Rhythmic activity in the medial and orbital frontal cortices tracks reward value and the vigor of consummatory behavior

Linda M. Amarante and Mark Laubach*

Department of Neuroscience, American University, Washington, DC, USA

*Corresponding author:
Mark Laubach, PhD
Department of Neuroscience
American University
4400 Massachusetts Avenue NW
Washington, DC 20016
mark.laubach@american.edu

Abbreviated title: Mechanisms of reward signaling in MFC and OFC

Acknowledgments: We thank Wambura Fobbs, Alexxai Kravitz, Catherine Stoodley, Steve Wise, and Samantha White for helpful feedback on the manuscript.

Conflict of Interest: None

Financial Support: NIH DA046375 to ML and a NSF Graduate Research Fellowship to LMA.

ABSTRACT

This study examined how the medial frontal (MFC) and orbital frontal (OFC) cortices process reward information to guide behavior. We simultaneously recorded local field potentials in the two areas as rats consumed liquid sucrose rewards and examined how the areas collectively process reward information. Both areas exhibited a 4-8 Hz “theta” rhythm that was phase locked to the lick cycle. The rhythm similarly tracked shifts in sucrose concentrations and fluid volumes, suggesting that it is sensitive to general differences in reward magnitude. Differences between the MFC and OFC were noted, specifically that the rhythm varied with response vigor and absolute reward value in the MFC, but not the OFC. Our findings suggest that the MFC and OFC concurrently process reward information but have distinct roles in the control of consummatory behavior.

INTRODUCTION

The medial and orbital frontal cortices (MFC and OFC) are two of the most studied parts of the cerebral cortex for their role in value-guided decision making, a process that ultimately results in animals consuming rewarding foods or fluids. There are extensive anatomical connections between the various parts of the MFC and OFC in rodents (Gabbott et al., 2003; Gabbott et al., 2005; Barreiros et al., 2020), and the regions are part of the medial frontal network (Öngür and Price, 2000). The MFC and OFC are thought to have specific roles in the control of behavior and specific homologies with medial and orbital regions of the primate frontal cortex (MFC: Laubach et al., 2018; OFC: Izquierdo, 2017). The extensive interconnections between MFC and OFC suggest that the two regions work together to control value-guided decisions. Unfortunately, few, if any, studies have examined concurrent neural processing in these regions of the rodent brain as animals perform behavioral tasks that depend on the two cortical regions.

In standard laboratory tasks, the action selection and outcome evaluation phases of value-guided decisions are commonly conceived as separate processes (Rangel et al., 2008). MFC and OFC may contribute independently to these processes or interact concurrently across them. Though there is some variation across published studies, most argue for MFC having a role in action-outcome processing (Alexander and Brown, 2011; Simon et al., 2015) and OFC having a role in stimulus-outcome (stimulus-reward) processing (Gallagher et al., 1999; Schoenbaum and Roesch, 2005; Simon et al., 2015). The present study directly compared neural activity in the MFC and OFC of rats as they performed a simple consummatory task, called the Shifting Values Licking Task, or SVLT (Parent et al., 2015a). Importantly, the task depends on the ability of animals to guide their consummatory behavior based on the value of available rewards, and performance of these kinds of tasks depends on both the MFC (Parent et al., 2015a,b) and OFC (Kesner and Glibert, 2007). The goal of the study was to use the SVLT

to determine if the MFC and OFC have distinct roles in processing reward information, e.g. varying with action (licking) in MFC and the sensory properties of the rewards in OFC.

Most published studies on reward processing used operant designs with distinct actions preceding different outcomes. For example, a rat might respond in one of two choice ports to produce a highly valued reward, delivered from a separate reward port. To collect the reward, the rat has to travel across an operant chamber and then collect a food pellet or initiate licking on a spout to collect the reward. In such tasks (Pratt and Mizumori, 2001; van Durren et al., 2009; van Wiingerden et al., 2010; Riceberg and Shapiro, 2017; Jarovi et al., 2018; Siniscalchi et al., 2019), neural activity during the period of consumption might reflect the properties of the reward, how the animal consumes it, and/or the behaviors that precede reward collection (e.g. locomotion). As such, it is difficult to isolate reward specific activity using such operant designs.

Several published studies have used simpler consummatory and Pavlovian designs, and found neural activity in the MFC is selectively modulated during active consumption (Petykó et al., 2009; Horst and Laubach, 2013; Petykó et al., 2015). None of these tasks used fluids with different reward values. Amarante et al. (2017) was the first study to examine if similar neural activity was associated with animals consuming different magnitudes of reward. The study used the SVLT and presented rats with rewards that differed in terms of the concentration of sucrose contained in the rewarding fluids. The study found that neural actiivty in the MFC is entrained the animals' lick cycle and the strength of entrainment varies with the value of the rewarding fluid, i.e. stronger entrainment with higher value reward. The study also used reversible inactivation methods to demonstrate the licking entrainment depends on neural activity in the MFC.

In the present study, we used the SVLT, and several variations on the basic task design, to study consumption related activity in MFC and OFC. A custom designed syringe pump was used to deliver different volumes of fluid over a common time period (Amarante et al., 2019).

Using the custom device, we were able to directly compare neural activity associated with differences in sucrose concentration and fluid volume. We further manipulated the predictability of changes in reward magnitude to assess how predictable and unpredictable rewards are processed and used a third, intermediate level of reward to assess if reward magnitudes are encoded in a relative or absolute manner. Our findings reveal several similarities – and key differences – in each cortical region across all behavioral tasks that may allude to specific roles for MFC and OFC in the control of consummatory behavior.

METHODS

All procedures carried out in this set of experiments were approved by the Animal Care and Use Committee at American University (Washington, DC). All procedures conformed to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were taken to minimize the number of animals used and to reduce pain and suffering.

Animals

Male Long Evans and Sprague Dawley rats weighing between 300 and 325 g were used in these studies (Charles River, Envigo). Rats were given one week to acclimate with daily handling prior to behavioral training or surgery and were then kept with regulated access to food to maintain 90% of their free-feeding body weight. They were given ~18 g of standard rat chow each day in the evenings following experiments. Rats were single-housed in their home cages in a 12h light/dark cycle colony room, with experiments occurring during the light cycle. A total of 12 rats had a 2x8 microwire array implanted into either the MFC (N=6), the OFC (N=2) or one array in each area contralaterally (N=4). Arrays consisted of 16 blunt-cut 50-µm tungsten (Tucker-Davis Technologies) or stainless steel (Microprobes) wires, separated by 250 µm within each row and 500 µm between rows. *In vitro* impedances for the microwires were ~150 kΩ.

Surgeries

Animals had full access to food and water in the days prior to surgery. Stereotaxic surgery was performed using standard methods. Briefly, animals were lightly anesthetized with isoflurane (2.5% for ~2 minutes), and were then injected intraperitoneally with ketamine (100mg/kg) and dexdomitor (0.25mg/kg) to maintain a surgical plane of anesthesia. The skull was exposed, and craniotomies were made above the implant locations. Microwire arrays were lowered into MFC (coordinates from bregma (AP: +3.2 mm; ML: + 1.0 mm; DV: -1.2 mm from the surface of the brain, at a 12° posterior angle; Paxinos and Watson, 2013) or into OFC (AP: +3.2 mm, ML: + 4.0 mm, DV: -4.0 mm; Paxinos and Watson, 2013). The part of the MFC studied here is also called “medial prefrontal cortex” in many rodent studies and the region is thought to be homologous to the rostral ACC of primates (Laubach et al., 2018). Four skull screws were placed along the edges of the skull and a ground wire was secured in the intracranial space above the posterior cerebral cortex. Electrode arrays were connected to a headstage cable and modified Plexon preamplifier during surgery, and recordings were made to assess neural activity during array placement. Craniotomies were sealed using cyanocrylate (Slo-Zap) and an accelerator (Zip Kicker), and methyl methacrylate dental cement (AM Systems) was applied and affixed to the skull via the skull screws. Animals were given a reversal agent for dexdomitor (Antisedan, s.c. 0.25 mg/ml), and Carprofen (5 mg/kg, s.c.) was administered for postoperative analgesia. Animals recovered from surgery in their home cages for at least one week with full food and water, and were weighed and monitored daily for one week after surgery.

Behavioral Apparatus

Rats were trained in operant chambers housed within a sound-attenuating external chamber (Med Associates; St. Albans, VT). Operant chambers contained a custom-made glass

drinking spout that was connected to multiple fluid lines allowing for multiple fluids to be consumed at the same location. The spout was centered on one side of the operant chamber wall at a height of 6.5 cm from the chamber floor. Tygon tubing connected to the back of the drinking spout administered the fluid from a 60 cc syringe hooked up to either a PHM-100 pump (Med Associates) for standard experiments, or to a customized open source syringe pump controller (Amarante et al., 2019) that is programmed by a teensy microcontroller to deliver different volumes of fluid with the same delivery time from one central syringe pump. A "light-pipe" lickometer (Med Associates) detected licks via an LED photobeam, and each lick triggered the pump to deliver roughly 30 µL per 0.5 second. Behavioral protocols were run though Med-PC version IV (Med Associates), and behavioral data was sent via TTL pulses from the Med-PC software to the Plexon recording system.

Shifting Values Licking Task

The operant licking task used here is similar to those previously described (Parent et al., 2015a,b; Amarante et al., 2017). Briefly, rats were placed in the operant chamber for thirty minutes, where they were solely required to lick at the drinking spout to obtain a liquid sucrose reward. Licks to the light-pipe lickometer would trigger the syringe pump to deliver liquid sucrose over 0.5 sec. Every 30 sec, the reward alternated between of high (16% weight per volume) and low (4% wt./vol.) concentrations of liquid sucrose, delivered in a volume of 30 µL. In volume manipulation sessions, the reward alternated between a large (27.85 µL) and small volume (9.28 µL) of 16% liquid sucrose. Rewards were delivered over a period of 0.5 sec for all levels of concentration and volume using a custom made syringe pump (Amarante et al., 2019). The animal's licking behavior was constantly recorded throughout the test sessions.

Blocked versus Randomly Interleaved Licking Task

The Shifting Values Licking Task was altered to allow for comparison of blocked versus interleaved presentations of reward values. The first three minutes of the task consisted of the standard Shifting Values Licking Task, with 30 second blocks of either the high or low concentration sucrose rewards delivered exclusively during the block. After three minutes, the rewards were presented in a pseudo-random order (e.g., high, high, low, high, low, low, high) for the rest of the test session. With rewards interleaved, rats were unaware of which reward would be delivered next. Behavioral and neural data were only analyzed from the first six minutes of each test session. We focused on manipulating sucrose concentration, and not fluid volume, in this task variation, as concentration differences provided the most effects of reward value on licking behavior (see Figure 1D below).

Three Reward Licking Task

The Shifting Values Licking Task was modified, using a third intermediate concentration of sucrose (8% wt./vol) to assess if reward value influenced behavior and neuronal activity in a relative or absolute manner. In the first three minutes of each session, rats received either the intermediate (8%) or low (4%) concentration of sucrose, with the two rewards delivered over alternating 30 second periods as in the SVLT. After three minutes, the rewards switched to the high (16%) and intermediate (8%) concentrations, and alternated between those concentrations for the rest of the session. Behavioral and neural data were only analyzed from the first six minutes of each test session.

Electrophysiological Recordings

Electrophysiological recordings were made using a Plexon Multichannel Acquisition Processor (MAP; Plexon; Dallas, TX). Local field potentials were sampled on all electrodes and

recorded continuously throughout the behavioral testing sessions using the Plexon system via National Instruments A/D card (PCI-DIO-32HS). The sampling rate was 1 kHz. The head-stage filters (Plexon) were at 0.5 Hz and 5.9 kHz. Electrodes with unstable signals or prominent peaks at 60 Hz in plots of power spectral density were excluded from quantitative analysis.

