.73 ug/ml (n=9), monkey NI 2.29 ± 2.45 ug/ml (n=4)). RTD significantly decreased plasma tryptophan levels in each monkey (monkey SH, ANOVA, p<0.01, F=17.2; monkey BR, p<0.001, F=27.5; monkey NI, ANOVA, p=0.016, F=16.0) and across the population (baseline mean ± s.e.m. = 8.97 ± 0.42 ug/ml, RTD mean ± s.e.m. = 3.04 ± 0.51 ug/mL, ANOVA, F=253.1, p<0.00001; subject effect n.s.). * indicates p<0.05, ** indicates p<0.01.
Monkeys less frequently chose the safe option following serotonin depletion than in baseline conditions (ANOVA of mean probability of safe choice per session vs tryptophan depletion condition, $F=5.38$, $p=0.028$; mean baseline preference for the safe choice $±s.e.m. = 53.4±3.2\%$ across 12 sessions with 2963 trials; mean RTD preference for the safe choice $42.9±3.0\%$ across 18 sessions with 5070 trials).

**Figure 4.3**: Serotonin depletion systematically decreases preference for the safe option in monkeys.
(b) Each monkey chose the safe option less frequently following RTD, despite differences in baseline preferences for each subject (Monkey BR, 4 baseline sessions with mean probability of safe choice ± s.e.m. 48.4% ± 7.0% and 9 RTD sessions with mean probability of safe choice 36.0% ± 4.7%; Monkey NI, 2 baseline sessions with 47.4% ± 2.8% and 4 RTD sessions with mean probability of safe choice 44.2% ± 0.5%; Monkey SH, 6 baseline sessions with mean probability of safe choice 58.7% ± 3.8% and 5 RTD sessions with mean probability of safe choice 54.3% ± 0.8%).

**Figure 4.3:** Serotonin depletion systematically decreases preference for the safe option in monkeys.
RTD reduced preference for the safe option whether both options had the same expected value (“Equal”; 12 baseline sessions with mean probability of the safe choice ± s.e.m. 35.5% ± 3.8% and 18 RTD sessions with mean probability of choosing the safe option 28.6% ± 2.8%) or unequal expected value (“Unequal”; 12 baseline sessions with mean probability of safe choice ± s.e.m. 73.6% ± 4.1% and 17 RTD sessions with mean probability of safe choice 61.3% ± 4.0%). Importantly, preference for the safe option always diminished following RTD, thus ruling out impaired reward discrimination. Error bars: 1 S.E.M. * indicates p < 0.05, ** p < 0.01.

Figure 4.3: Serotonin depletion systematically decreases preference for the safe option in monkeys.
Figure 4.4: Serotonin depletion increases the subjective value of the risky option in three macaques. To test whether serotonin depletion influences valuation of risky rewards, we calculated the amount of juice monkeys were willing to pay for the risky option. The payment difference between the two options when the monkeys were indifferent indicates the monkeys’ willingness to pay for risk, or safety premium (arrows: fringed arrow = baseline, filled arrow = RTD). In all cases, the subjective value of the safe option decreased following RTD. Lines shown were fitted by linear regression to the probability of choosing the safe choice at each safety premium in each session; each point represents the mean of these probabilities. Blue line, baseline; red, RTD.
Figure 4.5: Tryptophan depletion increases risky choices following sub-maximal rewards. Tryptophan depleted monkeys are more likely to select the risky choice following receipt of safe rewards (Baseline 69.8%±4.7%; RTD 57.4%±4.9%; ANOVA; logistic regression, choice vs depletion, Wald stat 35.9, p<0.000001) or small risky rewards (Baseline preference for the safe choice 53.3%±4.8%; RTD 43.3±3.6%; logistic regression, choice vs depletion, Wald stat 5.2, p<0.05), both of which represent losses relative to the large risky reward. In contrast, RTD did not affect preferences following receipt of the large risky reward (Baseline 11.2%±2.7%; RTD 11.5%±1.9%; logistic regression, choice vs depletion, Wald stat 1.0, n.s.). Bar height represents the mean safe choice likelihood across sessions following receipt of the indicated choice. Error bars: 1 S.E.M. * indicates p<0.05, ** p <0.01.
Low serotonin increases risky decisions in mice

5.1 Summary

Extensive clinical evidence implicates serotonergic dysfunction in psychiatric disorders associated with abnormal responses to risk (Deakin, 2003; Fletcher, 1995; Grados, 2009; Harrison et al., 1999; Higley et al., 1996; Mossner et al., 2007; Wong et al., 2008). Genetic variations in serotonergic effectors modulate probabilities of psychiatric illness (Caspi et al., 2003; Koenen et al., 2009; Nielsen et al., 1998), and serotonergic hypofunction is tied to pathological gambling, impulsivity and aggression (Apter et al., 1990; Dolan et al., 2001; Higley et al., 1996; Nordin & Sjodin, 2006). Previous work from our lab confirmed a causal role for low serotonin in increasing gambling behavior in monkeys offered a choice between a variable, or risky, reward and a guaranteed reward (Long et al., under review). To develop an assay to map the detailed serotonergic contributions to decisions made in the presence of economic risk, we trained 20 sham-injected C57BL/6J (wild-type) (C57BL/6J) mice to choose between a risky reward and a guaranteed reward. We then compared the behavior of 20 C57BL/6J mice treated with PCPA, an irreversible inhibitor of
the rate-limiting enzyme in serotonin synthesis (Koe & Weissman, 1968), to that of the wild-type (WT) controls. High performance liquid chromatography (HPLC) confirmed that PCPA treatment consistently lowered brain serotonin levels. WT mice were strongly averse to the risky option, but a significant subset of the PCPA-treated mice demonstrated risk-taking behavior. This result confirms that lowering brain serotonin increases individuals’ likelihood of risky choices relative to guaranteed alternatives.

5.2 Results and Discussion

To assay murine risk preferences, we trained mice to choose between a guaranteed, or safe, condensed milk reward (1uL) and a variable, or risky, option with 50% probability of reward (2uL) (Fig 5.1). Since the expected values of the two options are equal, measuring the frequency of risky choices in this context allows estimation of attitudes toward risk, or risk-preferences. In economic terms, choosing the risky option more frequently than chance (i.e. than indifference) is described as risk-seeking behavior; choosing the risky option less frequently than chance indicates risk-aversion. Such choices arouse strong biases across species (Bateson, 2002; Kacelnik & Bateson, 1996; Kahneman & Tversky, 1979), and evidence from social observation and behavioral pathologies implicates low serotonin in risk-seeking behaviors (Higley et al., 1996; Kuhnen & Chiao, 2009; Nordin & Sjodin, 2006; Nordstrom et al., 1994).

