Stony Brook University

Orbitofrontal Cortex and the Decision-Making Process

A Thesis Presented

by

Youran Wu

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Master of Science

in

Biochemistry and Cell Biology

Stony Brook University

May 2014

Stony Brook University

The Graduate School

Youran Wu

We, the thesis committee for the above candidate for the

Master of Science degree, hereby recommend

acceptance of this thesis.

Dr. Adam Kepecs, Ph.D.
Associate Professor, Cold Spring Harbor Laboratory

Dr. Dinu Florin Albeanu, Ph.D.
Assistant Professor, Cold Spring Harbor Laboratory

This thesis is accepted by the Graduate School

Charles Taber
Dean of the Graduate School

Abstract of the Thesis

Orbitofrontal Cortex and the Decision-Making Process

by

Youran Wu

Master of Science

in

Biochemistry and Cell Biology

Stony Brook University

2014

Orbitofrontal cortex (OFC) is situated at the ventral floor of the prefrontal cortex region. OFC and some of its related structure (ventral striatum, ventral tegmental area, basolateral amygdala) are considered to be part of the valuation system that is essential to the decision-making process. In order to understand the exact role OFC plays in the decision-making process, we sought to find out how decision variables are represented in different OFC projections, and what will happen if OFC projections were disrupted. The first specific aim of this project is to find out whether projections to OFC related structures are originated from non-overlapping population of neurons within OFC; our double labeling experiments did support this argument, which further implied the possibility that different decision variables are represented in different OFC projections. The second specific aim is to utilize morphine to induce structural changes in the OFC region, and assess how the changes affect the decision-making process; high dosage

of morphine was injected in rats for 8 days, and their performance in 2AFC tasks were compared between session data collected before and after the injection. It was shown that rats were more impulsive, and no longer able to optimally integrate information.

Dedication Page

I dedicate my work to my family, friends, and all laboratory animals.

TABLE OF CONTENTS

Figures

Tables

List of Abbreviations

BLA	Basolateral Amygdala
CTB	Cholera Toxin B Subunit
OFC	Orbitofrontal Cortex
PBS	Phosphate Buffered Saline
PFC	Prefrontal Cortex
vSTR	Ventral Striatum
VTA	Ventral Tegmental Area
2AFC	Two-Alternative Forced Choice Task

Acknowledgment

I would like to thank my PI Dr. Adam Kepecs, Dr. Junya Hirokawa, and the rest of the Kepecs Lab for mentoring and nurturing.

I would like to thank Professor Neta Dean for advice and support; Dr. Scott Powers for trainings on critical thinking.

I. Introduction

1.1 Decision-making process requires a valuation system

Decision-making is a cognitive process that results in the commitment to a categorical proposition[1]. It has long been treated as the hallmark of higher cognition, and is required in a spectrum of behaviors ranging from simple movements to complicated abstract reasoning. For example, a snake determining if what it sees is a mouse; a frustrated graduate student with limited stipend choosing between vanilla and chocolate flavored ice cream. Studies on the decision-making process have been performed in multiple disciplines; now with new advances in fields like molecular genetics, engineering, and statistics, researchers are able to bridge the gap and come to new conclusions on the nature of the process, and how it is implemented in the brain.

Neuroeconomics address value-based decision-making. Studies on the decision-making process have been traditionally focused on two domains: perceptual decision-making and preferential decision-making[2].

Perceptual Decision-making relies on sensory information acquired through experience[3]. Since the sense-perception system is internally noisy, the interpretation requires decision-making. Perceptual decision-making has been the subject of study in

the fields of experimental psychology and neurophysiology[4, 5]. Preferential decision-making refers to the commitment to one choice among a set of alternatives. It has been under the investigation of economics and social sciences, often in the format of choice preference reports[2].

It had been difficult to compare above two types of theories until recent studies on neuroeconomics emerged, which consist of neurophysiology studies on value-based decision-making in behaving animals, and computational models that emphasize on the process of value-based decision-making[2]. A full understanding requires description from economics, psychology, neuroscience, and computational level[6].

A valuation system. A framework has been proposed, which describes the value-based decision-making as a combination of five basic processes[6]: representation of decision problem and potential course of action; assignment of values to different actions; comparison of value; measurement of desirability of the outcome; incorporation of feedback to improve future decisions[6]. (Figure 1.1.1) These processes require a valuation system that translates environmental input to behavioral output, of which reward processing and association learning is detrimental.

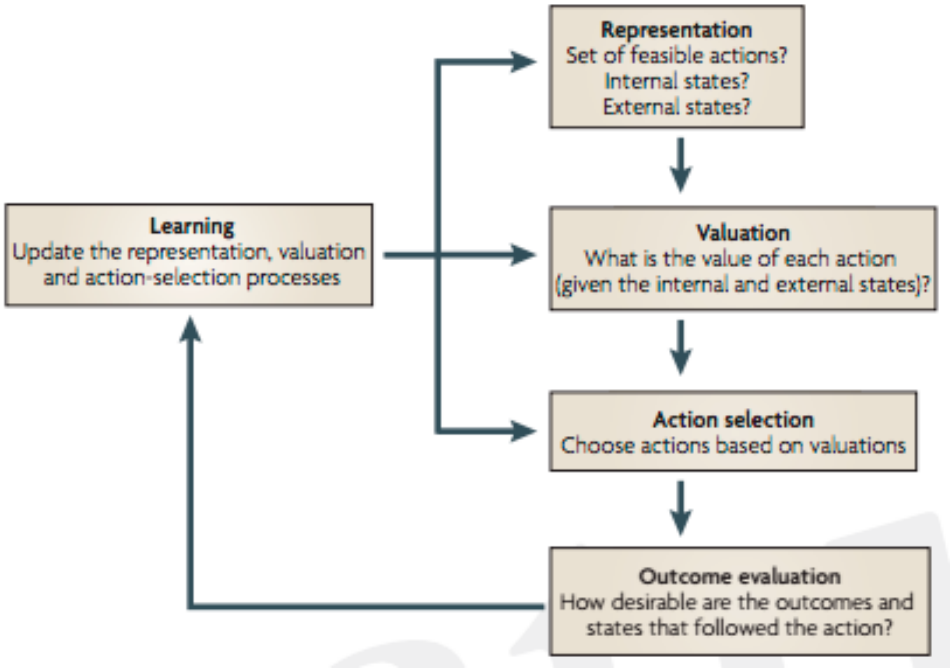

Figure 1.1.1 **Value-based decision-making requires five basic processes**. The representation of decision problem and potential course of action; assignment of values to different actions; comparison of value; measurement of desirability of the outcome; incorporation of feedback to improve future decisions. (Adapted from Rangel, 2008[6])

1.2 Orbitofrontal cortex (OFC) and related structures play an important role in the valuation system

Structure. OFC is anatomically situated at ventral side of the frontal region of the brain. According to definition given by Korbinian Brodmann, OFC is composed of Broadmann area 10, 11 and 47 in human; area 11, 12 and 13 in non-human primates[32]. Although the nomenclature remains consistent across species, and the details of the orbitofrontal cortex were not investigated, this was the first effort spent to comprehensively characterize brain architecture in human and primates. Further studies posted questions on the homogeneity that was present in Brodmann's map; recent advances in technology enabled researchers to address the subdivisions in the OFC region. (Figure 1.2.1) Presently, it is defined as the part of the prefrontal cortex that receives projections from the magnocellular medial nucleus of the mediodorsal thalamus[8].