Histology

After all experiments were completed, rats were deeply anesthetized via an intraperitoneal injection of Euthasol (100mg/kg) and then transcardially perfused using 4% paraformaldehyde in phosphate-buffered saline. Brains were cryoprotected with a 20% sucrose and 10% glycerol mixture and then sectioned horizontally on a freezing microtome. The slices were mounted on gelatin-subbed slides and stained for Nissl substance with thionin.

Data Analysis: Software and Statistics

All data were analyzed using GNU Octave (https://www.gnu.org/software/octave/), Python (Anaconda distribution: https://www.continuum.io/), and R (https://www.r-project.org/). Analyses were run as Jupyter notebooks (http://jupyter.org/). Computer code used in this study is available upon request from the corresponding author.

Statistical testing was performed in R. Paired t-tests were used throughout the study and one or two-way ANOVA (with the error term due to subject) were used to compare data for both behavior and electrophysiological measures (maximum power and maximum inter-trial phase coherence) for high and low value licks, blocked versus interleaved licks, and high-intermediate-low licks. For significant ANOVAs, the error term was removed and Tukey's post-hoc tests were performed on significant interaction terms for multiple comparisons. Descriptive statistics are reported as mean $\pm$ SEM, unless noted otherwise.

Data Analysis: Behavior

All rats were first run for at least five standard sessions in the standard Shifting Values Licking Task with differences in concentration (16% and 4% wt./vol.). Rats have been shown to acquire incentive contrast effects in the SVLT after this duration of training (Parent et al., 2015a). For the Blocked-Interleaved and Three Reward tasks, rats were tested after extensive experience in the SVLT and after two "training" sessions with the Blocked-Interleaved and Three Reward designs. The electrophysiological recordings reported here were from the animals' third session in each task.

Behavioral measures included total licks across the session, the duration and number of licking bouts, and the median inter-lick intervals (inverse of licking frequency). Bouts of licks were defined as having at least 3 licks within 300 ms and with an inter-bout interval of 0.5 sec or longer. Bouts were not analyzed in the Blocked-Interleaved Task; due to the unique structure of the task, bouts were all shortened by default due to a constantly changing reward in the interleaved phase of the task. While bouts of licks were reported in most tasks, electrophysiological correlates around bouts were not analyzed because there were often too few bouts (specifically for the low-lick conditions) in each session to deduce any electrophysiological effects of reward value on bout-related activity.

For analyzing lick rate, inter-lick intervals during the different types of rewards were obtained, and then the inverse of the median inter-lick interval provided the average lick rate in Hertz. Any inter-lick interval greater than 1 sec or less than 0.09 sec was excluded from the analysis. For licks during the randomly interleaved portion of the Blocked-Interleaved Task, more than two licks in a row were needed to calculate lick rate. To analyze behavioral variability of licks, we used coefficient of variation (ratio of the standard deviation to the mean) on high and low value inter-lick intervals that occurred within bouts.

Data Analysis: Local Field Potentials

Electrophysiological data were first analyzed in NeuroExplorer (http://www.neuroexplorer.com/), to check for artifacts and spectral integrity. Subsequent processing was done using signal processing routines in GNU Octave. Analysis of Local Field Potentials (LFP) used functions from the EEGLab toolbox (Delorme and Makeig, 2004) (Event-Related Spectral Power and Inter-Trial Phase Coherence) and the signal processing toolbox in GNU Octave (the peak2peak function was used to measure event-related amplitude). Circular statistics were calculated using the circular library for R. Graphical plots of data were made using the matplotlib and seaborn library for Python. Analyses were typically conducted in Jupyter notebooks, and interactions between Python, R, and Octave were implemented using the rpy2 and oct2py libraries for Python.

To measure the amplitude and phase of LFP in the frequency range of licking, LFPs were bandpass-filtered using eeglab's eegfilt function, with a fir1 filter (Widmann and Schröger, 2012), centered at the rat's licking frequency (licking frequency + inter-quartile range; typically around 4 to 9 Hz), and were subsequently z-scored. Analyses were performed with a pre/post window of 2 seconds, and the Hilbert transform was used to obtain LFP amplitude and phase.

For inter-trial phase coherence (ITC) and event-related spectral power (ERSP), LFP data was preprocessed using eeglab's eegfilt function with a fir1 filter and was bandpass filtered from 0 to 100 Hz. For group summaries, ITC and ERSP matrices were z-scored for that given rat after bandpass filtering the data. Peri-lick matrices were then formed by using a pre/post window of 2 seconds on each side, and the newtimef function from the eeglab toolbox was used to generate the time-frequency matrices for ITC and ERSP up to 30 Hz.

Since most of the lick counts from the Shifting Values Licking Task are generally imbalanced (with a greater number of licks for high versus low value rewards), we used permutation testing to perform analyses on amplitude and phase-locking in these studies. Licks

were typically down-sampled to match the lower number of licks. 80% of the number of lower value licks were randomly chosen from each session. For example, if a rat emitted 400 licks for the high concentration sucrose and 200 licks for the low concentration sucrose, then 160 licks would be randomly chosen from each of data type to compare the same number of licks for each lick type. This permutation of taking 80% of the licks was re-sampled 25 times and spectral values were recalculated for each permutation. The maximum ITC value was obtained through calculating the absolute value of ITC values between 2 to 12 Hz within a ~150 ms window (+1 inter-lick interval) around each lick. The maximum ERSP value was also taken around the same frequency and time window. Then, the average maximum ITC or ERSP value (of the 25x resampled values) for each LFP channel for each rat was saved in a data frame, and each electrode's maximum ITC and ERSP value for each type of lick (high-value or low-value lick) were used in the ANOVAs for group summaries. Group summary for the peak-to-peak Event-Related Potential (ERP) size recorded the average difference between the maximum and minimum ERP amplitude across all frequencies, using + 1 inter-lick interval window around each lick. The mean ERP size for each electrode for each rat was used in the ANOVAs for group summaries. These analyses were performed for all behavioral variations.

RESULTS

Shifting Values Licking Task: Effects of reward magnitude on consummatory behavior

The Shifting Values Licking Task (Amarante et al., 2017; Figure 1A) was used to assess reward encoding across the MFC and OFC as 12 rats experienced shifts in reward value defined by differences in sucrose concentration or fluid volume. Shifts in concentration were between 16% and 4% sucrose in a volume of 30 µL. Shifts in volume were between 30 µL and 10 µL containing 16% sucrose. Concentrations and volumes alternated over periods of 30 sec

(Figure 1B, left). LFP activity was recorded from 16-channel multi-electrode arrays in the MFC in 10 of the 12 rats and OFC in 6 of the 12 rats (Supplementary Figure 1).

Several measures of licking behavior varied with sucrose concentration or fluid volume: lick counts, inter-lick intervals, lick rate, and bout duration (Figure 1C). All rats licked more for the high concentration reward compared to the low concentration reward (paired t-test; $t(11)=10.76$, $p<0.001$) (Figure 1D). Rats also licked at a faster rate for the high concentration reward compared to the low concentration reward (paired t-test; $t(11)=6.347$, $p<0.001$) (Figure 1E). Additionally, rats had increased bout durations when licking for the high concentration reward compared to the low concentration reward (paired t-test: $t(11)=2.9439$, $p=0.013$) (Figure

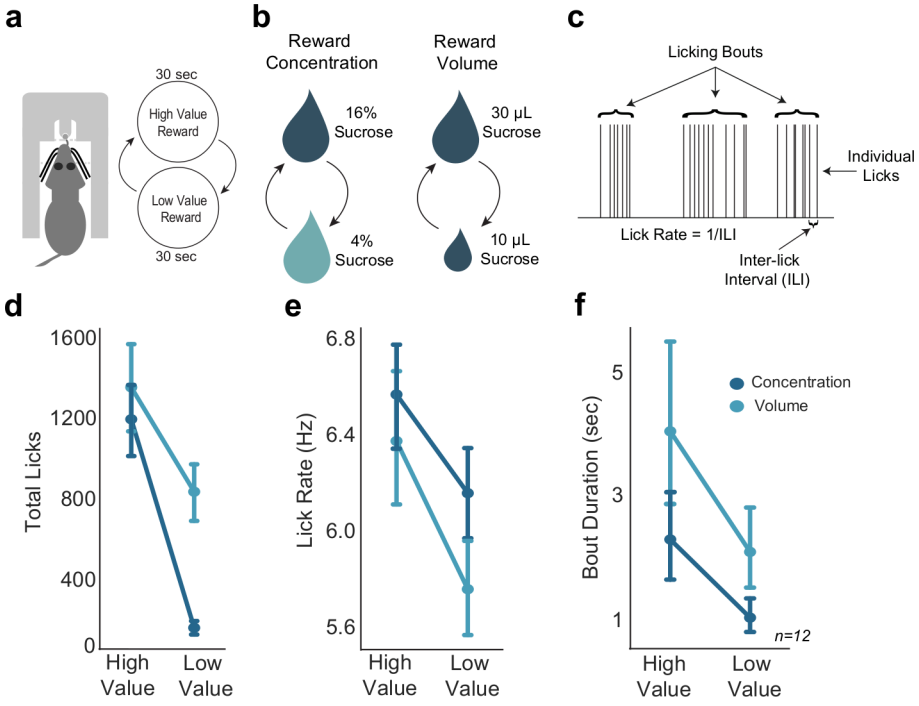


Figure 1. Consummatory behavior tracked shifts in sucrose concentration and fluid volume. A. In the Shifting Values Licking Task, rats received access to one of two values of reward, with rewards alternating every 30 sec. B. Manipulation of reward value by changing either concentration or volume. C. Types of behavioral licking measurements recorded in all licking tasks. D,E,F. Rats licked more (D), faster (E), and over longer bouts (F) for the high concentration and large volume rewards. Error bars represent SEM.

1F). There was no difference in variability of high or low concentration licks: the coefficient of variation for inter-lick intervals was the same (paired t-test: t(9)=0.864, p=0.41).

Rats behaved similarly when consuming the high concentration and large volume rewards. In volume manipulation sessions, rats emitted more licks for the large reward than the small reward (paired t-test; t(11)=4.99, p<0.001). However, this difference in lick counts was less robust than the difference in high and low concentration rewards during concentration manipulation sessions (Figure 1D). Rats licked at a faster rate for large rewards compared to