To test whether brain serotonin levels modulate risk preference in this simple decision task, we randomly sorted the population of 68 mice into two groups: the sham control group (33 mice) received vehicle injections while the experimental group (35 mice) received PCPA injections to block serotonin synthesis and lower brain serotonin. We confirmed the success of this manipulation by measuring frontal cortex serotonin levels in a subpopulation of mice (21 sham and 25 PCPA), and found that PCPA treatment significantly lowers frontal cortex serotonin concentrations.
relative to sham controls (Fig 5.2, one-way ANOVA brain serotonin per mouse vs group, F=26.0, p<0.01). Since PCPA may also decrease CNS dopamine (Dailly, Chenu, Petit-Demouliere, & Bourin, 2006), we also measured brain dopamine and dopaminergic metabolites. Since measures of brain dopamine were unaffected by PCPA treatment (Fig. B.1, one-way ANOVA, F=0.38, n.s.), and not predicted by brain 5-HT concentration (Fig. B.2, linear regression, dopamine fraction vs 5-HT $R^2 = 0.08$, n.s.), we concluded that potential behavioral differences between control and experimental mice would reflect serotonergic modulation.

Like most animal species (Bateson, 2002), mice strongly prefer a safe option over a risky option with equal expected value (Fig 5.3). However, PCPA-treated mice were significantly more likely than controls to choose the risky option overall (Fig 5.3, one-way ANOVA, risky choice frequency per mouse per day vs group, F=4.5, p<0.05), an effect that resulted from strong risk-seeking behavior in a significant subset of the PCPA-treated mice (Fig 5.4; one-sided t-test, number of risk-seeking or risk-neutral mice vs the predicted risk-averse population: sham mice n.s., PCPA group p<0.05).

Importantly, the two groups of mice had similar initial biases and, across the population, did not demonstrate initial biases distinct from indifference (Fig B.3, one-way ANOVA, risky choice frequency on the first day vs treatment group, F=0.08, n.s.). Notably, while vehicle-treated controls became risk-averse despite their initial risk-preferences, PCPA mice were more likely to continue to choose the risky option if their initial bias favored the risky option (Fig B.4), suggesting that low serotonin might enhance primacy effects.

These observations led us to question whether low 5-HT altered learning in this context, particularly given previous evidence that low 5-HT impairs learning and compromises memory consolidation (Riedel et al., 2003; R. D. Rogers et al., 1999). We found that even when mice differed in their overall risk attitudes, the behavior of both control (Fig 5.5) and PCPA-treated (Fig 5.6) mice was well fit by simple
exponential models. We therefore asked whether, as predicted by models that posit a role for serotonin in communicating information about negative rewards (Daw et al., 2002; Deakin, 2003), the observed shift in mouse preferences might be dependent on decreased sensitivity to relative losses. Our data did not support this interpretation, as risk-seeking PCPA-treated mice were more likely to choose the risky option after any of three possible reward outcomes (Fig B.5).

Finally, since lowering brain serotonin speeds behavioral responses in rats, consistent with increased motivation, attention or temporal impulsivity (Harrison et al., 1997; Scholes et al., 2007), we asked whether PCPA treatment induced similar behavioral changes in this task. We found that PCPA-treated mice nosepoked significantly more often for reward (Fig B.6; ANOVA, number of nosepokes for reward vs treatment group and choice: interaction term, F=22.8, p<0.01; post-hoc LSD t-test, groups significantly different (p<0.01) both on safe choice trials and on risky choice trials), consistent with the hypothesis that decreased serotonin might increase impulsive or perseverative behavior. Notably, this effect was stronger on trials where the mouse chose the safe option, indicating that this observation is not simply reflective of risky choices. However, the reaction time between trial initiation and choice indication did not differ significantly between groups even though mice were significantly slower to nosepoke for the risky option (ANOVA, reaction time vs group and choice: effect of group, F=3.4, p=0.06; choice, F=15.9, p<0.01; interaction, F=2.2, n.s.). Including these factors in our analysis (logistic regression, binomial safe/risky choice vs treatment, reward nosepokes and reaction time) confirmed the main effect that lowering brain serotonin increases risk preferences (Wald stat 42.5, p<0.01) even in the presence of a significant effect of reaction time (Wald stat 19.7, p<0.01); nosepoke frequency did not predict choices (Wald stat 1.0, n.s.).
5.3 Experimental procedures

5.3.1 Behavioral procedures

All procedures were approved by the Duke University Institutional Animal Care and Use Committee and were designed and conducted in compliance with the Public Health Services Guide for the Care and Use of Animals. Four to six week old wild-type C57BL/6J mice were habituated to the experimental room for a minimum of 1 week before commencing training. After reaching a minimum weight of 20g, the mice were food restricted to 90-95% of their age-adjusted baseline weights.

Each operant chamber (MED Associates) contained three nosepoke holes: an initiating nosepoke hole in the back wall of the chamber and two reward nosegokes on the opposite (front) wall of the operant chamber, which were positioned at the same height and symmetric around the center of the wall. A LED within each nosepoke was illuminated to cue availability for poking. Nosepokes were detected with infrared beams situated within each nosepoke. Each trial began with a tone and illumination of both a house light positioned above the chamber and the initiating nosepoke LED (Fig 2a). To initiate the trial, the mouse was required to nosepoke into the initiating nosepoke. Two reward nosepokes were immediately illuminated. Once the mouse poked into a reward nosepoke, a liquid dipper containing condensed milk automatically rose to an inferior opening in the nosepoke, and the mouse was able to consume the milk. After a 5 second interval for complete consumption, the dipper was lowered and the next trial began. Since inter-trial intervals affect risk-preferences in an analogous macaque task (B. Y. Hayden & Platt, 2007), there was no inter-trial interval. All trials were controlled and monitored by custom-written software (Med PC) that recorded each choice as well as the number of nosepokes per trial, latency to initiation and latency to collect reward after initiation.

Mice were initially habituated to the operant chambers and trained to perform
a simple, risk-less choice task. Side preferences were extinguished before mice were
exposed to the risky choice task. After this initial behavioral training, mice were di-
vided randomly into two groups: sham (initial injection 10ml/kg vehicle, thereafter
3.3ml/kg vehicle injection; n=33 mice) and PCPA (initial injection 300mg/kgPCPA,
thereafter 100mg/kg PCPA dissolved to allow a 3.3ml/kg bolus; n=35 mice). Injec-
tions were performed once daily following each experimental session. 24 hours after
the first injections, the reward values in each chamber were lateralized randomly
and counterbalanced across animals, so that one reward nosepoke offered 0.02 mL of
condensed milk (safe) and the other reward nosepoke provided either 0 or 0.04 mL of
condensed milk with equal probability (risky). Mice performed 30 risky choice trials
per session following completion of 4 forced choice trials (2 right, 2 left) included
to ensure daily sampling of each reward option. The mice were then tested for 14
sessions before they were euthanized painlessly.