Sensory input and reward. Prior studies has shown that OFC receives input from olfactory[4,5], visual[9], auditory[10], taste, and somatosensory cortex[10]. Taste studies have shown that rewards and punishment, ie. positive and negative reinforcers, are separately represented in the OFC[11,12]; in addition, information transmitted from the OFC often carries reward values, indicated by neuronal inactivity upon taste stimulating when the monkey is fed to satiety[33]. On the other hand, lesions of OFC lead to deficits in learning whether stimuli are rewarding or not[34].

Anatomy. OFC projects to regions including ventral striatum (vSTR), ventral tegmental area (VTA), and basolateral amygdala (BLA), all of which are considered to be part of the valuation system[6]. (Figure 1.2.1 D)

Ventral striatum is a part of the striatum, and is composed of the nucleus accumbens, the olfactory tubercle, and part of the caudate nucleus. Neurophysiological studies demonstrated that vSTR is involved in reward processing, expectation, and learning [17,18]. In addition, it was shown that vSTR is likely to be involved in early stages of trial and error learning rather than late stages[19]. Ventral tegmental area is located closely to the midline on the floor of midbrain[20]. Dopaminergic projections originated from VTA to vSTR are activated upon reward stimuli, which is important for recognizing and consuming rewards in the environment[21]. Basolateral amygdala is part of the amygdala. Its primary function is regulating fear response; VTA-BLA projection participates in avoidance and fear-conditioning.

Functions. OFC remains to be one of the least-understood areas in the forebrain region. Studies have shown that the firing rate of neurons in OFC region is correlated with identifying the nature of the reward, and, expected value of choice outcomes; more recent studies further implies that OFC might be representing values “in an abstract and context-independent manner that provide a ‘common currency’ for decision making”[35]. Immunofluorescence experiments shows that there are distinct subpopulation of neurons in the cerebral cortex, which includes OFC region[36]; however, it is not clear how different decision variables are represented in those neurons.

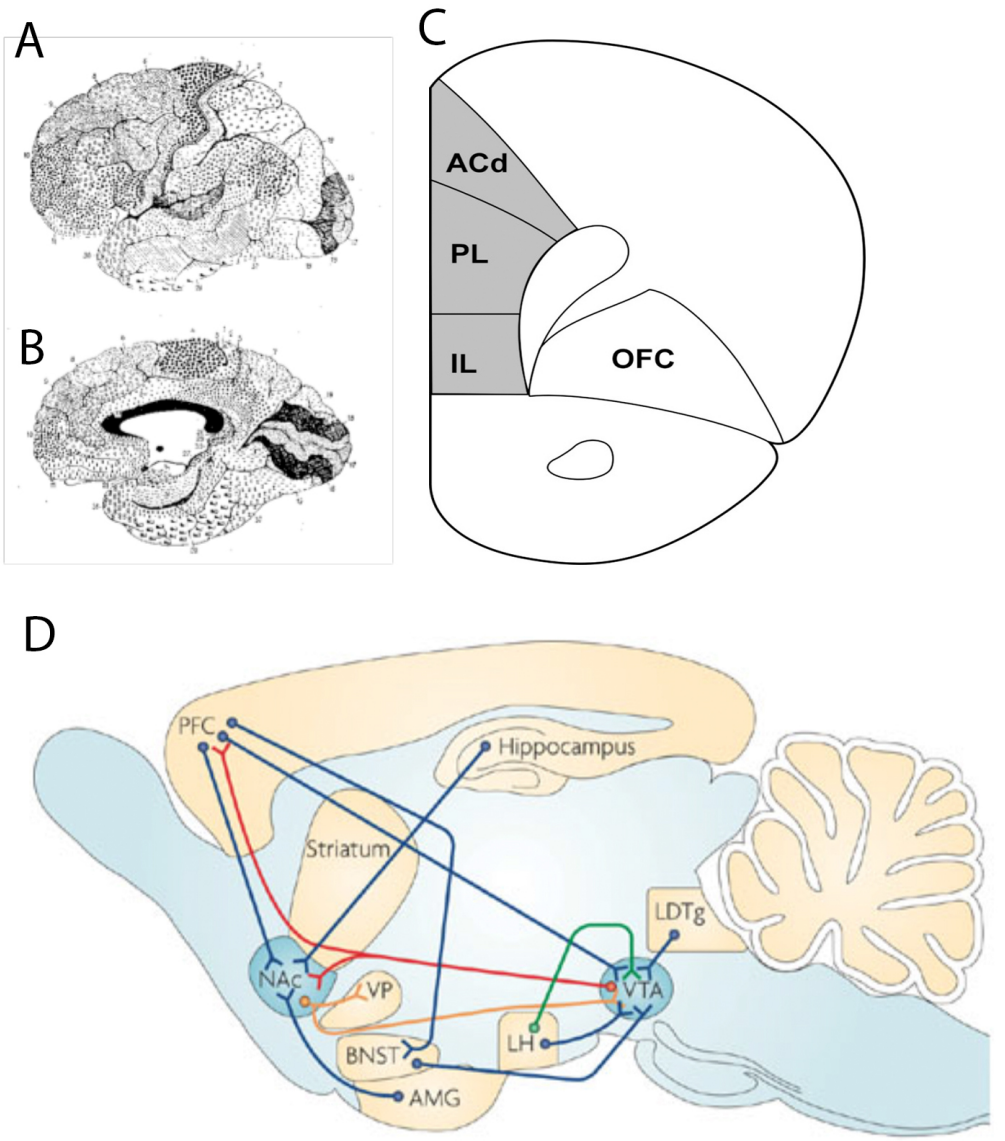

Figure 1.2.1 **Simplified scheme of brain structures**. A-B. Sample maps of cortical architecture using Brodmann numbers. A. Lateral view of left hemisphere. B. Medial view of right hemisphere. (Adapted from Brodmann[22]) C. Orbitofrontal cortex (OFC) is situated at the ventral site of prefrontal cortex, while above the olfactory bulb. D. Mesolimbic dopamine system circuitry in rat brain, which involves orbitofrontal cortex (ventral PFC), nucleus accumbens (ventral Striatum), basolateral amygdala (AMG), and ventral tegmental area (VTA). Dopaminergic pathways (in red) originated from VTA to vSTR and PFC, while glutaminergic projections (in blue) from PFC extend to vSTR, VTA, and BLA; in addition, GABAnergic projections (in orange) also connects vSTR and VTA (Adapted from Kauer, 2007[23])