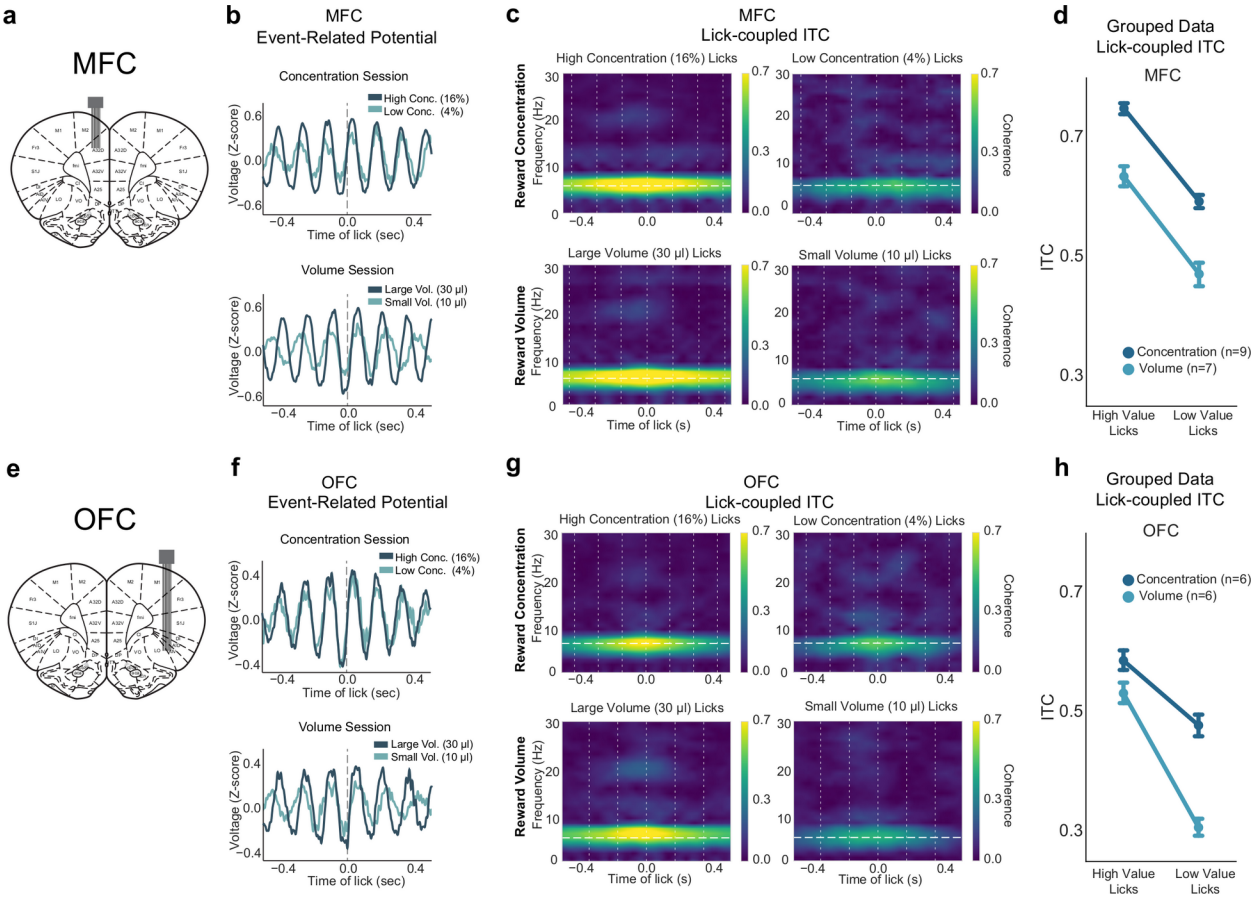


Figure 2. Lick-entrained neural activity in MFC and OFC tracked shifts in sucrose concentration and fluid volume. A,E. Rats were implanted with a 2x8 electrode array in either MFC (A) or OFC (E). B,F. Event-related potentials during concentration and volume manipulation sessions in the Shifting Values Licking Task for MFC (B) and OFC (F). C,G. Spectral ITC time-frequency plots revealed strong phase locking during licks for the high concentration and large volume (left sides) rewards in both MFC (C) and OFC (G). Plots are from one electrode from one individual animal. ITC is consistently strongest around 4-8 Hz. D,H. Grouped data from all rats in both concentration and volume sessions in MFC (D) and OFC (H) showed strongest ITC during licks for the high value reward. Error bars represent SEM.

small volume rewards (paired t-test; $t(11)=6.311$, $p<0.001$) (Figure 1E), and licking bouts were longer for large rewards compared to bouts to consume small rewards (Figure 1F), (paired t-test; $t(11)=2.569$, $p=0.027$).

Shifting Values Licking Task: Lick entrainment in MFC and OFC tracks reward magnitude

We next aimed to determine if there were electrophysiological differences in MFC and OFC during access to the different types of rewards. We focused on three key measurements of local field potential (LFP) activity: amplitude (as measured by the amplitude of the ERP; Supplementary Figure 2A), spectral power (as measured by ERSP; Supplementary Figure 2B), and phase (as measured by ITC, Supplementary Figure 2C). We recorded LFP activity from both cortical areas as rats consumed the different types of rewards (concentration or volume) in the Shifting Values Licking Task (Figure 2A,E).

Similar to Amarante et al. (2017), LFP activity in MFC was entrained to the lick cycle and varied with value (Figure 2B-D, top). Uniquely, we found similar lick-entrained activity in OFC that also varied with value (Figure 2F-H). Time-frequency analyses for both cortical areas revealed elevated ITC between 4 and 8 Hz for licks that delivered the high concentration liquid sucrose but not the low concentration sucrose (Figure 2C,G). That is, the phase angles of the LFP fluctuations were more consistent when rats consumed the high concentration fluid compared to the low concentration fluid. This result was observed in all rats that were tested (dark blue lines in Figure 2D,H) (MFC: $F(1,278)=443$, $p<0.001$; OFC: $F(1,177)=77.31$, $p<0.001$; one-way ANOVAs with an error term for within-subject variation). Analysis of phase coherence (Supplementary Figure 2D) and event-related power (Supplementary Figure 2E) revealed effects solely in the 4-8 Hz (theta) frequency range.

To assess differences in power, we used a peak-to-peak analysis of ERPs during licks for the high-value and low-value rewards. The measure calculates the difference in the maximum and minimum ERP amplitude using a window centered around each lick. The size of the window was twice each rat's median inter-lick interval. LFPs in MFC showed increased amplitudes for high concentration rewards, as opposed to low concentration rewards (one-way ANOVA: $F(1,278)=34.19$, $p<0.001$). Figure 2B shows MFC ERPs for high and low concentration rewards of an example rat. This effect was not significant in OFC ERPs, as seen in Figure 2F ($F(1,177)=0.557$, $p=0.456$). We also measured ERSP, and found a decrease in MFC power from licks for the high to low concentration rewards specifically in the 4-8 Hz range ($F(1,278)=18.72$, $p<0.001$; one-way ANOVA); there was no major difference in ERSP measures in OFC ($F(1,177)=0.039$, $p=0.843$).

We then manipulated the size of the reward and recorded LFP activity from MFC and OFC. Unlike the variable concentration session, event-related potentials in MFC or OFC alone did not distinguish between large versus small volume rewards (MFC: $F(1,216)=0.865$, $p=0.354$; OFC: ($F(1,179)=1.876$, $p=0.173$); one-way ANOVAs) (Figure 2B,F, bottom). There was no major difference in event-related spectral power during licks for large or small rewards in MFC or OFC (MFC: $F(1,216)=0.877$, $p=0.35$; OFC: $F(1,179)=1.76$, $p=0.186$); one-way ANOVAs). However, in both MFC and OFC, rats showed similar 4-8 Hz phase-locking for large rewards (Figure 2C,G, bottom), closely resembling what we observed with high concentration rewards (Figure 2C,G, top), which was significantly increased from phase-locking for small rewards (MFC: $F(1,216)=138.5$, $p<0.001$; OFC: $F(1,179)=280.8$, $p<0.001$; one-way ANOVA). This effect was consistent across all rats tested (light blue lines in Figure 2D,H).

These findings suggest that LFP activity in both MFC and OFC similarly encodes aspects of preferred, versus less preferred, reward options. 4-8 Hz phase-locking was strongest for both the high concentration and large volume rewards, which may be evidence that the

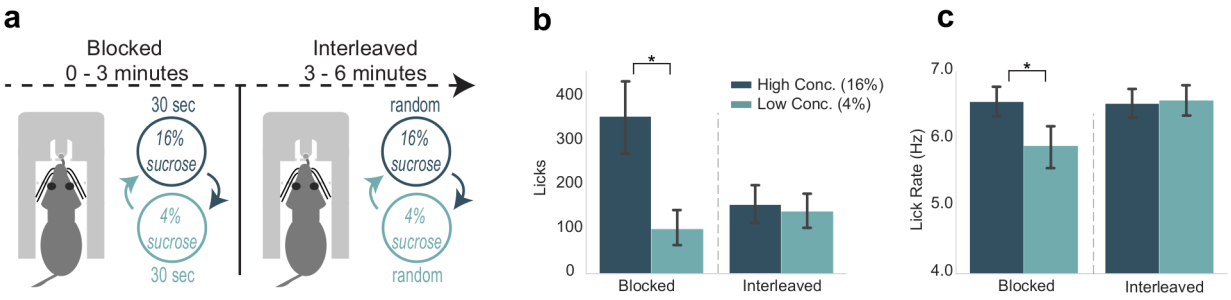


Figure 3. Engagement in and the vigor of licking varied with reward expectation. A. Rats participated in a modification of the Shifting Values Licking Task, called the Blocked-Interleaved Task, in which they received alternating access to high and low concentrations of liquid sucrose for three minutes and then received interleaved (and thus unpredictable) presentations of the two levels of sucrose for the rest of the session. B. Total licks emitted for both high and low concentration rewards during the blocked and interleaved portion of the task. Rats licked less for both rewards when rewards were randomly interleaved. C. Lick rate was similar for both rewards in the interleaved, but not blocked, portion of the task. Asterisk denotes $p<0.05$. Error bars represent SEM.

animal is acting within a preferred state with the goal of obtaining their most "valued" reward. These findings provided further evidence suggesting that the entrainment of neural activity in MFC and OFC to the lick cycle tracks reward magnitude.

Blocked-Interleaved Task: Engagement in and the vigor of licking vary with reward expectation

The same group of 12 rats were subsequently tested in an adjusted version of the Shifting Values Licking Task, which will be referred to as the Blocked-Interleaved Task (Figure 3A). In the first three minutes of the task, i.e. the "blocked" phase, rats behaviorally showed their typical differentiation of high versus low concentration rewards by emitting more licks for the high concentration reward (Figure 3B, left), and licked at a faster rate (Figure 3C, left). However, this pattern changed when the rewards were randomly presented in the "interleaved" part of the task. With a randomly interleaved reward presentation, rats licked nearly equally for high and low concentration rewards (Figure 3B, right). We performed a two-way ANOVA on the number of licks by each lick type (high or low concentration) and portion of the task (blocked or interleaved). There was a significant interaction between concentration of reward and the

blocked or interleaved portion of the task ($F(1,33)=24.51$, $p<0.001$). Post-hoc analyses revealed that while there was a significant difference in high and low concentration licks during the blocked portion ($p<0.001$), there was no difference between high and low concentration licks during the interleaved portion of the task ($p=0.98$).