5.3.2 Serotonin depletion

We depleted brain serotonin using the tryptophan hydroxylase inhibitor PCPA (Koe
& Weissman, 1968; Koe, 1971; Koe & Corkey, 1976), which effectively depletes brain
serotonin within 24 hours and maintains low serotonin levels for weeks when admin-
istered daily (Cahir, Ardis, Reynolds, & Cooper, 2007). Successful brain serotonin
depletion was confirmed by post-mortem analyses in a subpopulation of mice. Sham
injected and PCPA-treated mice were maintained on daily injections until 18-24 hours
preceding sacrifice. Immediately following euthanasia, the brains of each mouse were
removed and the frontal cortex, ventral striatum and dorsal striatum were dissected
and flash-frozen, with the exception of two lost dorsal striatal samples from PCPA-
treated mice. These regions were stored at -40C and 5-HT and dopamine (DA) levels
were analyzed using HPLC.
5.3.3 Analysis of mouse data

Behavioral data were translated using MedPC2XL and analyzed using custom R scripts and Statistica. We calculated the mean frequency of choosing the risky choice for each mouse and each experimental session, and used ANOVAs to assess the effect of PCPA treatment on the probability of choosing the safe target. In addition, we quantified the relationship between serotonin depletion and physiological metrics on a per-trial basis, using ANOVAs to compare the number of headpokes and latency to nosepoke for reward following trial initiation in the sham and PCPA-treated conditions. Finally, we coded choice as a binary variable (safe or risky) and included these behavioral indicators of motor activity and attention as factors in logistic regression to assess their potential contributions to behavioral preferences. Statistics were computed using Statistica (StatSoft).
5.4 Figures

Figure 5.1: Mouse gambling task. To begin each trial, a mouse nosepoked into an illuminated (LED) nosepoke centered at a low height on the back wall of an operant chamber. The associated LED was immediately extinguished and LEDs within two symmetrically positioned peripheral reward nosepokes on the opposite (front) wall of the chamber were illuminated. All choices of the safe reward nosepoke were rewarded with condensed milk; the risky nosepoke was rewarded stochastically with 50% probability of a reward twice the size of the safe reward.
Figure 5.2: PCPA lowers brain serotonin relative to vehicle-injected controls. To confirm the neural effects of PCPA treatment, we used HPLC to measure serotonin levels in the frontal cortex of vehicle-injected (blue) and PCPA-treated (red) mice. Error bars, S.E.M., double asterisk, p<0.01 relative to baseline.
Figure 5.3: Lowering brain serotonin increases the probability of a risky choice. Mice treated with PCPA (red) were significantly more likely to choose the risky option than sham controls (blue). Bar indicates the mean of the daily risky choice frequencies, error bars represent 1 S.E.M. Asterisk, p<0.05 relative to baseline.
Figure 5.4: Lowering brain serotonin increases the probability of a risky choice in a subset of mice. While the vehicle-treated mice overwhelmingly preferred the safe option, a significant number of PCPA-treated mice chose the risky option at or above chance. Points, mean of daily risky choice frequencies per mouse; error bars, 1 S.E.M. one-sided t-test, p<0.05
Figure 5.5: Example vehicle mouse learning curve. Control mouse preferences shift to the safe option over several days of experience.
Figure 5.6: Example PCPA mouse learning curve. This example PCPA mouse had a normal (though inverted) learning curve and, like the control mouse, its preferences strengthened over several days of experience.
6.1 Overview

Although this thesis is focally directed toward understanding the neural mechanisms of decision making, it describes two very different sets of experiments that use very distinct investigative techniques. Furthermore, the exact demarcation of the boundaries of these experimental groups may be questioned: are these experiments differentiated by the neural substrate (CGp vs serotonin) or by task (cross-contextual investigations vs decisions under risk)? These nested distinctions hamper attempts to summarize this data briefly and concisely; as with Russian Matryoshka dolls, it is difficult to decide which groupings work best. As I have previously suggested a model in which anatomically defined brain regions interact via neuronal projections and are modulated by contributions from neurochemicals such as serotonin (Section 1.3.5), I will use this distinction to organize this discussion. I will first summarize my studies of posterior cingulate cortex function before addressing serotonergic contributions to preferences expressed under risk and turning to the question of how serotonin might modulate CGp activity.
6.2 CGp, decisions and salience

6.2.1 Summary of results

In the Introduction of this thesis, I suggested that CGp linked outcome evaluation to action selection, and that its contributions to decisions included maintaining an integrated representation of stimulus value and future action value. The implications of these proposals — namely, that CGp neurons should respond to informationally relevant events whether task-relevant or surprising, and that CGp activity should track integrated measures of stimulus evaluation that predict future choices — led to the experiments described in Chapters 2 and 3. The results described in these chapters thus add supporting evidence to the hypothesis that CGp reports and tracks motivationally salient stimuli, and thus contributes to learning, memory and decision making.

Thus, the evidence described in this thesis, in combination with findings that CGp differentiates outcomes, maintains evaluative representations across time, and encodes stimulus-outcome associations, supports a model in which CGp links outcome evaluation and action selection. Figure 6.1 depicts such a model based on the anatomical connections between regions described in Sections 1.3.3 and 1.3.4. In this model, CGp transmits both immediate and remembered (or maintained) information about integrated outcome evaluation to regions such as OFC (and possibly ACC) that encode stimulus-outcome associations. CGp also contributes to rule-based decisions via projections to PFC. Recursive connections between ACC and CGp, and between LIP and CGp, contribute to refining evaluation-based (LIP) or strategic (ACC) action selection. In addition, recursive connections between CGp and the hippocampal formation contribute to updating and retrieving memory, thus ensuring that outcome evaluation will be stored in memory and later retrieved for prospective action selection. Finally, this model includes regions not directly con-
nected to CGp whose activity contributes to outcome evaluation (VTA, DRN) or encoding new information (BLA).

6.2.2 The breadth of relevant information: from novelty to memory

Comparing CGp responses to those in the BLA, a region that, like CGp, sends efferents to both ACC and OFC, reveals the importance of memory and encoding motivational salience for CGp function. BLA responses to novel and changing environmental conditions have led to the suggestion that the BLA, like CGp, contributes to ACC’s role in strategy selection (as well as to recognition of error or “conflict” signals) and to the formation of stimulus-outcome associations in OFC (Holland & Gallagher, 2004). Unlike CGp, whose responses maintain representations of reward even after stimulus novelty fades, BLA responses fade as stimulus-outcome associations are learned and encoded in OFC.

Thus, in information theoretic or learning theory terms, BLA responses are specific to surprising stimuli that strongly motivate learning (Courville et al., 2006; Rescorla & Wagner, 1972; Shannon, 1948). While CGp responses reflect the informational salience of surprise, they also encode predictive information that is as likely to maintain as to change stimulus-outcome associations. CGp contributions to decisions — whether through connections to outcome-evaluative regions such as OFC or to action-selection regions such as LIP, ACC and PFC — reflect both learned and surprising reward information. These experiments thus provide evidence for the possibility that CGp might appropriately be characterized as a salience map for motivationally relevant information such as reward; both the observation that CGp neurons report task-relevant and surprising events and the confirmed prediction that CGp neurons track the motivational information of available options are consistent with this description.