1.3 Chronic exposure to opiate drugs induce significant impact on brain including orbitofrontal cortex region

Morphine is a naturally occurring chemical found in opium poppy. It is primarily used in clinical settings as an analgesic agent. Nociceptive neurons deliver action potentials in response to pain stimulus, and studies have shown that microinjection of morphine into rat brain inhibit the firing of nociceptive neurons; evidence also support that morphine function through activating descending inhibitory pathways[24].

Chronic exposure to opiates has been demonstrated to induce antigens or increase DNA binding activity in certain brain areas[25], it also leads to significant morphological changes in neurons located in nucleus accumbens and prefrontal cortex area; the number of dendritic spines and complexity of dendritic branching was decreased when rats received repeated morphine treatment[26]. This type of structural changes is considered to be an indication for synaptic organization alteration. Although the exact mechanism of those morphological changes induced by morphine is unclear, several experiments have been conducted linking it to morphine's localized effects on cytoskeleton proteins[27, 28].

Studies conducted in human have linked chronic morphine usage with risk-taking and poor decision-making, animal studies further shows that the acute effect of morphine includes increase in impulsivity; all of these phenotypes resembles having abnormalities in the OFC region.

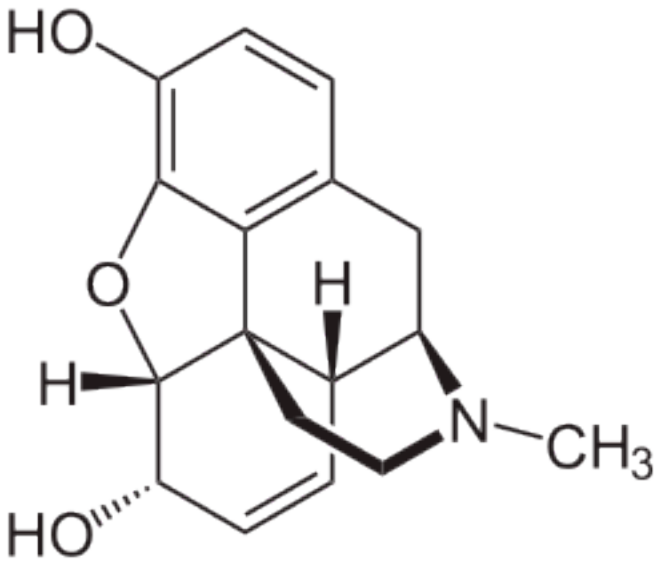

Figure 1.3.1 **The molecular structure of morphine.** Morphine is a naturally occurring chemical found in opium poppy. It is often used as analgesic drug to relive pain in clinical settings; it is also considered to be a psychoactive compound that induces addiction.

1.4 Rodent model is a reliable tool to investigate neuroeconomics

Although rat genome has reported to be slightly smaller than human genome (2.75 billion bp vs. 2.9 billion bp), it was found that rat genome contains roughly the same number of genes as human[29]; in addition, rat genome contains counterparts to almost all human genes known to be associated with diseases[29]. Therefore, rats, especially the *Long-Evans rat*, have long been used as animal models in behavioral research. Reliable methods have also been established to investigate neuroeconomics topics.

Retrograde tracing utilize different tracers that travel through synaptic terminals to cell bodies to provide details on neural circuits. Among commonly used tracers are cholera toxin B subunit (CTB), which is the non-toxic part of the protein complex cholera toxin, and microspheres (beads). When the protein or the beads are tagged with fluorescent markers, they enable double retrograde labeling that interrogates the sources of neuronal projections to two different brain regions simultaneously.

Two-alternative forced choice Task (2AFC) is a psychophysics method frequently used for the purpose of eliminating observer's bias due to criterion level of sensory activation[30]. In a classical 2AFC experiment, the experimental subject is presented with two stimuli, one of which is deemed to be the test stimulus and is to be detected. Noise is inherently correlated with stimuli presented, but is assumed to be independent of the stimuli according to the signal detection theory[31].

II. Materials and Methods

2.1 Retrograde labeling experiment

Animal handling. Six adult male *Longs-Evans* rats (C112, C130-C134) were pair-housed under a 12:12 hour light/dark cycle (light on at 6:00 A.M.) prior to retrograde tracer injection, and were individually house post injection. Animal handling and experimental procedures were approved by the Cold Spring Harbor Laboratory, Institutional Animal Care and Use Committee.

Tracer preparation. 100μg of CTB Alexa Fluor 488 (Life Technologies, C-34775) and 100μg of CTB Alexa Fluor 594 (Life Technologies, C-34777) were obtained and diluted in 20μl of distilled water individually. Vials of concentrated green and red fluorescent RetroBeads (Lumafluor Inc.) were obtained and used as is. Both CTB and RetroBeads solutions were stored in 4°C cold room, removed from light sources.

Injection. Rats were anesthetized with isofluorane (3% for induction and 2-2.5% for maintenance, oxygen was supplied at 0.5-0.8%). Glass capillary tubes (0.86mm, with filament, Warner Instrument G105F-3) were heated, pulled, and the tips were cut back to approximately 20μm diameter under microscopic control. These glass pipettes were then filled with 1μl of CTB or RetroBeads, and were injected stereotaxically into

vSTR, VTA, BLA, or right OFC (Table 1.1 and 1.2), by applying air pressure with picospritzer (Parker Picospritzer III). Glass pipette was left in place for 10 minutes for avoid leakage.

Perfusion and imaging. Fourteen days after CTB injection, or eight days after RetroBeads injection, animals were deeply anesthetized (0.5% oxygen and 3% isofuorane), euthanized (euthasol, Virbac 710101), and subsequently perfused through the ascending aorta with 40ml PBS, followed by approximately 400ml of paraformaldehyde (4%). The brain was removed and immersed in paraformaldehyde (4%) overnight, then transferred to PBS solution for another 24 hours at 4°C. The resulting brain was mounted onto a microtome (Leica VT1000S) and cut into 100μm thin slices, which were then preserved in 24-well plates filled with PBS solution. Brain slices were observed and gray-scale pictures were taken with microscope (Olympus MVX1000S); pictures were further processed with Photoshop to add color and adjust contrast.