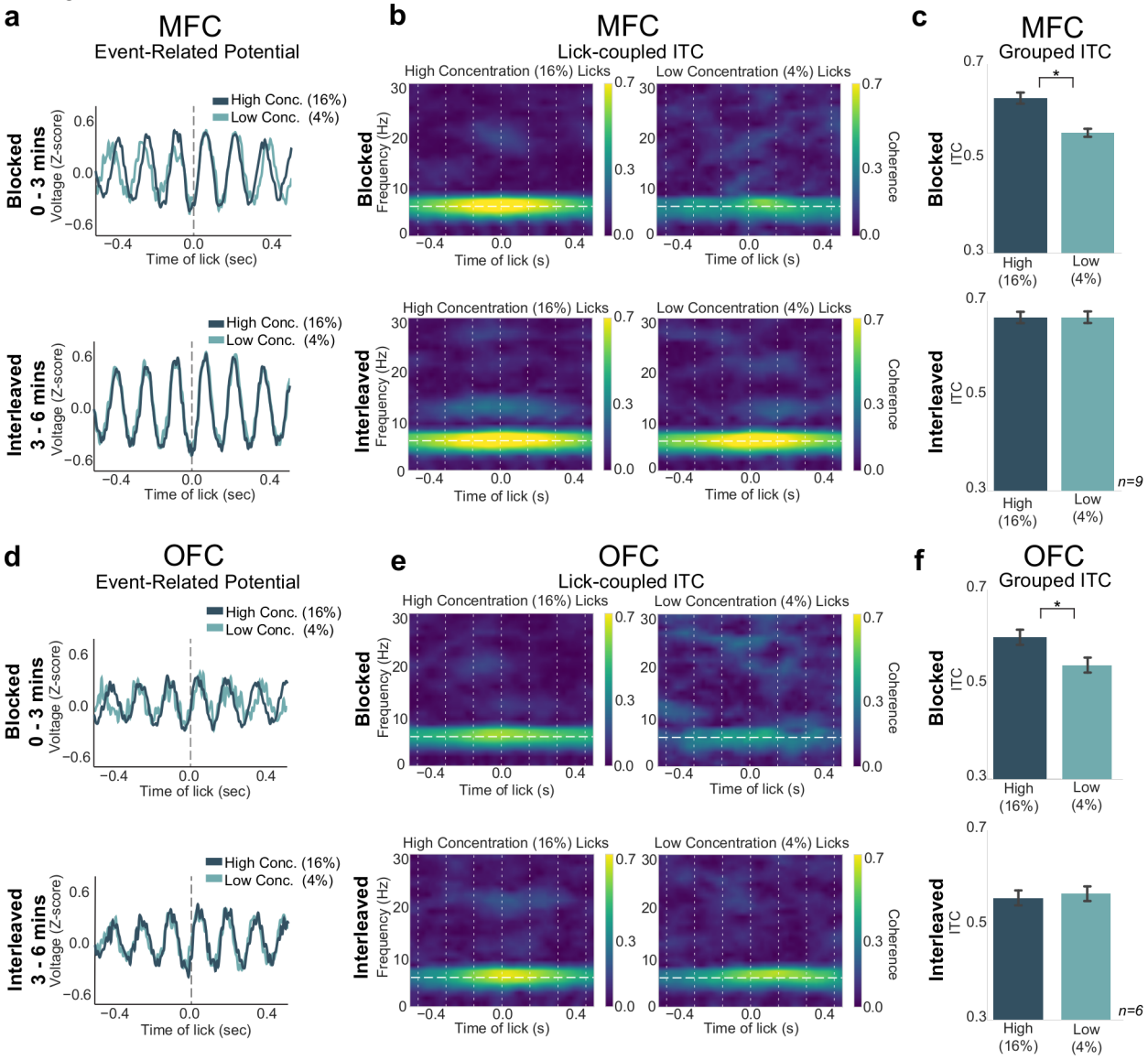


Figure 4. Lick-entrained neural activity varied with reward expectation. A,D. ERPs for licks of both rewards in MFC (A) and OFC (D) remain unchanged during the interleaved portion of the task. B,E. Spectral ITC plots revealed stronger 4-8 Hz phase-locking during licks for the high concentration reward in the blocked portion (top), but phase-locking during licks for high and low concentration rewards in the interleaved portion were indistinguishable from each other. C,F. Grouped data revealed no difference in ITC values during high or low concentration licks in the interleaved phase. Asterisk denotes $p<0.05$. Error bars represent SEM.

Additionally, there was a significant difference in lick rate by each lick type and portion of the task ($F(1,33)=23.13$, $p<0.001$; two-way ANOVA) (Figure 3C). Post hoc analyses revealed that rats licked significantly faster for high versus low concentration rewards during the blocked portion ($p<0.005$). Lick rates for high versus low concentration licks during the interleaved part of the task were not significantly different ($p=0.99$). Notably, lick rate during access to either high concentration ($p=0.005$) or low concentration ($p=0.002$) rewards during the interleaved portion was significantly increased from lick rate during access to the low concentration reward in the blocked portion of the task.

Blocked-Interleaved Task: Dissociation of MFC and OFC with regards to response vigor

Having established that the Blocked-Interleaved Task can reveal effects of reward expectation on task engagement and response vigor, we next examined how neural activity in the MFC and OFC varies with these behavioral measures. We assessed changes in lick-entrained ERPs and their amplitudes (Figure 4A,D), ERSP, and ITC (phase-locking) (Figure 4B-C,E-F). LFPs in MFC and OFC showed strong 4-8 Hz phase-locking during licks for the high concentration rewards in the blocked phase of the task (Figure 4B,E). We performed a two-way ANOVA on maximum ITC values (Figure 4C,F) from LFPs in both MFC and OFC for each rat and each electrode channel with interaction terms for lick type (high or low concentration reward) and portion of the task (blocked or interleaved reward access), and found a significant interaction of lick type by portion of the task (MFC: $F(1,572)=10.45$, $p=0.001$); OFC: $F(1,363)=12.119$, $p<0.001$). Post-hoc analyses revealed that while there was a significant difference in phase-locking of licks for high versus low concentration in the blocked portion (MFC: $p<0.001$; OFC: $p<0.036$), there was no difference in phase-locking of licks for high versus low concentration rewards in the interleaved portion of the task (MFC: $p=0.999$; OFC: $p=0.973$).

In MFC, a two-way ANOVA revealed a significant interaction of lick type by portion of the task with ERP peak-to-peak size (Figure 4A) as the dependent variable ($F(1,564)=6.232$, $p=0.013$). However, there were no differences between the ERP measures between high and low concentration licks during the blocked portion of the task ($p=0.887$) and between high and low concentration licks during the interleaved portion of the task ($p=0.938$). The same was true with ERSP measures for MFC LFPs; There was a significant interaction between lick type and portion of the task ($F(1,564)=30.17$, $p<0.001$; two-way ANOVA), but no significant difference between ERSP values between high and low concentration licks in the blocked ($p=0.213$) or interleaved ($p=0.743$) portions of the task. In OFC (Figure 4D), there was no significant interaction of lick type and portion of the task by the amplitude size of the lick's ERPs ($F(1,363)=0.131$, $p=0.718$; two-way ANOVA), and no difference in OFC ERSP values of lick type by portion of the task either ($F(1,363)=0.744$, $p=0.389$; two-way ANOVA).

We wanted to further investigate potential differences in MFC and OFC in the Blocked-Interleaved Task, since initial results show a general increase of ITC values from MFC in the interleaved portion of the task and a general decrease in ITC values from OFC. This was

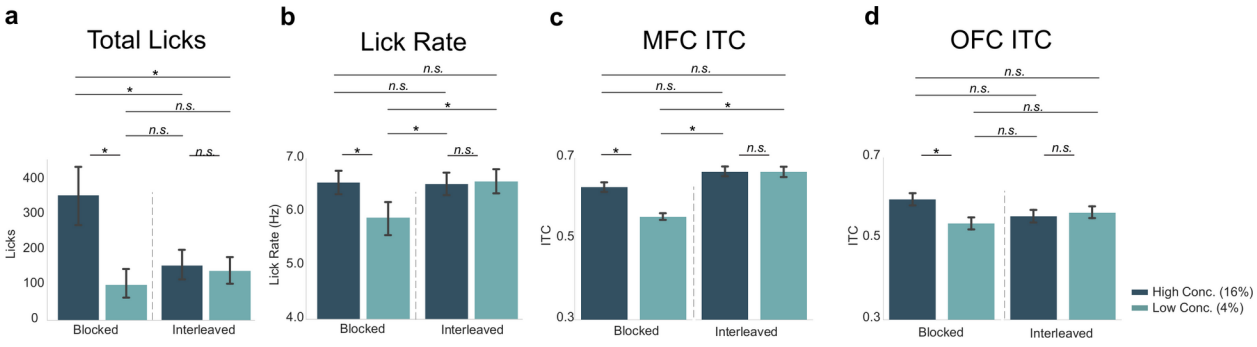


Figure 5. Neural activity in MFC, but not OFC, varied with the lick rate (vigor) and not task engagement (total licks). Post-hoc contrasts of statistically significant effects revealed by two-way ANOVA. Direct comparison of behavioral measures (A – total licks; B – lick rate) with MFC ITCs (C) and OFC ITCs (D) showed a similar pattern (and identical post-hoc statistical contrasts) between lick rate (B) and MFC ITCs (C). The pattern of post-hoc contrasts for OFC ITCs (D) did not match either total licks or lick rate. Asterisk denotes $p<0.05$. Error bars represent SEM.

of particular interest since MFC ITC values varied with the lick rate, which increased for both the high and low concentration licks in the interleaved portion of the task.

We directly compared ITC values in both regions with lick rate and total lick counts (Figure 5). Post-hoc analyses displayed in Figure 5C revealed that in MFC there was a significant difference between ITC values for the high versus low concentration licks (as also documented at the top of Figure 4C), but ITC values for high concentration licks during the blocked portion of the task did not differ from ITC values for either the high ($p=0.075$) or low concentration ($p=0.089$) conditions in the interleaved portion of the task. The pattern of post-hoc contrasts matches the lick-rate data (Figure 5B) for all paired comparisons. This match includes the finding (Figure 5C) that ITC values for low concentration licks in MFC differed from all three of the other conditions (high concentration blocked, high concentration interleaved, and low concentration interleaved licks; $p<0.001$ for each comparison). The MFC ITC post-hoc test results (Figure 5C) did not match the pattern for total licks (Figure 5A).

In OFC, ITC values (Figure 5D) did not match either the total-lick (Figure 5A) or lick-rate (Figure 5B) comparisons, despite the qualitative similarity with the total number of licks (compare Figure 5D with Figure 5A). The only significant difference in ITC values in OFC was between the high and low concentration licks in the blocked portion of the task (as also documented at the top of Figure 4F). All other comparisons were non-significant. This pattern of post-hoc comparisons did not match either total licks (compare Figure 5A with 5D) or lick rate (compare Figure 5B with 5D).

Together with the results summarized in Figure 4, these findings from post-hoc testing in Figure 5 provide evidence that MFC and OFC encode different aspects of licking and reward value. There was a clear match between the pattern of lick entrainment in the MFC, but not the OFC, with the animals' licking rates. The correspondence between lick entrainment in MFC and the animals' lick rates provides support for the idea that MFC plays a role in response vigor. By

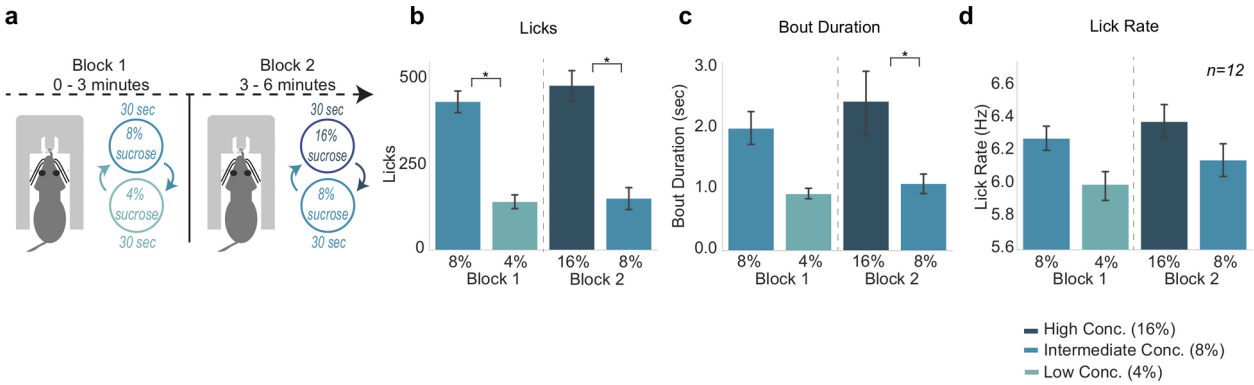


Figure 6. Consummatory behavior tracked relative differences in reward value. A. The Three Reward Task is a variation of Shifting Values Licking Task but with a third reward introduced. In the first block of the task, rats experience the intermediate (8%) reward and low (4%) reward. In block 2, rats experience the high (16%) reward paired with the intermediate (8%) reward. B. Rats licked more for the sweeter reward in each block. C. Rats showed greater bout durations for the sweeter reward. D. Lick rate showed a similar pattern to licks and bout duration, but was not statistically significant. Asterisk denotes $p<0.05$. Error bars represent SEM.

contrast, OFC might be involved in more general aspects of motivation, e.g. to lick or not (reward evaluation) based on reward magnitude or the predictability of the environment.