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Figure 6.1: Neural components of evaluative action selection. This figure highlights the anatomical connections between CGp and regions that evaluate outcomes (blue) or select actions (green). In addition, it highlights the reciprocal connections between CGp and the hippocampal formation (magenta), which is implicated in memory. Notably, CGp appears to be at a crossroads between memory, outcome evaluation and visuomotor regions that contribute to action selection. The orange arrows from the dorsal raphe nuclei (DRN) (small orange molecule: serotonin) depict the serotonergic projections from the DRN to other decision-related structures shown. (ACC, anterior cingulate gyrus; BLA, basolateral amygdala; CGp, posterior cingulate gyrus; DRN, dorsal raphe nucleus; HPC/PHG, hippocampus, parahippocampal gyrus; LIP, lateral intraparietal cortex; OFC, orbitofrontal cortex; PFC, prefrontal cortex; VTA, ventral tegmental area)
6.2.3 *CGp as a putative salience map*

These observations support the argument, initially proposed in the Introduction, that CGp’s function, like its anatomical location, resembles a crossroads in decision making and that CGp’s contributions to outcome evaluation and action selection can be described using a salience map framework (Figure 6.2). In Section 1.4, I briefly summarized evidence from a variety of studies that supports my hypothesis that CGp functions as a salience map. In this section, I will demonstrate that the data described in this paper provides evidence that CGp fulfills all five of the criteria proposed for a salience map (Section 1.2).

1. **CGp neurons respond to stimuli across a variety of contexts.** As described in Chapter 2, CGp neurons respond phasically to visual stimuli across three task contexts (decision, operant, Pavlovian). In addition, these neurons respond following saccades and rewards in each of these contexts and also respond to uncued rewards in a context without reward-relevant visual cues. Importantly, each of these events — cue, saccade and reward — is experientially linked to past, current or future rewards. The observed responses to these reward-related events across contexts are consistent with my suggestion that the putative CGp salience map deals with motivational information.

2. **CGp neuronal responses are not exclusively feature-selective.** Previous observations that CGp neuronal activity tracks reward magnitude in a reward discrimination task (A. N. McCoy et al., 2003) and risk coefficient of variance in a gambling task (A. McCoy & Platt, 2005b) allow the possibility that CGp responses are selective for unique features of reward within separate contexts. However, the data described in Chapter 3 indicates that these responses reflect an integrative measure of contextually relevant motivational information rather than unitary feature-selectivity:
Figure 6.2: A simple salience map. In this diagram, the rhesus monkey (left) receives cherry juice rewards while viewing stimuli presented on a computer monitor (right). Atop the monitor, a small videocamera monitors the monkey’s eye position. Each stimulus (red, yellow, green) is associated with differently sized rewards (large, medium, small). In this paradigm, motivational salience depends exclusively on reward magnitude. As shown in the greyscale salience map (center screen, scaled from black (low motivational salience) to white (high motivational salience)), regions of space associated with high reward have high salience (white) relative to both smaller rewards (greys) and the minimally salient background (black). The three representative PSTHs (left, thought bubble) show a possible neuronal encoding of the salience strength of each visual cue, i.e., a direct proportionality between the size of phasic responses and the motivational salience bound to the corresponding region of space.
neuronal activity reflects preferences within each of two decision contexts that dissociate risk from reward.

3. CGp neuronal activity reflects sensorimotor integration. As mentioned in the previous point, CGp neuronal activity reflects contextually determined preference, an integrative measure of reward probability and magnitude (Chapter 3). Furthermore, CGp neurons reflect a heuristically categorized measure of reward desirability (win/loss) and neuronal activity predicts heuristically categorized motor output (stay/shift) (Chapter 3). This pattern of activity suggests that CGp neuronal activity encodes both sensory input and motor output.

4. CGp neurons have spatial receptive fields. Phasic CGp responses to saccade depend on the spatial location of the chosen target in a decision task (Chapter 2). This observation is consistent with previous studies that demonstrated that the tonic activity of CGp neurons is modulated by target location and saccadic endpoints and that CGp neurons have broad visuospatial receptive fields following saccade (Dean & Platt, 2006; Dean et al., 2004; C. R. Olson et al., 1993; C. Olson & Musil, 1992; C. Olson et al., 1996).

5. CGp responses to stimuli are not exclusively spatial. CGp neurons respond phasically following uncued reward delivery, that is, in the absence of visuospatial information (Chapter 2). In addition, neuronal responses to visual cues and to rewards within the decision task context are not significantly modulated by spatial cue location (Chapter 2).

Thus, the data described in this paper supports my hypothesis that the posterior cingulate cortex may function as a salience map to direct attention toward desirable actions based on experienced and remembered outcomes. However, it is important to note that while the experiments described in this thesis involve stimuli that are
associated with reward, they do not conclusively demonstrate that CGp neurons respond only to reward-indicative cues. Further experiments will be required to determine whether CGp neurons respond exclusively to cues and events associated with gustatory rewards, or whether these neurons also encode the motivational value of visual social or auditory stimuli (Klein et al., 2008). Evidence that posterior cingulate cortex neuronal activity does reflect the motivational value of such non-gustatory stimuli, as suggested by functional neuroimaging (Maddock & Buonocore, 1997; Maddock et al., 2003; Pierce, Haist, Sedaghat, & Courchesne, 2004; Shah et al., 2001), would strongly support arguments that CGp integrates broadly defined motivational information across contexts, and that CGp neuronal responses are indeed integrative rather than feature-selective.

6.2.4 On the biphasic directionality of CGp responses

The studies described in this thesis reveal that posterior cingulate neurons respond phasically to events with either increases or decreases in firing rate. This observation, like the observation that CGp activity often increases (or decreases) after both large rewards and unexpected rewards (cf. (A. N. McCoy et al., 2003)), introduces difficulties in the interpretation of CGp responses. Unlike VTA neurons, which increase firing rate when rewards are unexpectedly large and decrease activity when rewards are unexpectedly omitted (Fiorillo et al., 2003; P. Tobler, Fiorillo, & Schultz, 2005), CGp neurons are unlikely to linearly code increases and decreases in reward size consistently across the population. However, other reward-evaluative regions of brain, including the OFC and striatum (Cromwell & Schultz, 2003; Hassani, Cromwell, & Schultz, 2001; Tremblay & Schultz, 1999), contain neurons that are both positively and negatively correlated with reward size (or that either increase or decrease activity following reward omission). Thus, it is not uncommon to find subpopulations of neurons with either increasing or decreasing correlations to reward outcomes.
Although explanations for this phenomenon are necessarily speculative, several reasonable options may be posited. First, neurons that increase or decreasing firing rate in response to relevant events may represent subpopulations of neurons with distinct patterns of anatomic connectivity: they may integrate information from different regions of the brain, and they may send projections to distinct anatomical endpoints. For example, CGp neurons with projections to the anterior cingulate cortex might respond to stimuli by decreasing firing while CGp neurons projecting to the LIP might respond by increasing activity. Second, these neurons may represent different subpopulations based on molecular expression patterns. For example, variability in the serotonergic receptors expressed across CGp may modulate valenced responses to reward: cells with 5-HT1A receptors might respond to external stimuli by decreasing activity (since these receptors are inhibitory) while cells without these receptors might increase activity. While this particular pattern of activity is highly speculative, the importance of genetic expression patterns for defining either anatomical regions and subregions or subpopulations of cells should not be underestimated (C. L. Thompson et al., 2008). A recent study demonstrated that within the hippocampus, genetic expression patterns define both classical anatomical regions and smaller subregions (C. L. Thompson et al., 2008). Notably, many of the molecules that defined the map described in this paper were cellular adhesion molecules that guide and maintain the connections that underlie neuronal circuitry: the molecular and circuit explanations for distinct subpopulations with differentially valenced responses may be closely linked.