Rat	Tracer	Injection site
C112	CTB-594	vSTR
	CTB-488	VTA
C130	CTB-594	VTA
	CTB-488	vSTR
C131	CTB-594	Right OFC
	CTB-488	vSTR
C132	CTB-594	vSTR
	CTB-488	Right-OFC
C133	RetroBeads-Green	BLA
	RetroBeads-Red	vSTR
C134	RetroBeads-Green	vSTR
	RetroBeads-Red	BLA

Table 2.1.1 **List of injection sites and tracers used in each experimental subject.** Six adult male *LE rats* (C112, C130-C134) were double injected with either CTB or RetroBeads for retrograde double labeling.

	AP (mm)	ML (mm)	Depth (mm)
Right OFC	3.7	3.0	3.0
vSTR	1.2	-3.0	5.4
BLA	-2.2	-4.8	6.3
VTA	-5.2	-0.7	7.0

Table 2.1.2 **List of injection site coordinates**.

2.2 Morphine experiment

Animal handling. Five adult male *Longs-Evans* rats (M01, M03, M06-M08) were individually housed under a 12:12 hour light/dark cycle (light on at 6:00 A.M.). Animal handling and experimental procedures were approved by the Cold Spring Harbor Laboratory, Institutional Animal Care and Use Committee. Rats were initially trained under one session of odor discrimination tasks for 180 minutes (approximately 900-11000 trials), 5 days a week for 16 days in total, in order to establish behavior baseline, and data collected during this period were deemed the control set. After morphine administration, animals were left undisturbed for 4 weeks before additional 8 sessions of

odor discrimination tasks were performed; behavior data from the post drug-intake-period were considered the experimental set.

Free access to food was allowed, while animals were water-deprived for at least 18 hours prior to the task session; free access to water were granted for 30 minutes during non-training days.

Odor discrimination task. In a behavior rig, a central poke hole is located on one side of the wall, flanked by two feeder holes on both sides. All three holes were monitored by infrared photo-sensors. Upon snout poking by the animal, the central poke hole will release an odor mixture consisting of D/L octanol at varying percentage; each compound dictate either the left or right choice. Rats were trained to decide which odor was the dominating component (>50%), and chose the corresponding feeder hole dictated by the odor; correct choices were rewarded with a small amount of water (25ul) after 1s delay, while wrong choices led to no reward. Tasks were given in blocks of 60-80 trials to avoid habituation (Figure 2.1.2 B). The percentage composition of the odor mixture was categorized into 6 groups (Table 2.1.1); after each block transition, the odor category changes randomly, while the amount of reward (water) was reduced to 1/3 on one side, in order to generate side-bias.

Morphine administration. Morphine (Sigma, Cat. No. M8777) was dissolved in saline to achieve final concentration of 20mg/ml. This stock solution of morphine was further diluted into working solution when needed. Rats were injected subcutaneously with increasing dosage of morphine for 8 days (Table 2.2.1).

	Morphine Dosage
Day 1	10mg/kg
Day 2	15mg/kg
Day 3	20mg/kg
Day 4	25mg/kg
Day 5	20mg/kg
Day 6	25mg/kg
Day 7	30mg/kg
Day 8	40mg/kg

Table 2.2.1 **Morphine dosage used in the chronic morphine experiment.** Rats were injected with increasing amount of morphine during an 8 day period.

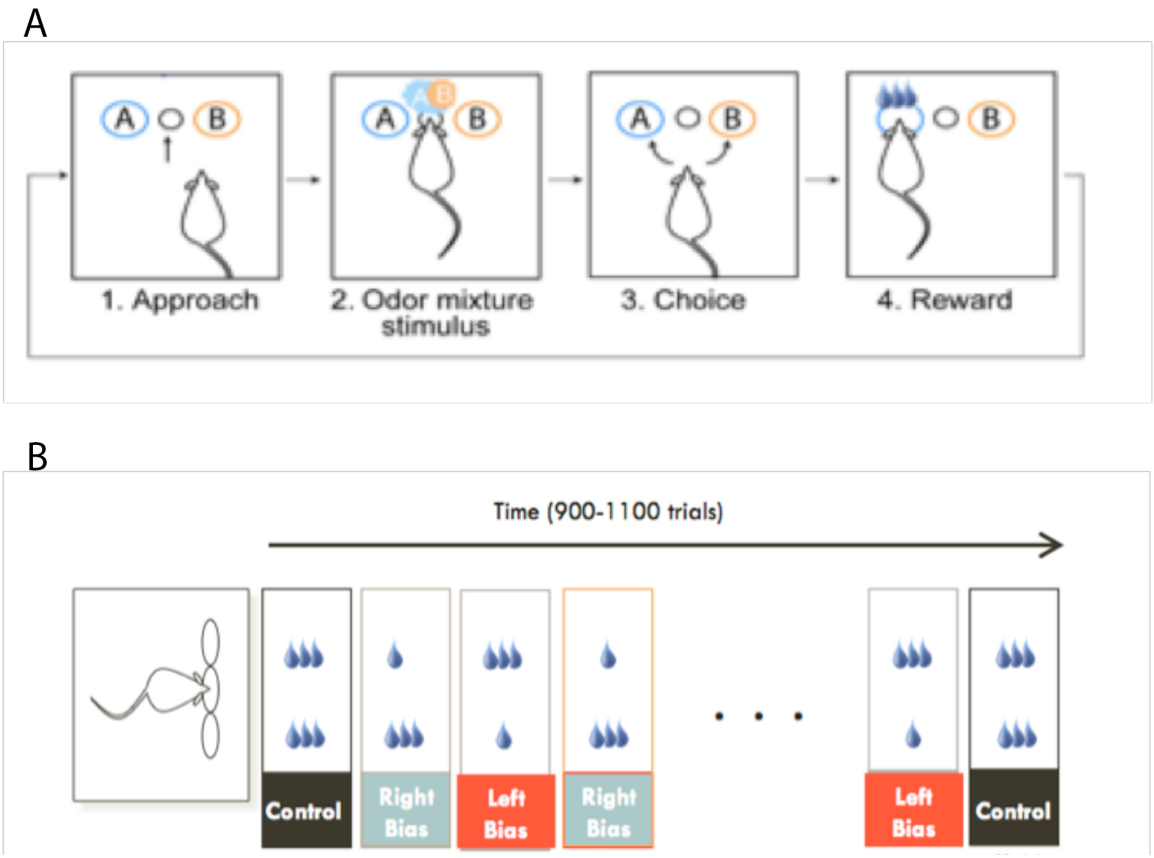

Figure 2.1.1 **Scheme of odor discrimination tasks.** A. Rats approach the center poke hole, which releases a mixture of D/L octanol at varying percentage, each compound dictate either the left or right choice. Rats were to decide which odor was the dominating component (>50%), and chose the corresponding feeder hole dictated by the odor; correct choices were rewarded with water, and incorrect choices led to no reward; B. The odor discrimination tasks were giving in blocks of 60-80 trials. After the initial control block (no bias); the odor category changes randomly at each block transition, while the amount of water rewarded was reduced to 1/3 on one side, in order to generate side-bias.