Three Reward Task: Behavioral evidence for effects of relative reward value

The previous experiments assessed comparison of two levels of rewards (either high/low concentration or large/small volume) in the Shifting Values Licking Task. After seeing clear behavioral and electrophysiological differences between two rewards, we aimed to investigate how animals process reward with contexts involving three different rewards. In this experiment, we assessed if rats process rewards in a relative manner or in an absolute manner by implementing a third intermediate (8% wt./vol. sucrose concentration) reward.

In the Three Reward Task (Figure 6A), the first block consists of the Shifting Values Licking Task with 30 sec shifts between the intermediate value (8% sucrose) reward and the low value (4% sucrose) reward. After 3 minutes the second block of the task begins, where rats then experience shifting values of reward from the high value (16% sucrose) reward to the

intermediate value (8% sucrose) reward. This allowed us to assess how rats would process the intermediate 8% sucrose reward when it is paired with a worse (4%) or better (16%) option within one session. Additionally, the design introduces a second context (just like in the Blocked-Interleaved Task previously) in which we could assess if animals are still processing a (temporally) local comparison of reward types.

Licking varied with both reward value and block, i.e. low vs intermediate and intermediate vs high ($F(3,33)=34.2$, $p<0.001$) (Figure 6B). Post-hoc analyses revealed that rats emitted significantly more licks for the intermediate value 8% reward as opposed to the low value 4% reward in block 1 ($p<0.001$). In block 2, rats also emitted significantly fewer licks for the intermediate value 8% reward when it was paired with the high value 16% reward ($p<0.001$). Rats also licked significantly less for the intermediate 8% reward in block 2 than they did in block 1 ($p<0.001$).

There was a more subtle effect for differences in bout duration across the different rewards ($F(3,33)=5.333$, $p=0.004$; two-way ANOVA) (Figure 6C). Post-hoc analyses revealed no significant difference in bout duration for the 4% versus 8% in block one ($p=0.098$), yet there was a significant decrease in bout durations during access to the 8% versus 16% in block two ($p=0.023$). Bout durations during access to the intermediate 8% reward in block 1 versus block 2 were not different ($p=0.20$). While there was a significant effect of lick type on lick rate ($F(3,33)=10.59$, $p<0.001$; two-way ANOVA), post-hoc analyses revealed no major differences in lick rate of the licks for rewards in block 1 ($p=0.17$) or block 2 ($p=0.31$) (Figure 6D),nor for the lick rate for 8% licks in block 1 versus block 2 ($p=0.76$).

Three Reward Task: Neural evidence for effects of absolute, not relative, reward value

The behavioral measures summarized above established that the Three Reward Task can reveal effects of relative value comparisons. We next analyzed electrophysiological signals

429 from MFC and OFC (Figure 7) to determine if they tracked the animals' behavior in the task, and
430 might encode relative differences in value, or some other aspect of value, such as the absolute
431 differences between the three rewards. We found a significant difference between ITC values
432 for the three different rewards in both MFC and OFC (MFC: $F(3,627)=154.4$, $p<0.001$; OFC:
433 $F(3,363)=13.29$, $p<0.001$; two-way ANOVAs). Tukey post-hoc analyses revealed a difference in

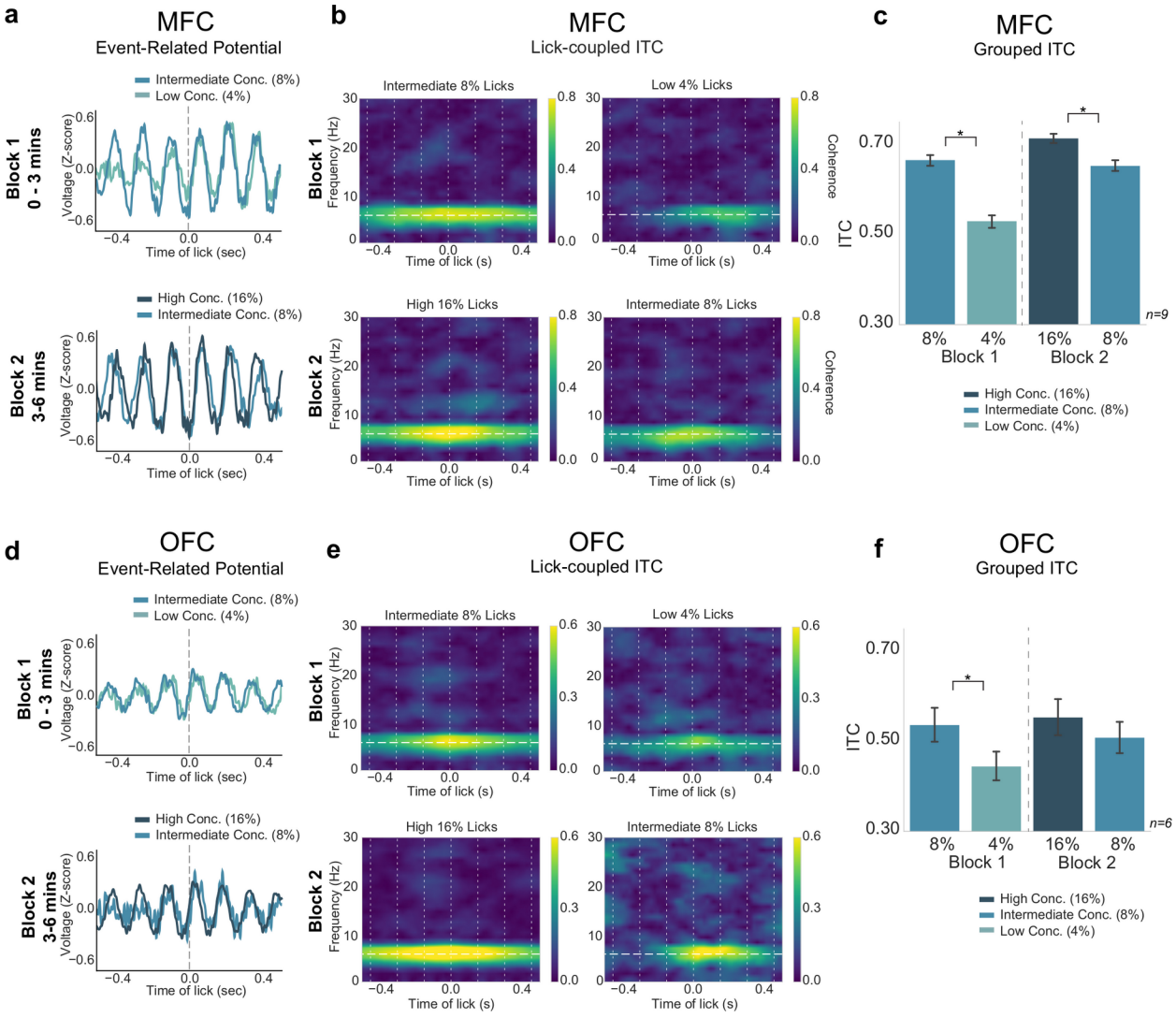


Figure 7. Neural activity in MFC, but not OFC, tracked absolute differences in reward value. A,D. ERPs for each block of the task from MFC (A) and OFC (D). B,E. ITC values in MFC (B) and OFC (E) showed strongest 4-8 Hz phase locking for the "high value" reward in each block. C,F. Group data revealed significantly greater ITC values for the high value reward in each block for MFC ITCs (C), and a similar pattern was found in OFC (F) but only block 1 rewards were significantly different. Asterisk denotes $p<0.05$. Error bars represent SEM.

ITC values between intermediate and low licks in block 1 (MFC: p<0.001; OFC: p=0.003), and a difference in ITCs between high and intermediate licks in block 2 for MFC only (MFC: p<0.005; OFC: p=0.313) (Figure 7B-C,E-F). There was no difference between ITC values from intermediate (8%) block 1 and intermediate block 2 licks in both regions (MFC: p=0.881; OFC: p=0.705).There was a significant difference between MFC ITC values for block 1 intermediate (8%) licks and block 2 high (16%) licks (p=0.028), as well as a significant difference between MFC ITC values for block 1 low (4%) licks and block 2 intermediate (8%) licks (p<0.001). Signals from the OFC did not differ across these conditions.

Peak to peak amplitude analysis of the Three Reward Task revealed a significant effect of block on MFC LFP amplitude across lick types (F(3,627)=15.56, p<0.001; two-way ANOVA) (Figure 7A). Tukey post-hoc testing revealed no relevant significant differences between ERP size in MFC (between block 1 intermediate and low licks: p=0.864; between block 2 high and intermediate licks: p=0.944). There was no difference in OFC amplitude size (F(3,363)=0.827, p=0.479, two-way ANOVA) (Figure 7D). While there was a significant effect for ERSP values in both MFC and OFC (MFC: F(3,627)=18.35, p<0.001; OFC: F(3,363)=5.108,

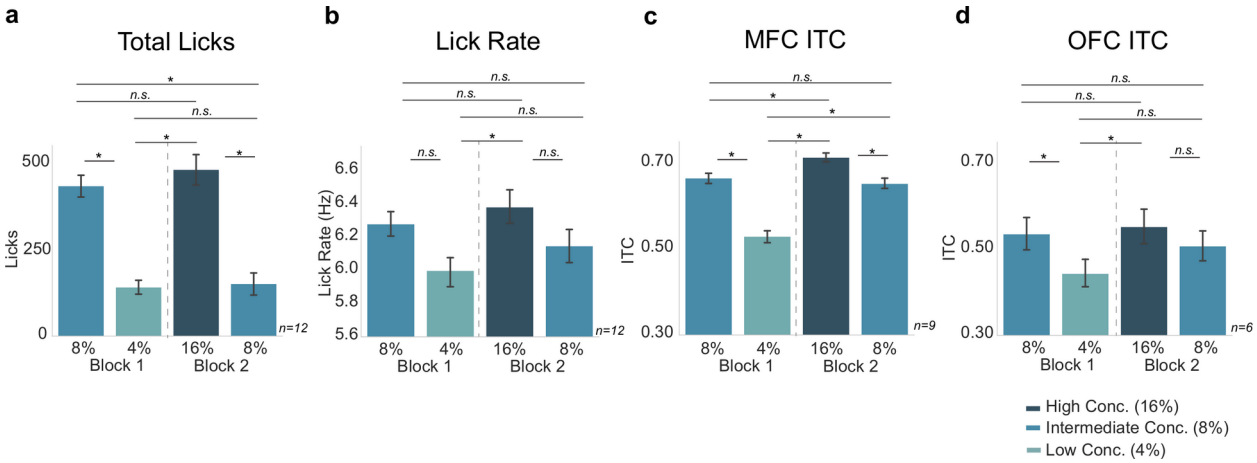


Figure 8. Neural activity in MFC, but not OFC, varied with effects of absolute reward value on lick rate (vigor) and task engagement (total licks). A,B. Behavioral measures replotted with significance bars for each combination reward. MFC ITCs (C) did not show the exact same pattern as lick rate, which is different from Figure 5. OFC ITCs (D) did not look like total licks or lick rate. Asterisk denotes p<0.05. Error bars represent SEM.

p=0.002; two-way ANOVAs), none of the relevant measures were significant (block 1 intermediate and low licks: MFC: p=0.875; OFC: p=0.492; block 2 high and intermediate licks: MFC: p=0.637; OFC: p=0.999).

The ITC findings, at least in MFC, support the idea that the "higher value" and "lower value" rewards in each context are being encoded differently across contexts. They indicate that MFC encodes absolute reward value. Qualitatively, the ITC values in MFC seem to have the same pattern as the lick rate (Figure 8B,C), similar to how MFC values reflected lick rate in the Blocked-Interleaved Task. However, post-hoc statistical testing revealed important differences. For example, the ITC in MFC differed significantly for high- vs. low-value rewards in both blocks 1 and 2, but lick rate did not. Importantly, post-hoc analyses revealed a significant difference in ITC values in MFC for every reward combination except for the intermediate block 1 and intermediate block 2 rewards, which is evidence for absolute encoding of value (see Supplementary Figure 3A-B).

