Good vibrations: Rhythms and plasticity in neural correlates of value in rat orbitofrontal cortex

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CHAPTER 2

THETA-BAND PHASE-LOCKING OF ORBITOFRONTAL NEURONS DURING REWARD EXPECTANCY

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ABSTRACT
The expectancy of a rewarding outcome following actions and cues is coded by a network of brain structures including the orbitofrontal cortex. Thus far, predicted reward was considered to be coded by time-averaged spike rates of neurons. However, besides firing rate, the precise timing of action potentials in relation to ongoing oscillations in local field potentials is thought to be of importance for effective communication between brain areas.

We performed multi-neuron and field potential recordings in orbitofrontal cortex of rats performing olfactory discrimination learning to study the temporal structure of coding predictive of outcome. After associative learning, field potentials were marked by theta oscillations, both in advance and during delivery of reward. Orbitofrontal neurons, especially those coding information about upcoming reward with their firing rate, phase-locked to these oscillations in anticipation of reward. When established associations were reversed, phase-locking collapsed in the anticipatory task phase, but returned when reward became predictable again after re-learning. Behaviorally, the outcome anticipation phase was marked by licking responses, but the frequency of lick responses was dissociated from the strength of theta-band phase locking. The strength of theta-band phase-locking by orbitofrontal neurons robustly follows the dynamics of associative learning as measured by behavior and correlates with the rat's current outcome expectancy. Theta-band phase-locking may facilitate communication of outcome-related information between reward-related brain areas and offers a novel mechanism for coding value signals during reinforcement learning.
INTRODUCTION
Constructing predictive representations of future reward is essential for goal-directed behavior. Goal-directed behavior appears to be guided by a network of brain structures encoding the predicted outcome of cues and actions as a result of associative learning (Schoenbaum et al., 1998; Padoa-Schioppa and Assad, 2006; Schultz, 2006; Rolls et al., 2008). Such predictive representations have been found in the amygdala, prefrontal cortex and ventral striatum and are a requirement for learning mechanisms that depend on the difference between actual and predicted reward (Rescorla and Wagner, 1972; Pennartz, 1997; Schultz et al., 1997; Sutton and Barto, 1998; Schultz, 2006). However, until now the neural coding of reward expectancy has been studied almost exclusively in the domain of firing rates, whereas a number of issues in reward processing require considering its temporal organization. First, the effect that a reward-predictive representation in a given brain structure exerts on its target areas will depend on the temporal alignment of the firing of contributing neurons (Salinas and Sejnowski, 2000; Engel et al., 2001). Second, the temporal phasing of activity in two connected brain areas may modulate the efficacy of their communication (Womelsdorf et al., 2007). Third, reward-dependent associative learning mechanisms likely involve long-term synaptic modifications depending on the exact timing of pre- and postsynaptic activity (Levy and Steward, 1983; Markram et al., 1997; Lisman and Spruston, 2005; Cassenaer and Laurent, 2007).

We investigated the relationship between single unit activity and local field potential (LFP) oscillations during an associative learning task in the orbitofrontal cortex (OFC), a prefrontal structure that is strongly connected with the amygdala, parahippocampal cortex, medial prefrontal cortex and ventral tier of the basal ganglia, and is crucial for flexibly encoding reward-value representations and adjusting goal-directed behaviors (Bechara et al., 1994; Carmichael and Price, 1995b; Öngür and Price, 2000; Fellows and Farah, 2003; Stalnaker et al., 2007; Schilman et al., 2008). Firing-rate changes of OFC neurons were shown to represent the predicted reward value of cues and actions, both at the single-cell and population level (Schoenbaum et al., 1998; Wallis and Miller, 2003; Padoa-Schioppa and Assad, 2006; van Duuren et al., 2008). Moreover, OFC representations can be updated when previously learned associations are no longer valid (Rolls et al., 1996; Schoenbaum et al., 1998). If the relationship between single
unit activity and field oscillations is of importance for associative learning, we would expect to find changes in this relationship that follow the dynamics of learning, expressed preferentially by those cells whose activity is informative about upcoming task outcomes. In addition to testing this hypothesis, we also examined whether rhythmic LFP-locked spiking activity was associated with licking behavior, as this was found especially in advance of and following reward delivery.

MATERIALS & METHODS

Subjects
Three adult male Wistar rats (Harlan CPB, Horst, the Netherlands), weighing 370-450 g at the time of surgery, served as subjects in these experiments. Prior to training and surgery, the rats were housed two to a cage on a reversed light/dark cycle (lights off: 7AM, lights on: 7PM) with *ad libitum* food and water. After surgery, animals were housed individually in a transparent cage (40x40x40 cm), with other rats present in the climate controlled colony room. During training and recording, rats were maintained on food restriction, with 5-15 grams of food available from >0.5 hr after training, depending on the amount of reward collected in the session, amounting to 90% free-feeding intake.

All experiments were conducted according to the National Guidelines on Animal Experiments and with approval of the Animal Experimentation Committee of the University of Amsterdam.