	Left Choice (D-octanol)	Right Choice (L-octanol)
Category 1	5%	95%
Category 2	30%	70%
Category 3	45%	55%
Category 4	55%	45%
Category 5	70%	30%
Category 6	95%	5%

Table 2.1.1 **List of odor compositions in each task category.** Odor mixtures given in the odor discrimination tasks were grouped into categories, according to percentage compositions.

III. Results

Retrograde Labeling

3.1 Retrograde injections provide some reliable information

The retrograde injection process proven to be quite a challenge, especially to someone with limited experience; at times it was difficult to estimate the amount of reagents delivered, when glass pipettes were inserted deeply into the brain and solution surface was not visible. Although slight variation was observed in certain injection sites (Figure 3.1.1), they provide us with valuable information nonetheless.

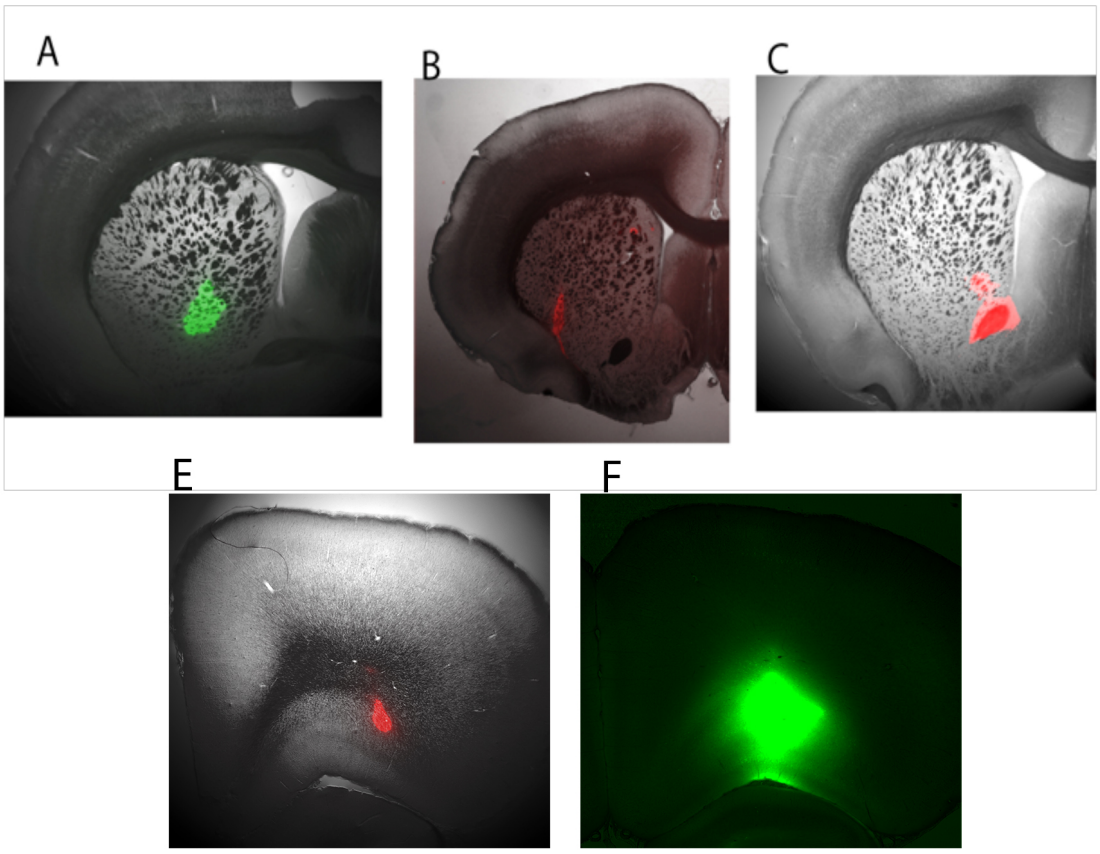

Figure 3.1.1 **Injection site comparison.** A-C: Injection site at vSTR (C131-C133). E-F: Injection site at OFC (C131-C132). The medial-lateral coordinates appear to vary but are within reasonable range.

The labeling in the OFC resulted from vSTR labeling differentiate slightly in the lateral OFC, while both showing strong signals in the ventral OFC area (Figure 3.1.2). The variation in vSTR signals might also resulted from the differences in nature of reagents injected.

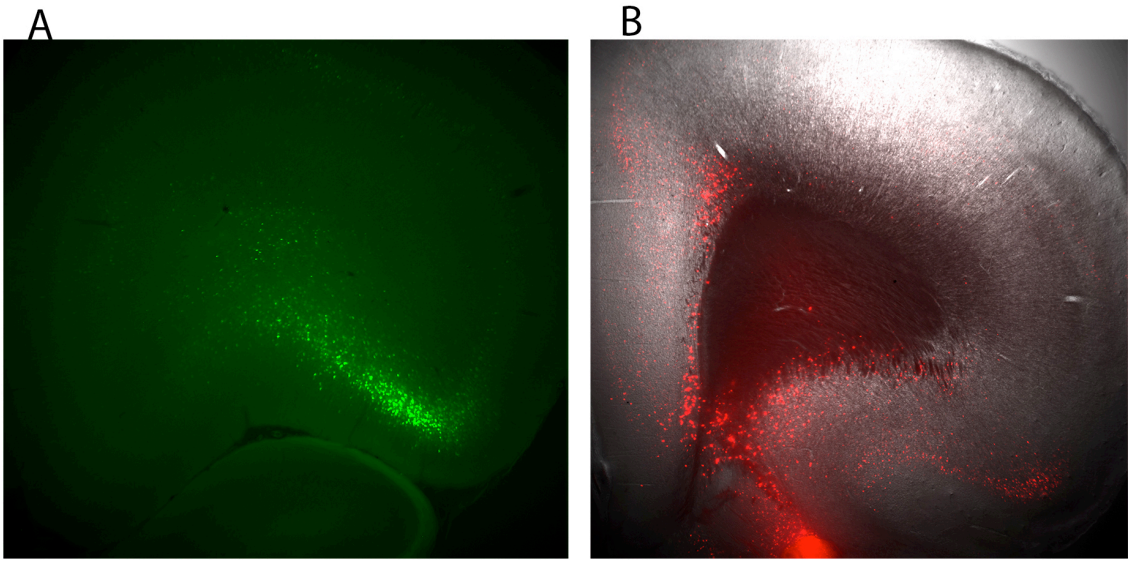

Figure 3.1.2 **OFC retrograde result comparison.** A. OFC-vSTR labeling with CTB in C131. B. OFC-vSTR labeling with RetroBeads in C133. Both show strong signals in the ventral OFC region.