The encoding of value was less clear based on ITC measures from the OFC. These values did not directly match the licking behavior (in either rate, total licks, or bout duration) (compare Figure 8A,B with 8D), and did not show clear evidence for either absolute or relative encoding of reward. Instead, the results from Figure 8D indicate that OFC encodes reward value in a mixed absolute/relative manner (as in Supplementary Figure 3C).

DISCUSSION

We investigated the role of MFC and OFC in processing reward information as rats participated in various consummatory licking tasks. Rats process and express changes in reward size in roughly the same manner as with reward concentration, both behaviorally and electrophysiologically. LFP activity in both MFC and OFC is sensitive to changes in reward type (both volume and concentration). Our results reveal context-dependent value signals in both

474 regions through randomly presented rewards and by introducing a third reward in the task.
475 Behaviorally, rats show evidence for a relative expression of rewards, while neural activity in
476 MFC, but not OFC, shows an absolute encoding of reward value. Together, our findings suggest
477 that rats sample rewards and commit to consuming a given reward when they are able to
478 predict its value, and this behavior is coupled to neural activity in MFC and OFC that encode
479 both the value of the reward and the animal's consummatory strategy. The subtle differences
480 between the two regions follow the hypothesis that MFC represents action-outcome
481 relationships and OFC represents stimulus-outcome relationships. MFC activity may provide the
482 "value of the action" information to OFC, while OFC may evaluate the reward and provides
483 feedback to MFC.

484 *Rhythmic Activity and Reward Processing*

485 Similar to our previous studies (Horst and Laubach, 2013; Amarante et al., 2017),
486 neural activity was entrained to the lick cycle across all tasks in both MFC and OFC.
487 Entrainment was strongest for the high-value reward (either of size or sweetness) and varied
488 with the animals consummatory strategy (persistently lick a highly preferred option or sample
489 fluid and wait for better option). Previous studies have viewed this rhythmic activity as being
490 driven by the act of licking, as rats naturally lick at 6-7 Hz (Travers et al, 1997; Weijnen, 1998;
491 Host and Laubach, 2013). However, the activity cannot be explained solely by licking, as there
492 are instances where phase-locking and behavior do not show the same pattern (e.g. the
493 Blocked-Interleaved experiment), and the variety of studies reported here and in Amarante et al.
494 (2017) suggest a higher order role for the neural activity in the control of consummatory
495 behavior.

496 Specifically how the rhythmic activity contributes to the control of behavior might be
497 best understood by considering how this general frequency range has been interpreted in other

types of behavioral tasks. Rhythms between 4 and 8 Hz are commonly referred to as "theta activity" and those found in the frontal cortex have been referred to as "frontal theta" (Cavanagh and Frank, 2014). There have been several proposals for the role of frontal theta in information processing. One idea is that the rhythm acts to break up sensory information into temporal chunks (Uchida and Mainen, 2003), and is related to the notion of a global oscillatory signal to synchronize neural activity across multiple brain structures throughout the taste-reward circuit (Gutierrez and Simon, 2013). Another idea is that frontal theta acts as an action monitoring signal (Cavanagh et al, 2012; Narayanan et al., 2013; Laubach et al., 2015), which can be generated through simple recurrent spiking network models (Bekolay et al., 2014). Finally, instead representing a specific function, frontal theta may act as a convenient "language" for distant brain regions to exchange information with each other (Womelsdorf et al., 2010). Our general findings contribute to this literature by suggesting that frontal theta acts as a value signal to guide consummatory behavior, which is the ultimate consequence of many goal-directed actions in natural environments.

A Common Code for Reward Magnitude

A major finding in the present study (Figures 1 and 2) was the similar electro-physiological signals in MFC and OFC are associated with the consumption of high and low concentration liquid sucrose rewards and large and small volume rewards. Although other studies have found either decreases (Kaplan et al., 2001) or increases in behavior with increases in concentration and volume rewards in the same study (Hulse et al., 1960; Collier and Myers, 1961; Collier and Wills, 1961), these studies did not investigate the electrophysiological correlates of consuming rewards. Our study is the first to show a generalized "value signal" in the frontal cortex that scales with increased size and increased concentration of liquid sucrose. These signals might underlie the computation of a common

currency (Montague and Berns, 2005; Levy and Glimcher, 2011; Levy and Glimcher, 2012; Strait et al., 2014) for the amount of nutrient available in a given food item and contribute to value-guided control of consumption.

Evidence for the Contextual Control of Consumption

In the Blocked-Interleaved Task (Figure 3A), rats who licked more, longer, and faster for the high concentration reward when rewards were blocked did not continue to do so during interleaved portion of the task (Figure 3B-C). Instead, they licked nearly equally for the high and low concentration solutions, a result that is suggestive of the loss of positive contrast effects for the higher value fluid that is commonly found in the blocked design (Parent et al., 2015a). Despite these differences in behavior, the rats' LFPs in MFC and OFC showed high levels of lick-entrained activity, essentially equal to that found during consumption of the higher value fluid in the blocked part of the session. This finding is hard to reconcile with enhanced lick entrainment reflecting reward contrast effects. If positive contrast engenders entrainment, then LFPs should have shown reduced phase locking to the lick cycle in the interleaved portion of the task. Instead, the results might suggest that LFPs in MFC and OFC are entrained to licking when rats engage in persistent licking, as was found in the periods with high concentration access in the blocked part of the sessions and across the entire interleaved part of the session, and entrainment is reduced when rats switch to sampling the fluid during periods with low value access in the blocked part of the session. By this view, LFP entrainment to the lick cycle could serve as a contextual marker for reward state and the behavioral strategy deployed by the rat to sample and wait or persistently consume the liquid sucrose. This contextual information would depend on knowledge of the temporal structure of the reward deliveries. That is, when reward values are blocked, the rats have learned to expect alternative access to higher and lower reward values over extended periods of time (30 sec). By contrast, when reward values are

546 interleaved, the changes in values occur rapidly and are unpredictable. The reduction in lick
547 entrainment might therefore reflect the animal's sampling strategy.

548 Contextual coding of reward value was also apparent in the Three Reward Task
549 (Figures 6-8), where lick entrainment was stronger when the higher value option was available
550 (Figure 7). In this case, the strength of engagement, for MFC but not OFC, tracked reward value
551 in an absolute manner, with entrainment being higher for the 16% sucrose solution compared to
552 the 8% solution when both were the "best" option (Figure 8C,D). These electrophysiological
553 results were notably distinct from behavioral measures such as total licking output and lick rate
554 (Figure 8A,B), which provided evidence for relative value comparisons. Our electrophysiological
555 results support theories of absolute reward value (Hull, 1943; Spence, 1956; Flaherty, 1982), as
556 opposed to theories of relative reward value (Crespi, 1942; Black, 1968; Webber et al., 2015).
557 Our findings might also fit with the neuro-economics idea of menu invariance versus menu-
558 dependent goods (Padoa-Schioppa, 2011), both of which have been supported by
559 electrophysiological studies on OFC (Padoa-Schioppa and Assad, 2006; Padoa-Schioppa and
560 Assad, 2008; Tremblay and Schultz, 1999; Saez et al., 2017).

561 It is not clear from our studies if the reduction in entrainment when low value rewards
562 are available is an active or passive process. For example, it is possible that some active input
563 to the MFC and OFC denotes the temporal context (e.g. dopamine, hippocampus) and
564 enhances entrainment when the higher value option is available. Alternatively, signals from
565 sensorimotor regions of the frontal cortex, which sit in between the MFC and OFC, the oral
566 sensory and motor cortices (Yoshida et al., 2009), might be reduced during periods with less
567 intense licking, leading to a passive reduction in overall frontal lick entrainment. Future studies
568 are needed to address these neural mechanisms of licking-related synchrony in the rodent
569 frontal cortex.

570 *Subtle Differences between MFC and OFC*

571 MFC and OFC are ideal locations for representing several aspects of value-based
572 decision making, since the cortical regions receives sensory input, project to motor planning
573 areas, and are connected with dopamine-rich areas either directly or through the striatum
574 (Sugrue et al., 2005). Both OFC and MFC play a role in processing and evaluating rewards, and
575 show activity modulated around the receipt and consumption of reward as well as the execution
576 of rewarding behaviors. These areas may be contributing to value-based decision making in a
577 goal-directed system (Balleine and Dickinson, 1998; Rangel et al., 2008; Rangel, 2013), where
578 the value of a given reward is computed, and information about previous outcomes can be used
579 to update values of predicted future outcomes (i.e., predicted rewards). This idea agrees with
580 our findings of increased theta phase-locking in MFC and OFC LFPs during licks for the high
581 value rewards, whether that reward is sweeter or larger in volume, which can be viewed as
582 subjective value.

583 The electrophysiological results from the Blocked-Interleaved Task and Three Reward
584 Task suggest that MFC and OFC, while showing similar results overall, may be contributing to
585 processing reward information in different ways. In accord with a previous theory on proposed
586 MFC and OFC functions (Balleine and Dickinson, 1998, Balleine and Dickinson, 2000;
587 Schoenbaum et al., 2009; Sul et al., 2011; Passingham and Wise, 2012), MFC activity may be
588 acting to maintain and optimize licking behavior in an action-centric manner, as reflected in
589 measures such as the licking rate, a measure associated with vigor and sensitive to inactivation
590 of the same cortical area in a progressive ratio licking task (Swanson et al., 2019). By contrast,
591 OFC activity generally reflected differences in reward value, perhaps due to the different
592 sensory properties of the fluids (Gutierrez et al., 2006), and was not sensitive to licking rate
593 (vigor) or task engagement (total licks).

REFERENCES

Alexander, W. H., & Brown, J. W. (2011). Medial prefrontal cortex as an action-outcome predictor. *Nature Neuroscience*, *14*(10), 1338–1344. https://doi.org/10.1038/nn.2921

Amarante, L. M., Caetano, M. S., & Laubach, M. (2017). Medial frontal theta is entrained to rewarded actions. *Journal of Neuroscience*, *37*(44), 10757–10769. https://doi.org/10.1523/JNeurosci.1965-17.2017

Amarante, L. M., Newport, J., Mitchell, M., Wilson, J., & Laubach, M. (2019). An open source syringe pump controller for fluid delivery of multiple volumes. *eNeuro*, *6*(5).https://doi.org/10.1523/eNeuro.0240-19.2019

Balleine, B. W., & Dickinson, A. (1998). Goal-directed instrumental action: Contingency and incentive learning and their cortical substrates. *Neuropharmacology*, *37*(4–5), 407–419. https://doi.org/10.1016/S0028-3908(98)00033-1

Balleine, B. W., & Dickinson, A. (2000). The effect of lesions of the insular cortex on instrumental conditioning: evidence for a role in incentive memory. *Journal of Neuroscience*, *20*(23), 8954–8964. https://doi.org/10.1523/JNeurosci.20-23-08954.2000

Barreiros, I.V.., Panayi, M.C., and Walton, M.E. (2020) Organisation of afferents along the anterior-posterior and medial-lateral axes of the rat OFC. bioRxiv. https://doi.org/10.1101/2020.08.28.272591

Bekolay, T., Laubach, M., & Eliasmith, C. (2014). A spiking neural integrator model of the adaptive control of action by the medial prefrontal cortex. *Journal of Neuroscience*, 34(5), 1892–1902. https://doi.org/10.1523/JNEUROSCI.2421-13.2014

Black, R. W. (1968). Shifts in magnitude of reward and contrast effects in instrumental and selective learning: A reinterpretation. *Psychological Review*, *75*(2), 114–126. https://doi.org/10.1037/h0025563

Cavanagh, J. F., Zambrano-Vazquez, L., & Allen, J. J. B. (2012). Theta lingua franca: A common mid-frontal substrate for action monitoring processes: Omnipresent theta. *Psychophysiology*, *49*(2), 220–238. https://doi.org/10.1111/j.1469-8986.2011.01293.x