6.3 Serotonin, decisions and risk

6.3.1 Summary of results

The experiments described in monkeys and mice confirm that low serotonin shifts choice tendencies toward risky options, suggesting that the low serotonin observed
in multiple behavioral disorders may in fact be causal. Importantly, these results are consistent across two species, consistent with observations that the serotonergic system is widely conserved (K. P. Lesch et al., 1997; Murphy, Lerner, Rudnick, & Lesch, 2004). Furthermore, the demonstration that mice learn in this simplified, economically-interpreted foraging task encourages me to think that future work could expand on this task to probe the behavioral effects of neuromodulatory systems in more detail. While rodents are commonly trained to perform ratio- and interval-based psychological tasks, few studies of economic and behavioral decisions have been performed in mice. In fact, although the literature on rat decisions is extensive, my initial literature reviews identified only one paper that demonstrated that mice could discriminate liquid reward magnitudes in a similar operant task (Isles, Humby, & Wilkinson, 2003). Similar tasks are under development elsewhere (Bari, Theobald, Mari, & Robbins, 2008; Watabe-Uchida & Uchida, 2008), but to my knowledge, the work I report in this paper is the first demonstration of economic risk-sensitivity in mice using an operant assay.

6.3.2 Caveats

The experiments that I have described using serotonergic manipulations in monkeys and mice depend on techniques that globally lower serotonergic activity. While these techniques have been well characterized and are widely used, it is worth noting three caveats that apply to these experiments.

First, the use of rapid tryptophan depletion (RTD) requires subjects that are willing to tolerate the dietary depletion mix. When this technique is used in human studies, most subjects only consume this protein-heavy, unpleasant-tasting mixture once or twice. In addition, the reimbursements offered for experimental participation effectively serve as bribes for tolerating moderately unpleasant experiences. The situation is somewhat different with macaques, as the available subject pool is
smaller and the number of experiments performed with each subject larger (these are, it should be noted, strategic experimental decisions that are consistent with responsible use of animals in research). In addition, it is difficult to bribe a macaque to consume an unpleasant mix now in order to receive extra treats later. More importantly, since macaques do not verbalize discomfort using human language, ethical considerations necessitate careful observation for even small signs of discomfort. While we found that we were able to modify experimental constraints to minimize potential discomfort without sacrificing experimental rigor (see Appendix A), we also found that monkeys lost interest in consuming the mix after repeated experiences. While we were not able to determine whether this disinterest was due to a discomfort resulting from satiety or due to anhedonic or unpleasant aftereffects of consuming the mix (Moore et al., 2000; Young & Leyton, 2002), these possibilities are worth noting when considering experimental designs that involve repeated dietary depletion experiments in individual animals. One simple alternative would be to perform only small numbers of experiments in a much larger set of subjects. In this regard, I noted that several monkeys seemed to find the depletion mix interesting and novel when they first experienced it, so a larger subject pool might allow sufficient data collection while avoiding habituation. In addition, pharmacological treatments like the parachlorophenylalanine (PCPA) used in mice offer an alternative to dietary depletion methods. I initially chose to avoid using PCPA in monkeys due to the potential for kidney damage (Ikonomov, Stoynev, Goranova-Stoyneva, Popov, & Minkova, 1990). However, after extensive observations of mice treated with PCPA, I suspect that while chronic PCPA treatment would make monkeys irritable, it would be safe to treat monkeys with PCPA acutely with careful observation and veterinary monitoring. However, these concerns should be addressed carefully given the importance of repeated recording sessions for electrophysiology.

Second, RTD and PCPA modulate global rather than focal serotonin levels. Using
methods that globally manipulate serotonin levels provided an important demonstration of the causal impact of low serotonin on economically risky decisions. In addition, it allowed me to bypass the serotonergic system’s extraordinary complexity (Baumgarten & Gothert, 1997). However, this global manipulation may be analogous to hitting a circuit board with a hammer – it will probably break the system (and this is in fact an important discovery), but it won’t tell you which components do what. Further experiments using genetic modifications, receptor-specific pharmacological agents, or locally applied agonists and antagonists will help clarify the functions of specific receptors. Additionally, in combination with electrophysiology, such techniques should clarify the effect of serotonergic signaling on neuronal activity. Notably, given the availability of genetic techniques for rodent models and the relative ease of increasing the numbers of individuals studied, these experiments will likely be most profitable if performed using rodent rather than primate models.

Finally, one notable difference between mouse and monkey behavior under hypo-serotonergic conditions is that while the preferences of all three individual monkeys tested shifted away from the safe option, toward the risky gamble, only a subset of mice within the population switched their preferences toward the gamble. This observation may reflect the strength of mouse aversion to the risky option, since null reward sizes induce much stronger aversion than small, non-zero outcomes. Alternatively, it may result from behavioral inflexibility associated with a limited menu of reward options or from early overtraining on safe options. Indeed, we were unable to perform within-subject testing in the mice since initial behavioral testing revealed that inbred C57BL/6J mice were unable to perform simple reversal learning in this context. Although the between-subject experimental design used in mice allowed us to test and compare learning rates across control and low-serotonin conditions, the strong perseverative tendencies observed in this task prompt consideration of alternative experimental designs, particularly of designs that allow presentation of
variably sized rewards, variable reward schedules, gambles with non-zero rewards, and flexible location-outcome contingencies.

Of note, the differences in training regimes associated with the two species may have contributed to the inconsistent evidences for impulsivity. Since the monkeys were extensively trained, perhaps even overtrained, the minimal changes in visuosaccadic parameters revealed little evidence for increased impulsivity; it is likely that the monkeys’ saccades were so thoroughly stereotyped that even the exquisite sensitivity of the visual monitoring techniques used could detect no changes. In contrast, the mice had relatively little exposure to the task. In fact, since each mouse only performed 30 trials per day, the mice had less experience at the end of the experiment than the monkeys had after a single session. This naivety allowed detection of behavioral changes that revealed increased impulsivity.