3.2 OFC outputs to vSTR, BLA, and VTA seem to originate from largely non-overlapping population of neurons

Cross section image obtained from C130 shows OFC-vSTR and OFC-VTA neurons were largely non-overlapping, with very few cells exhibiting both labels (Figure 3.2.1 A, B). Signals from right OFC injections shows up in very confined areas of the opposite hemisphere (Figure 3.2.1 C), and seem to be overlapping with vSTR signals in

those areas (Figure 3.2.1 D). Slices from C134 shows OFC-STR and OFC-BLA neurons are non-overlapping (Figure 3.2.1 E, F).

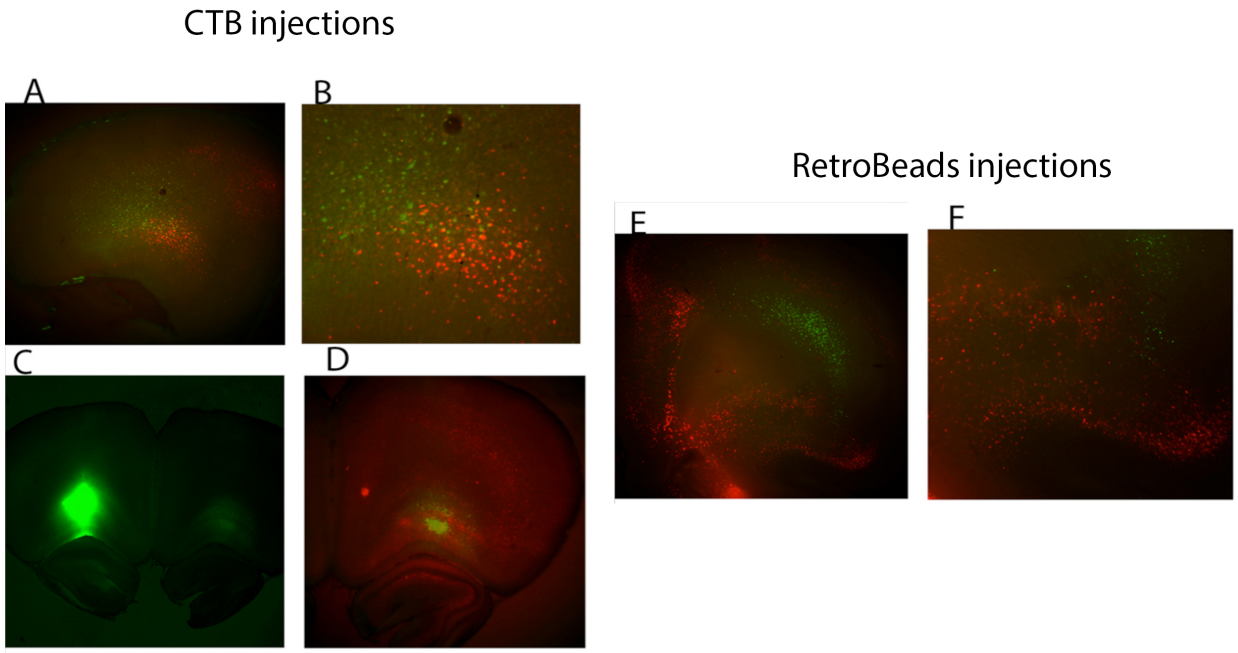

Figure 3.2.1 **Double labeling result shows non-overlapping population of neurons.** A-B. Slice showing PFC region of C130. OFC-vSTR (in red) and OFC-VTA (in green) were shown to be largely non-overlapping; zoom in picture shows a few cells exhibiting a mixture of red and green labeling. C. OFC injection (in green) in C132. Contra-lateral labeling shows the signal was localized. D. Slice from C132 shows OFC-vSTR (in red) overlapping with OFC-OFC (in green) neurons. E-F. Slice from C134 shows OFC-vSTR (in red) and OFC-BLA (in green) neurons are non-overlapping.

Chronic Morphine Exposure

3.3 Chronic exposure to morphine induce impulsivity, while rendering animals unable to optimally integrate information

It has been known that individual animals within the same species have different tolerance to drug intake. The total amount, as well as the concentration, of morphine used in our experiment was carefully determined by cross-examining processes and results from multiple literature[26, 37,38,39]. Despite our effort, one rat (M03) shown phenotypes of under stress (e.g. blood in urine) after high dosage of morphine injection; therefore was removed from further experiment. Control and experimental data set of the rest of the four rats (M01, M06-M08) were pooled and compared under multiple conditions.

We hypothesized that when a choice is associated with bigger reward, rats tend to pick said choice when the there is little evidence present; such bias would lessen significantly when enough evidence is present. This is manifested as the shifting of the psychometric function curve towards the biased side. Control data closely resembles our prediction (Figure 3.3.1 B, C); although experimental data does exhibit the characteristic shifting, the bias towards the big reward size seems to be uniform regardless of the level of evidence presented. (Figure 3.3.1 B, D) Further analysis supports this finding. When subtracting the biased and unbiased psychometric curve, the control data shows that choice-bias difference is increasing when the evidence level is low; the experimental data does not show this tendency. (Figure 3.3.1 E, F)

We found that normal rats spend longer time sampling odor mixture when the percentage compositions of two odors are close to each other (odor category 3/4 vs. odor category 1/2/5/6), regardless of reward size. After the rats receive high dosage of morphine, they do not seem to spend longer time on more difficult tasks. (Figure 3.3.1 G, H)