Cavanagh, J. F., & Frank, M. J. (2014). Frontal theta as a mechanism for cognitive control. *Trends in cognitive sciences*,*18*(8), 414–421. https://doi.org/10.1016/j.tics.2014.04.012

Collier, G., & Myers, L. (1961). The loci of reinforcement. *Journal of Experimental Psychology*, *61*(1), 57–66. https://doi.org/10.1037/h0048851

Collier, G., & Willis, F. N. (1961). Deprivation and reinforcement. *Journal of Experimental Psychology*, *62*(4), 377–384. https://doi.org/10.1037/h0047144

Crespi, L. P. (1942). Quantitative variation of incentive and performance in the white rat. *The American Journal of Psychology*, *55*(4), 467. https://doi.org/10.2307/1417120

Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*(1), 9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009

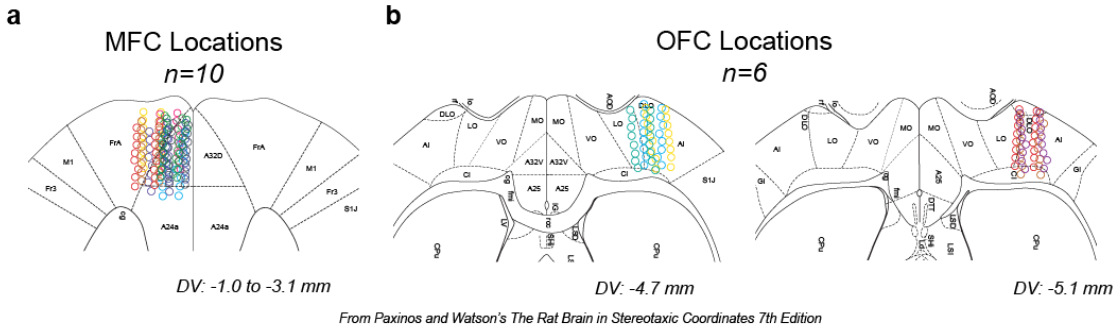
Flaherty, C. F. (1982). Incentive contrast: A review of behavioral changes following shifts in reward. *Animal Learning and Behavior*, *10*(4), 409–440. https://doi.org/10.3758/BF03212282

635 Gabbott, P. L. A., Warner, T. A., Jays, P. R. L., & Bacon, S. J. (2003). Areal and synaptic
636 interconnectivity of prelimbic (area 32), infralimbic (area 25) and insular cortices in the
637 rat. *Brain Research*, *993*(1–2), 59–71. https://doi.org/10.1016/j.brainres.2003.08.056
638 Gabbott, P. L. A., Warner, T. A., Jays, P. R. L., Salway, P., & Busby, S. J. (2005). Prefrontal
639 cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. *Journal*
640 *of Comparative Neurology*, *492*(2), 145–177. https://doi.org/10.1002/cne.20738
641 Gallagher, M., McMahan, R. W., & Schoenbaum, G. (1999). Orbitofrontal cortex and
642 representation of incentive value in associative learning. *Journal of Neuroscience*,
643 *19*(15), 6610–6614. https://doi.org/10.1523/JNeurosci.19-15-06610.1999
644 Gutierrez, R., Carmena, J. M., Nicolelis, M. A. L., & Simon, S. A. (2006). Orbitofrontal ensemble
645 activity monitors licking and distinguishes among natural rewards. *Journal of*
646 *Neurophysiology*, *95*(1), 119–133. https://doi.org/10.1152/jn.00467.2005
647 Gutierrez, R., & Simon, S. (2013). Increases in spike timing precision improves gustatory
648 discrimination upon learning. In P. DiLorenzo & J. Victor, *Spike Timing* (Vol. 20135633,
649 pp. 321–342). CRC Press. https://doi.org/10.1201/b14859-16
650 Horst, N. K., & Laubach, M. (2013). Reward-related activity in the medial prefrontal cortex is
651 driven by consumption. *Frontiers in Neuroscience*, *7*.
652 https://doi.org/10.3389/fnins.2013.00056
653 Hull, C.L. (1943). *Principles of Behavior* (Vol. 422). New York: Appleton-Century-Crofts.
654 Hulse, S. H., Snyder, H. L., & Bacon, W. E. (1960). Instrumental licking behavior as a function of
655 schedule, volume, and concentration of a saccharine reinforcer. *Journal of Experimental*
656 *Psychology*, *60*(6), 359–364. https://doi.org/10.1037/h0045841
657 Izquierdo, A. (2017). Functional heterogeneity within rat orbitofrontal cortex in reward learning
658 and decision making. *Journal of Neuroscience*, *37*(44), 10529–10540.
659 https://doi.org/10.1523/JNeurosci.1678-17.2017
660 Jarovi, J., Volle, J., Yu, X., Guan, L., & Takehara-Nishiuchi, K. (2018). Prefrontal theta
661 oscillations promote selective encoding of behaviorally relevant events. *eNeuro*, *5*(6).
662 https://doi.org/10.1523/Eneuro.0407-18.2018
663 Kaplan, J. M., Baird, J. P., & Grill, H. J. (2001). Dissociation of licking and volume intake
664 controls in rats ingesting glucose and maltodextrin. *Behavioral neuroscience, 115*(1),
665 188.
666 Kesner, R. P., & Gilbert, P. E. (2007). The role of the agranular insular cortex in anticipation of
667 reward contrast. *Neurobiology of Learning and Memory*, *88*(1), 82–86.
668 https://doi.org/10.1016/j.nlm.2007.02.002
669 Laubach, M., Amarante, L. M., Swanson, K., & White, S. R. (2018). What, if anything, is rodent
670 prefrontal cortex? *Eneuro, 5*(5). https://doi.org/10.1523/Eneuro.0315-18.2018
671 Laubach, M., Caetano, M. S., & Narayanan, N. S. (2015). Mistakes were made: Neural
672 mechanisms for the adaptive control of action initiation by the medial prefrontal cortex.
673 *Journal of Physiology-Paris*, *109*(1–3), 104–117.
674 https://doi.org/10.1016/j.jphysparis.2014.12.001

675 Levy, D. J., & Glimcher, P. W. (2011). Comparing apples and oranges: using reward-specific
676 and reward-general subjective value representation in the brain. *Journal of*
677 *Neuroscience*, *31*(41), 14693–14707. https://doi.org/10.1523/JNeurosci.2218-11.2011
678 Levy, D. J., & Glimcher, P. W. (2012). The root of all value: A neural common currency for
679 choice. *Current Opinion in Neurobiology*, *22*(6), 1027–1038.
680 https://doi.org/10.1016/j.conb.2012.06.001
681 Lin, J.-Y., Roman, C., & Reilly, S. (2009). Insular cortex and consummatory successive negative
682 contrast in the rat. *Behavioral Neuroscience*, *123*(4), 810–814.
683 https://doi.org/10.1037/a0016460
684 Montague, P. R., & Berns, G. S. (2002). Neural economics and the biological substrates of
685 valuation. *Neuron*, *36*(2), 265–284. https://doi.org/10.1016/S0896-6273(02)00974-1
686 Narayanan, N. S., Cavanagh, J. F., Frank, M. J., & Laubach, M. (2013). Common medial frontal
687 mechanisms of adaptive control in humans and rodents. *Nature Neuroscience*, *16*(12),
688 1888–1895. https://doi.org/10.1038/nn.3549
689 Öngür, D., & Price, J. L. (2000). The organization of networks within the orbital and medial
690 prefrontal cortex of rats, monkeys and humans. *Cerebral cortex, 10*(3), 206-219.
691 https://doi.org/10.1093/cercor/10.3.206
692 Padoa-Schioppa, C. (2011). Neurobiology of economic choice: A good-based model. *Annual*
693 *Review of Neuroscience*, *34*(1), 333–359. https://doi.org/10.1146/annurev-neuro-
694 061010-113648
695 Padoa-Schioppa, C., & Assad, J. A. (2006). Neurons in the orbitofrontal cortex encode
696 economic value. *Nature*, *441*(7090), 223–226. https://doi.org/10.1038/nature04676
697 Padoa-Schioppa, C., & Assad, J. A. (2008). The representation of economic value in the
698 orbitofrontal cortex is invariant for changes of menu. *Nature Neuroscience*, *11*(1), 95–
699 102. https://doi.org/10.1038/nn2020
700 Parent, M. A., Amarante, L. M., Liu, B., Weikum, D., & Laubach, M. (2015a). The medial
701 prefrontal cortex is crucial for the maintenance of persistent licking and the expression of
702 incentive contrast. *Frontiers in Integrative Neuroscience*, *9*.
703 https://doi.org/10.3389/fnint.2015.00023
704 Parent, M. A., Amarante, L. M., Swanson, K., & Laubach, M. (2015b). Cholinergic and
705 ghrelinergic receptors and KCNQ channels in the medial PFC regulate the expression of
706 palatability. *Frontiers in behavioral neuroscience, 9*, 284.
707 https://doi.org/10.3389/fnbeh.2015.00284
708 Passingham, R. E., & Wise, S. P. (2012). *The neurobiology of the prefrontal cortex: Anatomy,*
709 *evolution, and the origin of insight*. Oxford University Press.
710 Paxinos, G., & Watson, C. (2013). *The rat brain in stereotaxic coordinates*. Seventh Edition.
711 Elsevier Academic Press.
712 Petykó, Z., Gálosi, R., Tóth, A., Máté, K., Szabó, I., Szabó, I., Karádi, Z., & Lénárd, L. (2015).
713 Responses of rat medial prefrontal cortical neurons to pavlovian conditioned stimuli and
714 to delivery of appetitive reward. *Behavioural Brain Research*, *287*, 109–119.
715 https://doi.org/10.1016/j.bbr.2015.03.034

716 Petykó, Z., Tóth, A., Szabó, I., Gálosi, R., & Lénárd, L. (2009). Neuronal activity in rat medial
717 prefrontal cortex during sucrose solution intake: *NeuroReport*, *20*(14), 1235–1239.
718 https://doi.org/10.1097/WNR.0b013e32832fbf30
719 Pratt, W. E., & Mizumori, S. J. Y. (2001). Neurons in rat medial prefrontal cortex show
720 anticipatory rate changes to predictable differential rewards in a spatial memory task.
721 *Behavioural Brain Research*, *123*(2), 165–183. https://doi.org/10.1016/S0166-
722 4328(01)00204-2
723 Rangel, A., Camerer, C., & Montague, P. R. (2008). A framework for studying the neurobiology
724 of value-based decision making. *Nature Reviews Neuroscience, 9*(7), 545-556.
725 Rangel, A. (2013). Regulation of dietary choice by the decision-making circuitry. *Nature*
726 *Neuroscience*, *16*(12), 1717–1724. https://doi.org/10.1038/nn.3561
727 Riceberg, J. S., & Shapiro, M. L. (2017). Orbitofrontal cortex signals expected outcomes with
728 predictive codes when stable contingencies promote the integration of reward history.
729 *Journal of Neuroscience*, *37*(8), 2010–2021. https://doi.org/10.1523/JNeurosci.2951-
730 16.2016
731 Saez, R. A., Saez, A., Paton, J. J., Lau, B., & Salzman, C. D. (2017). Distinct roles for the
732 amygdala and orbitofrontal cortex in representing the relative amount of expected
733 reward. *Neuron*, *95*(1), 70-77.e3. https://doi.org/10.1016/j.neuron.2017.06.012
734 Schoenbaum, G., & Roesch, M. (2005). Orbitofrontal cortex, associative learning, and
735 expectancies. *Neuron*, *47*(5), 633–636. https://doi.org/10.1016/j.neuron.2005.07.018
736 Schoenbaum, G., Roesch, M. R., Stalnaker, T. A., & Takahashi, Y. K. (2009). A new
737 perspective on the role of the orbitofrontal cortex in adaptive behaviour. *Nature Reviews*
738 *Neuroscience*, *10*(12), 885–892. https://doi.org/10.1038/nrn2753
739 Simon, N. W., Wood, J., & Moghaddam, B. (2015). Action-outcome relationships are
740 represented differently by medial prefrontal and orbitofrontal cortex neurons during
741 action execution. *Journal of Neurophysiology*, *114*(6), 3374–3385.
742 https://doi.org/10.1152/jn.00884.2015
743 Siniscalchi, M. J., Wang, H., & Kwan, A. C. (2019). Enhanced population coding for rewarded
744 choices in the medial frontal cortex of the mouse. *Cerebral Cortex, 29*(10), 4090-4106.
745 Spence, K.W. (1956). *Behavior Theory and Conditioning*.
746 Strait, C. E., Blanchard, T. C., & Hayden, B. Y. (2014). Reward value comparison via mutual
747 inhibition in ventromedial prefrontal cortex. *Neuron*, *82*(6), 1357–1366.
748 https://doi.org/10.1016/j.neuron.2014.04.032
749 Sugrue, L. P., Corrado, G. S., & Newsome, W. T. (2005). Choosing the greater of two goods:
750 Neural currencies for valuation and decision making. *Nature Reviews Neuroscience*,
751 *6*(5), 363–375. https://doi.org/10.1038/nrn1666
752 Sul, J. H., Jo, S., Lee, D., & Jung, M. W. (2011). Role of rodent secondary motor cortex in
753 value-based action selection. *Nature Neuroscience*, *14*(9), 1202–1208.
754 https://doi.org/10.1038/nn.2881
755 Swanson, K., Goldbach, H. C., & Laubach, M. (2019). The rat medial frontal cortex controls
756 pace, but not breakpoint, in a progressive ratio licking task. Behavioral neuroscience,
757 133(4), 385–397. https://doi.org/10.1037/bne0000322