6.4 Future directions: Serotonergic contributions to CGp function

Since the experiments described in this thesis support roles for both CGp and serotonin in risky decision making, they raise an obvious question: do serotonin and CGp interact, and if so, how? Anatomical evidence suggests that such an interaction is probable: DRN projections target CGp and, although there are far fewer serotonin receptors in CGp than in ACC (Varnas et al., 2004), the unusually high concentration of 5-HT1A receptors in posterior cingulate cortex suggests the potential for unique serotonergic modulation of CGp neurons (Barnes & Sharp, 1999; Baumgarten & Gothert, 1997; Roth et al., 2004).

6.4.1 5HT1A receptors in CGp

5-HT1A receptors are inhibitory receptors that are generally found on the presynaptic terminals of serotonergic synapses. Thus, as inhibitory autoreceptors, they stereotypically cause feedback inhibition of serotonergic release (Baumgarten & Gothert,
1997; Stahl, 2008). Their presence in CGp as post-synaptic receptors is therefore particularly intriguing, although it should be noted that these receptors are also present in high concentrations in “limbic” structures such as the amygdala and the hippocampus (Varnas et al., 2004). Presumably, increasing serotonergic activity in CGp would result in inhibition of CGp neurons via 5-HT1A responses, dampening CGp activity and responsiveness. In contrast, diminishing serotonin release in CGp (for example, by RTD or PCPA treatment), might release baseline inhibition and increase responsiveness to reward information and external stimuli.

It is difficult to predict the exact relationship between serotonergic contributions to CGp and behavior, particularly given the minute effects of CGp microstimulation on behavior (B. Y. Hayden et al., 2008). The complexity of local interactions, upstream effectors and downstream contributions make such speculation even more challenging. It is possible, however, that increased CGp responsiveness would affect both phasic and tonic activity. Increased phasic activity might strengthen the signal of salient events, thus heightening attention to external events. If the tonic activity that distinguishes between large and small rewards is similarly upregulated, and we assume that the resulting gain modulation resembles a linear multiplier, then the difference between neuronal activity reflecting large and small rewards will increase. At first glance, this change should strengthen the distinction between large and small rewards, sharpening preferences for large rewards (notably, if we assume that the gain modulation resembles a logarithmic process, then this effect becomes even stronger). However, the observation that CGp microstimulation biases monkeys away from a preferred option after large rewards (B. Y. Hayden et al., 2008) suggests that increased CGp activity may instead blunt preferences by increasing the probability of choosing otherwise low probability options. This latter speculation would better explain the blunted preferences observed in depression, a disorder which is associated with increased CGp activity (Mayberg et al., 1997; Milak et al., 2005).
Notably, functional imaging studies have only infrequently indicated that RTD significantly changes activity in posterior cingulate cortex. More frequently, studies identify RTD-induced changes in PFC, ACC, the basal ganglia and the inferior parietal region (Fusar-Poli et al., 2006; Lamar et al., 2007). Studies of both attention and executive function have demonstrated changes in these regions but not in CGp following RTD (Bremner et al., 1997; Evers, Veen, Deursen, et al., 2006; Evers, Veen, Jolles, Deutz, & Schmitt, 2006; Horacek et al., 2005). However, RTD does modulate posterior cingulate cortical activity in depressed patients relative to controls (Neumeister et al., 2004) and in normal subjects performing memory tasks (Allen et al., 2006). Thus, it is possible that the putative CGp contributions to the effects of RTD on risk-sensitive behavior are modulated by memory-related functions — a hypothesis not inconsistent with the supposition that increased interest in the risky option may reflect decreased sensitivity to (or memory of) less desirable outcomes (see Chapters 4 and 5).

6.4.2 5HT2A receptors in CGp

The observation that RTD only infrequently modulates CGp activity may be due to conflicting interactions of serotonergic receptors found in CGp. While the inhibitory 5-HT1A receptors comprise the majority of serotonergic receptors in CGp, two excitatory receptors have been found in this region: 5-HT1F and 5-HT2A (Barnes & Sharp, 1999; Baumgarten & Gothert, 1997; Jakab & Goldman-Rakic, 1998; Morilak, Garlow, & Ciaranello, 1993). Since the 5-HT1F receptors are poorly understood and have been studied primarily as potential targets for migraine treatments (Ramadan, Skljarevski, Phebus, & Johnson, 2003; Agosti, 2007), I will not discuss them further. The 5-HT2A receptors are well-known since anti-psychotic drugs, in particular the atypical anti-psychotics that ameliorate the negative symptoms of schizophrenia, antagonize these receptors (Jakab & Goldman-Rakic, 1998; B. J. Jones & Blackburn, 148
5-HT2A receptor function has been implicated in impulsivity and aggression as well as anxiety (J. L. Evenden, 1998; Johnson, Gallagher, & Holland, 2009; Nomura & Nomura, 2006; Weisstaub et al., 2006; Winstanley, Theobald, Dalley, Glennon, & Robbins, 2004). Increasing 5-HT2A activity decreases aggression (Johnson et al., 2009) and selectively antagonizing 5-HT2A receptors decreases impulsive premature responses (Winstanley, Theobald, et al., 2004). Since the behavioral and neuronal mechanisms that underlie aggression, impulsivity and economic risk-seeking may not be identical (J. Evenden, 1999a), speculation about behavior on a simple gambling task on the basis of these results must necessarily be limited. However, measures of aggression are correlated with the probability of exceedingly risky behaviors such as suicide (Malkesman et al., 2009). If aggression and economic risk-preferences are related, one might speculate that up-regulating 5-HT2A receptors in posterior cingulate would decrease preference for a risky option. A more obvious prediction is that locally antagonizing 5-HT2A receptors in CGp would increase willingness to wait for larger rewards in a temporal delay task (for potential tasks, see (B. Y. Hayden & Platt, 2007; Long & Platt, 2005)).

6.5 Future directions: other decision regions with serotonergic input

The suggestion that serotonergic manipulations should affect CGp activity is a specific instance of a larger hypothesis: that serotonergic manipulations should modulate activity in brain regions that receive extensive DRN projections. Thus, neuronal activity in regions such as the prefrontal cortex and anterior cingulate cortex should also be modulated by serotonergic manipulations.
6.5.1 Prefrontal cortex

The prefrontal cortex is richly supplied with excitatory 5-HT2A receptors (Roth et al., 2004). Focal serotonin depletion in PFC inhibits global ability to reverse learned associations (Clarke, Dalley, Crofts, Robbins, & Roberts, 2004). However, this treatment appears to affect specific types of behaviors. Prefrontal serotonin depletion does not impair shifting attention between different dimensional qualities of compound visual stimuli (Clarke et al., 2005). While this example confirms that serotonin does contribute to normal PFC function, it also suggests that the interaction between neuromodulator and neuro-anatomical substrate may contribute to specialized regional functionality.