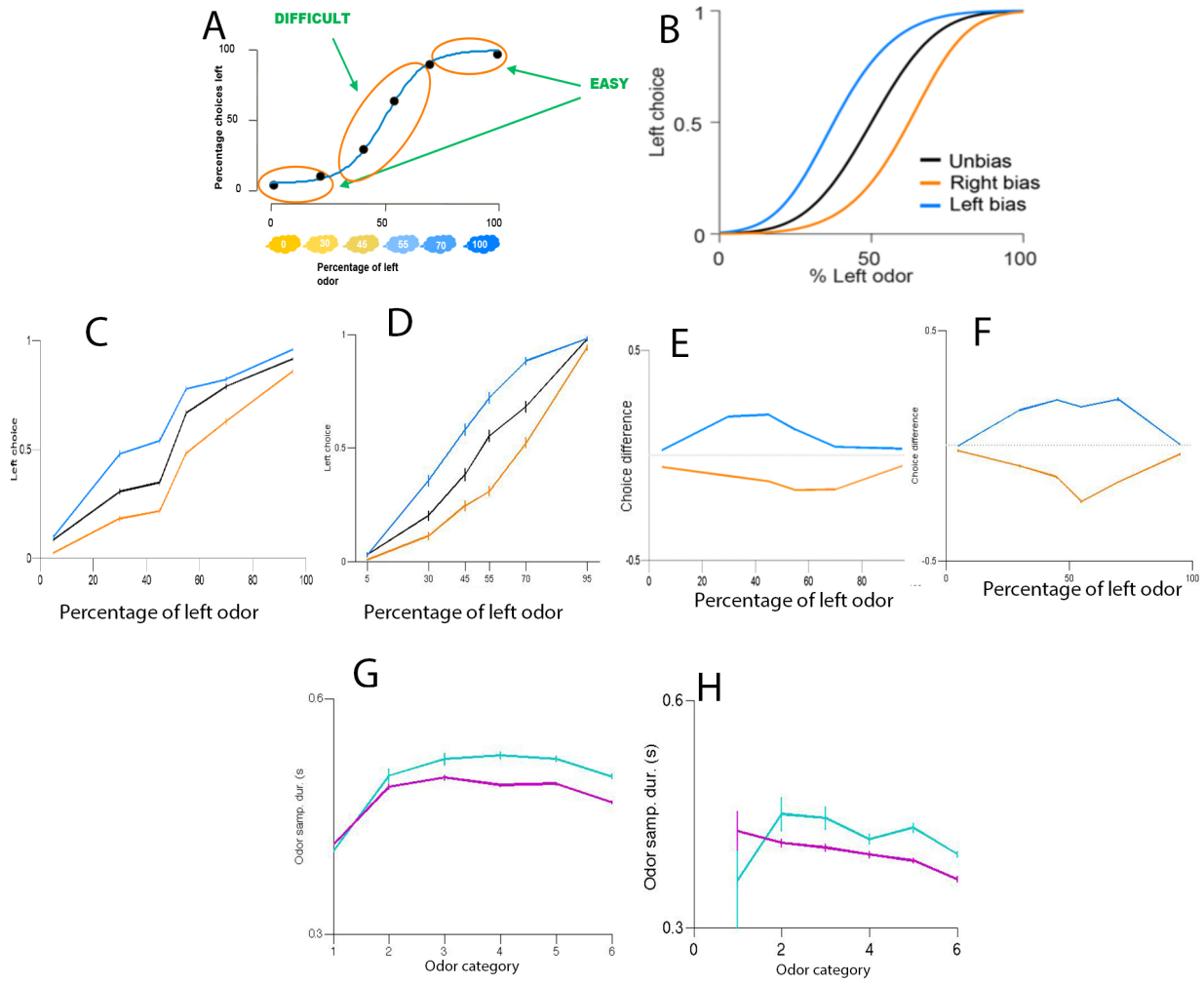

Figure 3.3.1 **Effects of chronic exposure of morphine on decision-making.** A. Psychometric curve of a typical rat. Percentage of left choice correspond to the percentage composition of left odor in the odor mixture; B. Predicted psychometric function for each reward block. Choice-bias is resulted from block-wise changes in reward size; C. Psychometric function of normal rats matches the predicted model; D. Psychometric function of morphine rats does not exhibit typical choice-bias; E. The differences between the psychometric curve (control set) between biased and control blocks. Choice difference increases when the percentage composition of corresponding odor is less than 50%; F. The differences between the psychometric curves (experimental set) from the biased and control blocks; G. Odor sampling duration of normal rats increases when the tasks are difficult (purple: choices associated with big reward; cyan: small reward choices); H. When rats are exposed to morphine, odor sampling duration does not increase when the tasks are difficult.

IX. Discussion

One of the overall goals of our lab is to understand the role OFC plays in the process of decision-making. Since it has been shown that projection from OFC to vSTR, BLA, and VTA are part of the valuation system, we sought to explore the specific decision variables encoded in each of the projection, and the outcome if the those projections are disrupted. Although my project is only a small part of the overall goal of the lab, it provides some valuable information for future studies.

The first specific aim of my project is to find out if OFC-vSTR, OFC-BLA, and OFC-VTA projections are originated from non-overlapping populations of neurons, or homogeneous population of neurons. It was shown by retrograde labeling that OFC-vSTR and OFC-VTA , OFC-vSTR and OFC-BLA projections were originated from largely non-overlapping population of neurons. These information points out the potential that OFC projections to different areas of the brain carry distinct decision variabels. Additional retrograde injections are needed to be done on OFC-VTA and OFC-BLA, in order to determine if those two neuronal pathways might carry different decision variables. Furthermore, by correlating decision variables with firing rate of individual neurons, it will be possible to find out what information is encoded in each OFC projections.

Not being able to accurate estimate the amount of reagent injected was very problematic in terms of obtaining consistent signal strength; subsequent image process inevitably introduced more noise, due to effort on normalizing differences in signal strength; in addition, a few weeks after dilution CTB 594 usually becomes sticky and

require larger glass pipette diameter for injection, which often result in leakages. In future experiments, I would suggest inject with Hamilton syringe instead of solely glass pipette, and pick up small amount of solution post-injection, in order to minimize leakage.

The second specific aim of my project is to understand the outcome when OFC projections were disrupted. Morphine was utilized in the experiment, since it has been demonstrated to be able to induce structural change in the OFC region. Animals were exposed to high dosage of morphine; their behavior was assessed by odor discrimination tasks that were previously designed in our lab. It was evident that under the chronic exposure of morphine, animals lost the tendency to spend long time sampling odor when the tasks were difficult, which means the increase in impulsivity. Furthermore, while rats under normal condition exhibit bias towards large reward choices providing that evidence level is now, chronic morphine treatment renders rat biased towards large reward choices regardless of the amount of evidence presented. We expected to see bias towards previously rewarded choices in normal rats, as well as the reduction in such bias after the morphine treatment; however, our data did not support this postulation. It is possible that there are other circuitry involved that functionally compensate disruptions in OFC.