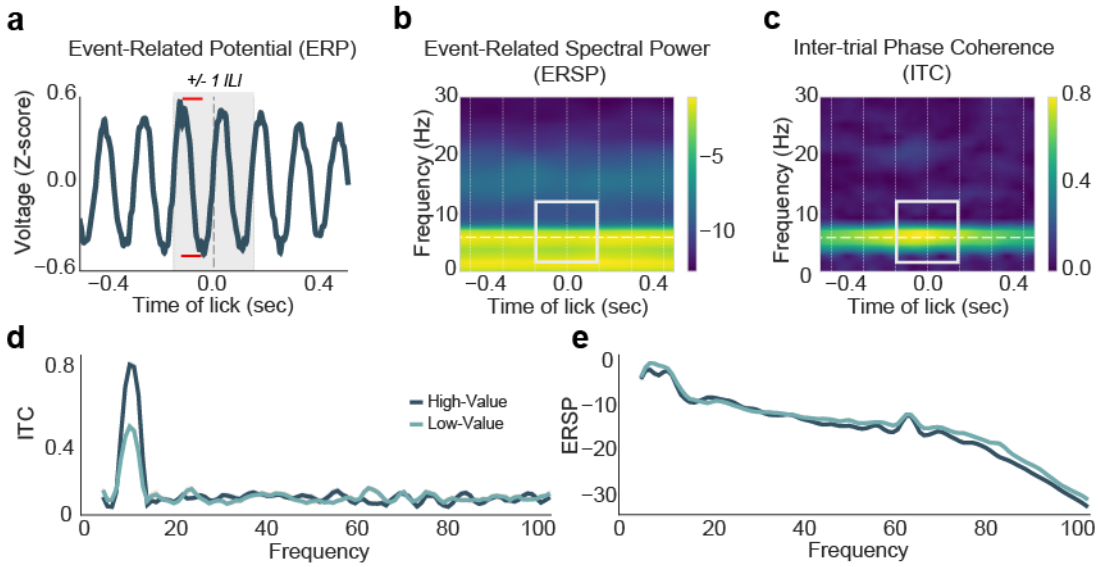
758 Travers, J. B., Dinardo, L. A., & Karimnamazi, H. (1997). Motor and premotor mechanisms of
759 licking. *Neuroscience and Biobehavioral Reviews*, *21*(5), 631–647.
760 https://doi.org/10.1016/S0149-7634(96)00045-0
761 Tremblay, L., & Schultz, W. (1999). Relative reward preference in primate orbitofrontal cortex.
762 *Nature*, *398*(6729), 704–708. https://doi.org/10.1038/19525
763 Uchida, N., & Mainen, Z. F. (2003). Speed and accuracy of olfactory discrimination in the rat.
764 *Nature Neuroscience*, *6*(11), 1224–1229. https://doi.org/10.1038/nn1142
765 van Duuren, E., van der Plasse, G., Lankelma, J., Joosten, R. N. J. M. A., Feenstra, M. G. P., &
766 Pennartz, C. M. A. (2009). Single-cell and population coding of expected reward
767 probability in the orbitofrontal cortex of the rat.. *Journal of Neuroscience*, *29*(28), 8965–
768 8976. https://doi.org/10.1523/JNeurosci.0005-09.2009
769 van Wingerden, M., Vinck, M., Lankelma, J., & Pennartz, C. M. A. (2010). Theta-band phase
770 locking of orbitofrontal neurons during reward expectancy. *Journal of Neuroscience*,
771 *30*(20), 7078–7087. https://doi.org/10.1523/JNeurosci.3860-09.2010
772 Webber, E. S., Chambers, N. E., Kostek, J. A., Mankin, D. E., & Cromwell, H. C. (2015).
773 Relative reward effects on operant behavior: Incentive contrast, induction and variety
774 effects. *Behavioural Processes*, *116*, 87–99.
775 https://doi.org/10.1016/j.beproc.2015.05.003
776 Weijnen, J. (1998). Licking behavior in the rat: Measurement and situational control of licking
777 frequency. *Neuroscience and Biobehavioral Reviews*, *22*(6), 751–760.
778 https://doi.org/10.1016/S0149-7634(98)00003-7
779 Widmann, A., & Schröger, E. (2012). Filter effects and filter artifacts in the analysis of
780 electrophysiological data. *Frontiers in Psychology*, *3*.
781 https://doi.org/10.3389/fpsyg.2012.00233
782 Womelsdorf, T., Vinck, M., Leung, L. S., & Everling, S. (2010). Selective theta-synchronization
783 of choice-relevant information subserves goal-directed behavior. *Frontiers in Human*
784 *Neuroscience*, *4*. https://doi.org/10.3389/fnhum.2010.00210
785 Yoshida, A., Taki, I., Chang, Z., Iida, C., Haque, T., Tomita, A., Seki, S., Yamamoto, S.,
786 Masuda, Y., Moritani, M., & Shigenaga, Y. (2009). Corticofugal projections to trigeminal
787 motoneurons innervating antagonistic jaw muscles in rats as demonstrated by
788 anterograde and retrograde tract tracing. The Journal of comparative neurology, 514(4),
789 368–386. https://doi.org/10.1002/cne.22013



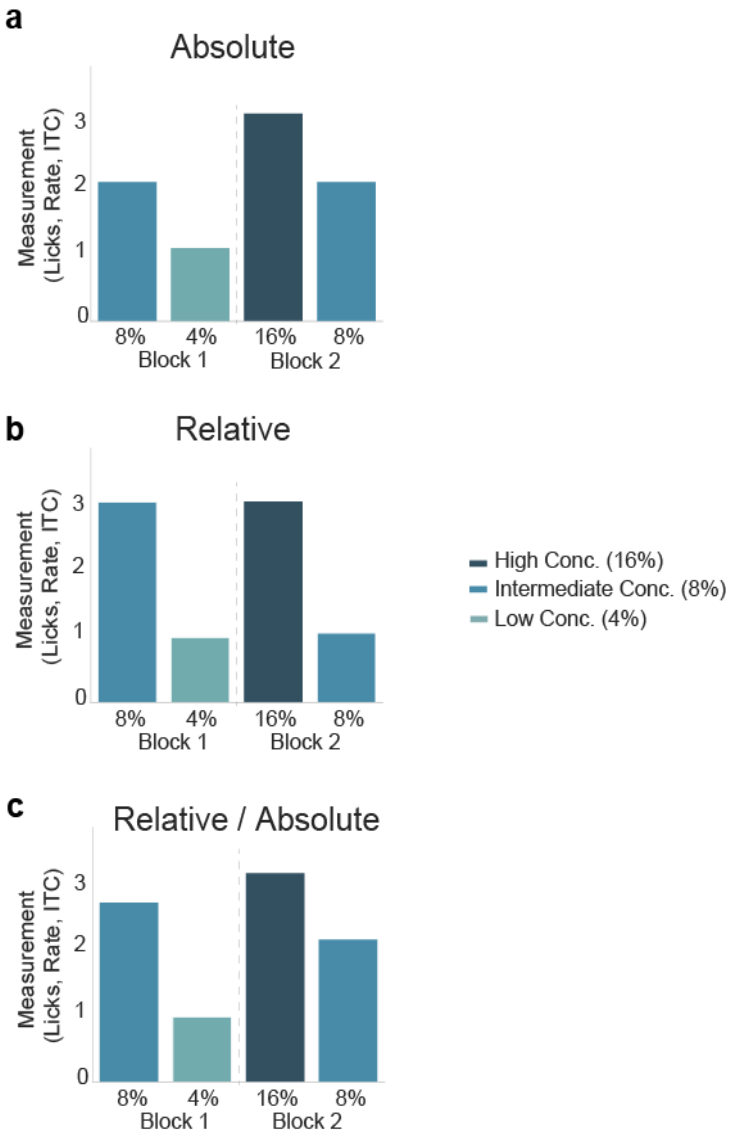
Supplementary Figure 1. Electrode localization. Locations of all electrodes plotted. A. MFC (n=10 rats; 160 electrodes) electrode arrays were localized around area 32 (A32D) and M2 (FrA) from 1 to 3 mm ventral from the brain's surface. B. OFC (n=6 rats; 96 electrodes) electrode arrays were localized around agranular insular (AI) and lateral orbital (LO) areas of OFC from 4.7 to 5.1 mm ventral from the brain's surface. Reconstructions were plotted over atlas figures from Paxinos and Watson's The Rat Brain in Stereotaxic Coordinates, 7th edition (2013).

790

Supplementary Figures



Supplementary Figure 2. Types of Electrophysiological Measures Used to Assess LFP Activity Related to Behavior. A. Event-related potentials (ERPs) were recorded around licks (time 0) after LFP activity was filtered and z-scored. Peak-to-peak analysis was performed on the ERP centered around each lick with a +1 inter-lick interval (ILI) window to calculate the amplitude size (red limits = maximum minus the minimum amplitude of the ERP). B,C. Spectral measures of power (B) and phase (C). Grouped statistics were based on the mean maximum Event-Related Spectral Power (ERSP) and Inter-Trial phase Coherence (ITC) value from 2-2 Hz and around +1 ILI (grey window). Vertical lines denote the rat's average ILIs. Horizontal line denotes the rat's median lick rate. D. Maximum ITC values over frequencies from 0-100 Hz from all 16 MFC electrodes from one example rat. E. Maximum ERSP measures over frequencies from 0-100 Hz in all 16 MFC electrodes from one example rat.



Supplementary Figure 3. Hypothesis for Relative versus Absolute Encoding of Reward Value. A. If rewards are processed in an absolute manner, we expected to see a graded expression (in lick counts, lick rate, bout duration, or ITC values) of reward value where the high (16%) concentration reward expression is greatest, followed by equal expression of the intermediate (8%) reward and then low expression of the low (4%) concentration reward. B. If rewards are processed in a relative manner, we expected to see a comparative process of rewards, where the “high value” (8% in block 1 or 16% in block 2) are processed similarly, and the focus is on the comparison within each block or context. C. An alternative hypothesis which incorporates a combination of relative and absolute processing of reward value, with partially mixed results of each process in A and B.