Correlational studies of the relationship between neuronal activity and serotonin transporter (5HTT) genotype have provided additional evidence for the importance of serotonergic contributions to neuronal function. The 5HTT gene is found in two common forms (polymorphisms), the short (S) and long (L) alleles, that influence the probability of depression (Canli & Lesch, 2007; Caspi et al., 2003; Hariri et al., 2002; Heinz et al., 2007; K. P. Lesch & Gutknecht, 2005; K.-P. Lesch, 2007; Lenze et al., 2005). Subjects with the S-allele, which predicts higher likelihood of depression, have stronger functional connectivity between the PFC and the amygdala (Heinz et al., 2005). This observation suggests that the form of the serotonin transporter contributes to the strength of coupling between brain regions. In addition, it implicates PFC-amygdalar connections in the pathology of depression, although it does not specify whether this increased functional connectivity is causal or symptomatic.

6.5.2 Anterior cingulate cortex

The ACC contains particularly high concentrations of the 5HTT (Roth et al., 2004). We might therefore speculate that modifying 5HTT activity could modulate both observed behavior and neuronal activity in ACC. In fact, ACC activity in subjects with
S-allele $5HTT$ polymorphisms exceeds that observed in subjects with L-allele polymorphisms (Canli et al., 2005). Additionally, since $5HTT$ polymorphisms are linked to the strength of functional coupling between the PFC and the amygdala (Heinz et al., 2005), $5HTT$ polymorphisms might modulate coupling between ACC and other decision-related regions. If connectivity is generally increased in people with the $5HTT$ S-allele, then the functional correlation between ACC activity and activity in regions such as CGp and OFC should be stronger in the presence of the S-allele.

6.5.3 Summary

These examples demonstrate that anatomically segregated serotonergic projections impact both behavior and localized neuronal activity. They also hint at the variety of techniques available to test serotonergic contributions to activity in a brain region. Both local and global serotonin depletion can be used to test the effects of low serotonin levels; alternatively, receptor-specific pharmacological agents can be used to inhibit or excite specific serotonin receptors (again, either locally or globally); and global or site-directed mutagenesis can, similarly, be used to probe the contributions of individual serotonin receptors. Teasing apart the mechanisms and interactions of the serotonergic system and its interactions with anatomical substrates, including the posterior cingulate cortex, will require a mélange of techniques and experimental approaches.
Appendix A

Supplementary Information for Rapid Tryptophan Depletion

A.1 RTD mix composition

Rapid Tryptophan Depletion (RTD) mixes were composed of 0.83g alanine, 0.48g glycine, 0.48g histidine, 1.2g isoleucine, 2.03g leucine, 1.65g lysine, 0.85g phenylalanine, 1.8g proline, 1.04g serine, 1.04g threonine, 1.04g tyrosine, 1.35g valine, 18.7mg methionine, 17mg cysteine, 30.6mg arginine. To confirm that tryptophan levels were not affected by the flavor included in the mix, in a subset of experiments the RTD mix was replaced by a volume-matched mix of flavor and water. Since plasma tryptophan levels did not differ significantly between these control experiments and the normal baseline measurements (ANOVA n.s.), we combined the behavioral data from these experiments in our analysis. Based on the distribution of plasma tryptophan measurements (Figure 4.3a), we divided the data into two categories for analysis, a normal tryptophan category with plasma tryptophan $<6.5$ ug/mL and a low tryptophan category with plasma tryptophan $\leq 6.5$ ug/mL. To control for the possibility that consuming the amino acid mixture might cause behavioral changes indepen-
dent of lowered serotonin levels, we included 0.35g tryptophan in the depletion mix for a separate subset of experiments (balanced mix). Unlike the RTD mix, this balanced mixture did not significantly lower plasma tryptophan levels (Supplementary Fig A.1; ANOVA, plasma tryptophan vs treatment (baseline, balanced, RTD): $F=36.3, p<0.01$; baseline vs balanced, post-hoc Bonferroni test, n.s.; baseline vs RTD, Bonferroni $p<0.01$; balanced vs RTD, Bonferroni $p<0.01$); furthermore, the monkeys’ behavior following administration of the balanced mix could not be distinguished from baseline (Supplementary Figure A.2; ANOVA, choice vs treatment (baseline, balanced, RTD) and reward context: effect of treatment $F=4.98, p<0.01$, effect of context, $F=56.3, p<0.01$, interaction term n.s. with both contexts in same direction; post-hoc Bonferroni t-test, choices after RTD vs baseline, $p<0.01$; choices after RTD vs balanced mix, $p<0.05$; post-hoc Bonferroni t-test, choices after balanced mix vs baseline, n.s. ($p>0.5$)).

A.2 Plasma tyrosine measurements

Since tryptophan competes with large neutral amino acids (LNAA), like tyrosine, for access to transport across the blood-brain barrier, the decreased plasma tryptophan/LNAA ratio that results from dietary tryptophan depletion contributes to RTD’s effect on brain serotonin. Since we were able to acquire only limited volumes of serum for analysis, we only measured plasma tyrosine levels as a proxy for LNAA concentrations. Tyrosine was measured by HPLC followed by electrochemical detection using a modification of literature methods (Le Masurier, Oldenzeil, Lehman, Cowen, & Sharp, 2006). Samples were injected directly onto a C18 reverse-phase ODC column and eluted with a mobile phase comprised of 0.1 M Na2HPO4, 0.1 mM EDTA, 2.7 mM octane sulfonic acid and 12% methanol, pH 3.8. Samples were detected with a BAS LC-4B amperometric detector with a dual 3 mM glassy carbon electrode at a potential of .8V vs. an Ag/AgCl reference electrode. Samples were
quantitated in comparison to external standards.

We then calculated the plasma tryptophan/tyrosine ratio. As shown in Supplementary Figure A.3, this ratio decreased significantly in RTD experiments relative to baseline and balanced amino acid sessions (ANOVA, tryptophan/tyrosine ratio vs experiment, F=14.2, p<0.01; Fischer/Bonferroni post-hoc t-test, baseline vs Tryp+, n.s. but RTD vs baseline or Tryp+, p<0.01).

A.3 Mix administration

Since the amino acid mixture used may be unpleasant, we carefully monitored the monkeys’ behavior for signs of discomfort. We found over the course of experiments that the monkeys drank the RTD mix more slowly as they approached completion of large (e.g. 200mL) mixes, suggesting satiety. In order to minimize potential discomfort to the monkeys while maintaining consistent amino acid intake, we lowered the volume of liquid used in the RTD mix and found that the monkeys consumed the mix more quickly. Our analysis indicated no effect of fluid volume, independent of treatment condition, on preferences, consistent with previous observations using this task (A. McCoy & Platt, 2005b).

In addition, to ensure that the monkeys consumed the full amino acid mix, we considered giving them access to the mix earlier. Since previously published experiments demonstrated that RTD decreases plasma tryptophan for at least 18 hours (Carpenter et al., 1998), we reasoned that allowing the monkey to consume part of the mix the night before testing would minimize potential discomfort while ensuring consistent tryptophan depletion. We carefully monitored amino acid and water intake and monitored both behavioral and biochemical results to determine whether this procedural variation affected either biochemical depletion or behavior. Since our analysis demonstrated that this procedural accommodation did not affect either behavioral or biochemical results (Results, Chapter 4), we concluded that this data
was acceptable to include in our analysis.