References:

1. Gold, Joshua I., and Michael N. Shadlen. "The neural basis of decision making." *Annu. Rev. Neurosci.* 30 (2007): 535-574.

2. Usher, Marius, Anat Elhalal, and James L. McClelland. "The neurodynamics of choice, value-based decisions, and preference reversal." *The probabilistic mind: Prospects for Bayesian cognitive science* (2008): 277-300.

3. Heekeren, H. R., et al. "A general mechanism for perceptual decision-making in the human brain." *Nature* 431.7010 (2004): 859-862.

4. Baylis LL, Rolls ET, Baylis GC (1994) Afferent connections of the orbitofrontal cortex taste area of the primate. Neuroscience 64: 801–812.

5. Bechara A, Damasio AR, Damasio H, Anderson SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 50:7–15.

6.Rangel, Antonio, Colin Camerer, and P. Read Montague. "A framework for studying the neurobiology of value-based decision making." *Nature Reviews Neuroscience* 9.7 (2008): 545-556.

7. Kringelbach, M.L. (2005) “The orbitofrontal cortex: liking reward to hedonic experience.” *Nature Reviews Neuroscience* **6:** 691-702

8. Fuster, J. M. *The Prefrontal Cortex* (Raven, New York, USA, 1997).

9. Booth MCA, Rolls ET (1998) View-invariant representations of familiar objects by neurons in the inferior temporal visual cortex. Cerebral Cortex 8:510–523.

10. Barbas H (1988) Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. J Comp Neurol 276:313–342.

11. Rolls ET, Yaxley S, Sienkiewicz ZJ (1990) Gustatory responses of single neurons in the orbitofrontal cortex of the macaque monkey. J Neurophysiol 64:1055–1066.

12. Rolls ET (1999a) The brain and emotion. Oxford: Oxford University Press

13. Rolls ET, Sienkiewicz ZJ, Yaxley S (1989) Hunger modulates the responses to gustatory stimuli of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. Eur J Neurosci 53–60.

14. Iversen SD, Mishkin M (1970) Perseverative interference in monkey following selective lesions of the inferior prefrontal convexity. Exp Brain Res 11:376–386.

15. Rushworth, Matthew FS, and Timothy EJ Behrens. "Choice, uncertainty and value in prefrontal and cingulate cortex." *Nature neuroscience* 11.4 (2008): 389-397.

16. Watakabe, Akiya, et al. "Area-specific substratification of deep layer neurons in the rat cortex." *Journal of Comparative Neurology* 520.16 (2012): 3553-3573.

17. Schultz, Wolfram, et al. "Neuronal activity in monkey ventral striatum related to the expectation of reward." *The Journal of Neuroscience* 12.12 (1992): 4595-4610.

18. Cardinal, Rudolf N., et al. "Impulsive choice induced in rats by lesions of the nucleus accumbens core." *Science* 292.5526 (2001): 2499-2501.

19. Delgado MR, Miller MM, Inati S, Phelps EA. An fMRI study of reward-related probability learning. Neuroimage 2005; 24:862–873.

20. Wikipedia, Ventral tegmental area, http://en.wikipedia.org/wiki/Ventral_tegmental_area#cite_note-1(as of 4/12/2014, 08:00 GMT)

21. Koob, G. F. & Le Moal, M. Addiction and the brain antireward system. Annu. Rev. Psychol. 59, 29–53 (2008).

22. Brodmann K. Vergliechende lokalisationlehre der grosshirnrinde in ihren prinziien dargestellt auf grund des aellenbaues. Leipzig: Barth;1909.

23. Kauer, Julie A., and Robert C. Malenka. "Synaptic plasticity and addiction."*Nature reviews neuroscience* 8.11 (2007): 844-858.

24. Kandel Eric R, Schwartz James H, and Jessell Thomas M. "Principles of neural science." (2000).

25. Nye, Heather E., and Eric J. Nestler. "Induction of chronic Fos-related antigens in rat brain by chronic morphine administration." *Molecular Pharmacology* 49.4 (1996): 636-645.

26. Robinson, Terry E., and Bryan Kolb. "Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats." (1999).

27. Boronat, M. Assumpció, Gabriel Olmos, and Jesús A. García-Sevilla. "Attenuation of tolerance to opioid-induced antinociception and protection against morphine-induced decrease of neurofilament proteins by idazoxan and other I2-imidazoline ligands." *British journal of pharmacology* 125.1 (1998): 175-185.

28. Sklair-Tavron, Liora, et al. "Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons." *Proceedings of the National Academy of Sciences* 93.20 (1996): 11202-11207.

29. Gibbs, Richard A., et al. "Genome sequence of the Brown Norway rat yields insights into mammalian evolution." *Nature* 428.6982 (2004): 493-521.

30. Chaplin, James Patrick. *Dictionary of psychology*. Random House LLC, 2010.

31. Tyler, Christopher W., and Chien-Chung Chen. "Signal detection theory in the 2AFC paradigm: Attention, channel uncertainty and probability summation."*Vision research* 40.22 (2000): 3121-3144.

32. Barbas H (1988) Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. J Comp Neurol 276:313–342

33. Heeger, David. "Signal detection theory." *Dept. Psych., Stanford Univ., Stanford, CA, Teaching Handout* (1997).

34. Carpenter, R. H. S., and M. L. L. Williams. "Neural computation of log likelihood in control of saccadic eye movements." *Nature* 377.6544 (1995): 59-62.

35. Sugrue, Leo P., Greg S. Corrado, and William T. Newsome. "Matching behavior and the representation of value in the parietal cortex." *science* 304.5678 (2004): 1782-1787.

36. Olds, James, and Peter Milner. "Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain." *Journal of comparative and physiological psychology* 47.6 (1954): 419.

37. Cadoni, C., and G. Di Chiara. "Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate–putamen in rats sensitized to morphine." *Neuroscience* 90.2 (1999): 447-455

38. Kumar, R., E. Mitchell, and I. P. Stolerman. "Disturbed patterns of behaviour in morphine tolerant and abstinent rats." *British journal of pharmacology* 42.3 (1971): 473-484.

39. Mickiewicz, Amanda L., and T. Celeste Napier. "Repeated exposure to morphine alters surface expression of AMPA receptors in the rat medial prefrontal cortex." *European Journal of Neuroscience* 33.2 (2011): 259-265.

40. Smith, Philip L., and Roger Ratcliff. "Psychology and neurobiology of simple decisions." *Trends in neurosciences* 27.3 (2004): 161-168.