Behavioral training: Apparatus
Odor discrimination training was conducted in an operant chamber (56 x 30 x 40 cm LxWxH) equipped with an odor sampling port and trial light on a front panel and a tray for delivery of fluids placed at the opposing wall (Fig. 2.1). The front panel was slanted at 45° (with respect to ground level) above the odor sampling port to allow unhindered nose poking into the odor port by implanted animals. Entries by the animals into the odor port and into the fluid well were recorded by photobeam interruptions and stored on a computer dedicated to behavioral data acquisition. Licking responses were detected by a separate photobeam situated within the fluid well. During recording sessions, behavioral events were synchronized with electrophysiological data acquisition running on a separate computer. Odors were delivered via
Figure 2.1 Task-related theta oscillations and phase-locking

(A) Operant chamber; impression of rat making odor poke (left) and fluid poke (right). (B) Raw LFP segments recorded during different task periods (black) and band-pass filtered LFP (4-12 Hz) (green trace, Hit trial; red, False Alarm trial) and spike trains from one unit, recorded on a different tetrode (vertical green/red bars); iti, intertrial-interval; psd, pre-stimulus-delay. (C) LFP power modulation, synchronized in time on sucrose delivery. Pseudocolors indicate averaged percentual increase in LFP power compared to the intertrial interval period. Green dashed line, median time of odor onset; red dashed line, median time of odor offset; white dashed line, start of waiting period; white solid line, sucrose delivery. Theta oscillations (9 Hz) found during odor sampling are not visible here due to synchronization on reward delivery. (D) Spike-field phase-locking spectrum averaged across all task-segments and all units. Shaded regions indicate 95% bootstrapped confidence intervals on the mean. (E) Polar histogram of mean phase values for all cells. Shaded part corresponds to number of significantly phase-locked units (270 out of 525, Rayleigh test, P<0.05). Mean phase: 11.3 degrees. Distribution of phases deviated significantly from uniform distribution (p<0.001).
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separate glass vials and tubing to avoid mixing. Upon entering the application system, they were mixed 1:1 with clean air and released into the compartment directly behind the odor port by way of computer-controlled valves. Likewise, quinine and sucrose solutions were delivered to the fluid well via separate fluid lines and electronically controlled by valves (van Duuren et al., 2007a). Training and task performance were devoid of human interference.

Training procedure
The animals were trained on a 2-odor go/no-go discrimination task (Schoenbaum et al., 1998). After habituation to the chamber and pre-training, rats were confronted with odor discrimination problems. Each session, one novel odor was associated with reward (100 μl of 15% sucrose solution), and a second novel odor with an aversive outcome (100 μl of 0.01 M quinine solution). Training sessions consisted of blocks of 5+5 pseudo-randomly ordered positive and negative odor trials. When a trial light was illuminated, rats could initiate a trial by poking their snout in the odor sampling port. After a 500 ms delay, air flow through the odor sampling port was switched from clean air to the selected clean air/odorant mixture. A correct nose poke in the odor port (wait for odor 500 ms, constituting a pre-stimulus delay, and sample odor for at least 750 ms) was indicated by the trial light turning off. After sampling, the rats could move over to the fluid well, into which they were required to make a nose poke for ≥ 1000 ms before the outcome (sucrose or quinine solution) was presented. We refer to the invariant 1000 ms delay period as the ‘waiting’ or anticipatory period. This waiting period allows sampling of neural activity during the waiting period that is not confounded by whole-body movement or novel sensory input (Schoenbaum et al., 1998; van Duuren et al., 2007a). When the rats left the fluid well, an intertrial interval (ITI) of 10-15 sec was observed before the next trial started. A Correct Rejection was scored if the rat refrained from entering the reinforcement tray for ≥ 5 s following sampling of the negative odor. Responses during the ITI had no programmed consequences, while premature responses (i.e. short pokes) during the odor sampling or waiting period resulted in immediate termination of the current trial and the start of a new trial.

Rats were implanted after they reached behavioral criterion, viz. scoring > 85% Hits and Correct Rejections over a moving block of 20 trials. Following surgery, animals were retrained on the familiar odor pair with which initial
training took place until performance was back at criterion level. Recording of neural activity started in the subsequent session. On each recording session, rats were confronted with a new odor pair. Furthermore, a reversal schedule of odor-outcome contingencies was appended to the session in case the rat reached criterion performance. To assess learning in the reversal phase, average performance over all sessions as a function of post-reversal trial was compared against chance level using a binomial test with expected performance of 0.5 (p<0.05).