We further note that, while we based the composition of our amino acid mix on that found in the primate (monkey and human) literature, experiments in both vervet monkeys and rats have used a minimal RTD mix composed of only 7 amino acids (Moja et al., 1989; Young et al., 1989). The persistence and specificity of serotonergic modulation found across these experiments, despite the differences in methodology, suggests that dietary tryptophan depletion is robust across a variety of procedural variations.

A.4 Safety premiums

To calculate each monkey’s safety premium, we used regression to identify the best-fit line describing the relationship between the choice likelihood (y) and the EV difference between the safe and risky options (x):

Monkey SH
Baseline, \( y = 0.34 + 0.008x (r = 0.91, p < 0.00001) \)
RTD, \( y = 0.31 + 0.007x (r = 0.98, p < 0.0000001) \)

Monkey NI
Baseline, \( y = 0.31 + 0.005x (r = 0.73, p = 0.016) \)
RTD, \( y = 0.34 + 0.003x (r = 0.65, p = 0.002) \)

Monkey BR
Baseline, \( y = 0.37 + 0.005x (r = 0.65, p = 0.012) \)
RTD, \( y = 0.24 + 0.005x (r = 0.61, p < 0.001) \)

A.5 Effect of reward and choice histories

We assessed the dependence of monkeys’ choices on reward history using a model in which the relative probability of risky (\( p_R \)) and safe (\( p_S \)) choices depends on the
weighted sum over n past trials of reward outcomes ($r^j_R = $ reward received from a risky choice on trial $j$, $r^j_S = $ reward received from a safe choice on trial $j$) and a constant term ($\gamma$):

$$\log(p_R/p_S) = \sum_{j=1:n} (\alpha_j (r^j_R - r^j_S)) + \gamma$$

To determine the number of past rewards (n) that best predicted choices, we used Aikake’s Information Criterion (AIC) to determine the explanatory power of models including reward history from the first to tenth trial preceding the current trial in every session. We then compared the kernels formed by weighting the reward coefficients (alpha) with the Akaike weights; these distributions were not significantly different (t-test n.s.).

A.6 Figures
Figure A.1: Plasma tryptophan levels diminish following RTD but not after a balanced amino acid mix. Plasma tryptophan levels measured after administration of a balanced amino acid mixture (RTD mix + tryptophan; n=5 measurements) are not statistically different from baseline measurements. After RTD, plasma tryptophan decreased significantly relative to either baseline or balanced conditions.
Figure A.2: RTD, unlike a balanced amino acid mix, shifts choice frequencies relative to baseline. Monkeys choose the safe option less frequently following RTD than at baseline or after administration of a balanced (RTD + tryptophan) mixture. However, choice frequencies following consumption of a balanced mixture were indistinguishable from baseline frequencies.
Figure A.3: The plasma tryptophan/tyrosine ratio, like plasma tryptophan, confirms successful tryptophan depletion. After RTD (n=7 measurements), the ratio of plasma tryptophan/tyrosine dropped significantly relative to both baseline (n=4 measurements; Bonferroni post-hoc t-test, p<0.01) and balanced (n=5 measurements; Bonferroni post-hoc t-test, p<0.01) conditions. However, the tryptophan/tyrosine ratio did not change significantly in the balanced condition relative to baseline (Bonferroni post-hoc t-test, n.s.).
To determine whether RTD diminishes monkeys’ ability to distinguish reward sizes, we measured their preferences with a simple discrimination task. As in the gambling task, monkeys initially foveated (±1-2°) a central yellow LED (200-800ms) to begin each trial, after which two peripheral LEDs (one red, one green) were illuminated diametrically opposite each other. Extinguishing the fixation LED cued a gaze shift to either target (± 1-2°) within 350 ms. Successful trials were rewarded with juice and a 300ms noise. The color and location of the peripheral LEDs were randomly matched on each trial so that each color appeared in each location on approximately half of the trials in a given block, but each color indicated a consistent, guaranteed reward within a block of 25-30 trials. We assigned one color to a large reward size and one to a small reward size within each block and tested discriminatory ability across a range of reward differences. We describe the choice of the larger option as the correct choice. The monkeys’ performance did not differ between baseline and depleted conditions (ANOVA, correct frequency per block vs condition, F=1.4, p=0.23; baseline 68.8±3.7% correct choices across 38 blocks; RTD 75.8±4.7% correct choices across 33 blocks).
Appendix B

Supplemental Information: Low serotonin increases risky decisions in mice
Figure B.1: Fractional dopamine is unaffected by PCPA treatment. For each mouse, we calculated the ratio of brain dopamine to dopamine and its metabolite DOPAC: \[
\frac{[DA]}{[DA]+[DOPAC]}
\]. Comparing the resulting fractional dopamine metric between conditions did not reveal a significant difference between treatment groups.
Figure B.2: Fractional dopamine levels do not depend on changes in brain serotonin. In case comparing fractional dopamine across treatment conditions failed to reveal a subtle relationship between dopamine and serotonin levels, we regressed the fractional dopamine vs brain serotonin. Frontal cortex serotonin levels did not significantly predict fractional dopamine (linear regression, $R^2 = 0.08$, n.s.).
Figure B.3: Vehicle and PCPA mice have indistinguishable initial preferences. Across the population, vehicle and control mice are approximately indifferent on the first day of testing (i.e. when naive to risky and safe options; one-way ANOVA, risky choice frequency per mouse vs treatment group, $F=0.08$, n.s.).
Figure B.4: Vehicle and PCPA risk preferences differ despite similar early experiences. Mice that initially choose the risky target less than chance (lower left quadrant) are likely to become risk averse. Similarly, control mice that choose the risky target more often than chance are likely to become risk averse (lower right quadrant). In contrast, a significant subset of the PCPA mice — mostly mice that initially choose the risky option more than chance — become risk seeking (upper right quadrant). Vertical dashed line demarcates indifference between safe and risky options on the first day of testing; horizontal dashed line, indifference across days.
Figure B.5: Shifted risk preferences reflect global changes in choice probability. We sorted mice by both treatment group and overall risk attitude. Blue, control mice; red, PCPA treated mice; solid lines, mice with overall safe preferences; dashed lines, mice more likely to choose the risky option overall.
Figure B.6: PCPA-treated mice nosepoke more frequently than sham controls, a difference that is enhanced on trials when mice choose the safe option. Data are similar when mice are sorted by overall risk attitudes (data not shown). Asterisk, p<0.01, post-hoc Fisher LSD (or Bonferroni, for that matter) t-test.
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Biography

Arwen Long was born in Evanston, IL on May 6, 1981. She wrote this document faster than you might think, slept less than you might imagine, and defeated a few pink robots along the way. Someday Arwen will become a retired hippie and move to Vermont, where she will run an organic food-n-flower farm when she is not joy-riding past the fields of fragrant bovine beasts.

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