**Surgical procedures**

Animals were anesthetized by i.m. injection of 0.08 ml/100 g Hypnorm (0.2 mg/ml fentanyl, 10 mg/ml fluanison; VetaPharma Ltd., Leeds, UK), followed by 0.04 ml/100 g Dormicum (5 mg/ml midazolam; Roche Nederland B.V., Woerden, the Netherlands) s.c. and mounted in a stereotact. Body temperature was maintained between 35-36 °C. A microdrive, holding 14 individually moveable electrode drivers, was chronically implanted onto a craniotomy (diameter: 2 mm) in the left hemisphere dorsal to the OFC at 3.4 -3.6 mm anterior and 3.0-3.2 mm lateral to bregma. The drivers were loaded with 12 tetrodes and 2 reference electrodes. Using dental cement, the drive was anchored to six stainless steel screws, one of which was positioned in the left parietal bone and served as ground. Immediately after surgery, all tetrodes and reference electrodes were advanced 0.8 mm into the brain. Next, the animal was allowed to recover for 7 days with *ad libitum* food and water, during which the 12 recording tetrodes were advanced in daily steps to the upper border of the OFC according to a standardized rat brain atlas (Paxinos and Watson, 2007). The reference electrodes were lowered to a depth of 1.2-2.0 mm and adjusted to minimize spiking activity on the reference channel.

After surgery, saline was injected s.c. (2 ml per flank), and pain relief was provided by 0.1 ml / 100 g pre-surgical weight of a 10% Finadyne (flunixin meglumine 50 mg/ml; Schering-Plough, Brussels, Belgium) solution administered in saline s.c.

**Electrophysiology**

Using tetrodes (Gray et al., 1995), neural activity was recorded by a 64-channel Cheetah setup (Neuralynx, Bozeman MT). Signals were passed
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through a unity-gain pre-amplifier headstage, a 72-channel commutator (Dragonfly, Ridgeley, West Virginia, USA), amplified 5000x and bandpass filtered between 600-6000 Hz for spike recordings. If a signal on any of the leads of a tetrode crossed a pre-set threshold, activity on all four leads was sampled at 32 kHz for 1 ms and stored for off-line analysis. Local field potentials recorded on all tetrodes were amplified 1000x, continuously sampled at 1874 Hz, and bandpass filtered between 1-475 Hz. Events in the behavioral task were co-registered and time-stamped by the Cheetah system.

**Data Analysis: Isolation of single-unit activity**

Spike trains were sorted to isolate single units using a semi-automated clustering algorithm (BubbleClust) followed by manual refinement using MClust. Automated and manual clustering of spikes was done using the waveform peak amplitude, area, squared amplitude integral and the first three principal components. Clusters were accepted as single units when having no more than 0.1% of inter-spike intervals shorter than 2 ms.

**Spike-LFP phase-locking**

All analyses were done with Matlab (The MathWorks, Inc., Natick, MA) and using FieldTrip (http://www.ru.nl/fcdonders/fieldtrip/; Oostenveld et al., 2011), an open source toolbox for the analysis of neurophysiological data. Briefly, for each spike of a given cell, we cut out a 1.0 sec data segment of the LFPs recorded simultaneously on the other tetrodes (such that we never included a spike-LFP pair from the same tetrode). Several methods exist to measure spike-LFP phase consistency (Fries et al., 1997; Jarvis and Mitra, 2001; Pesaran et al., 2002; Womelsdorf et al., 2006). The basis of all of these methods is to determine the phase of a spike or a spike train relative to an LFP trace in a particular frequency band. Here, we multiplied each unfiltered LFP data segment by a Hanning window and Fourier transformed the windowed data segment of length T, so that the spike-triggered LFP frequency (f) spectrum is given as

\[ X_i(f) = \sum_{t=1}^{T} w(t)x_i(t)e^{-2\pi jft} \]  

(1)
where $x_i(t)$ is the LFP time segment around the $i$th spike ($i = 1,2,...,N$) and $w(t)$ the Hanning window. Using a 1.0 sec data segment length set the Rayleigh frequency at 1 Hz, allowing a frequency resolution of 1 Hz. We determined the complex average spike-triggered LFP spectrum across the $C$ different tetrodes as

$$
\overline{X_i}(f) = \frac{1}{C} \sum_{c=1}^{C} \frac{X_{i,c}^e(f)}{|X_{i,c}^e(f)|}
$$

Equation (2) ensures that the power of each LFP segment is ignored in the computation of the average spike phase. The spike phase is now simply given by $\theta_i = \arg(\overline{X_i}(f))$. We measured phase consistency by means of the spike-LFP phase-locking value, which is defined as the resultant vector length across all spikes $N$,

$$
PLV(f) = \left| \frac{1}{N} \sum_{i=1}^{N} \frac{X_i^e(f)}{|X_i^e(f)|} \right|
$$

The resultant vector length is a real number in the range of 0 (low phase consistency) to 1 (high phase consistency). The more spikes we use to obtain our statistical estimate of the resultant length, the more reliable it is. Spike-LFP phase locking is a biased measure with respect to the number of spikes that are entered in the computation. In the method used here (Womelsdorf et al., 2008; cf. Lachaux et al., 2002), we controlled for the bias by always entering the same fixed number of spikes (N=50) into equation (3) when we compared between samples with a different number of elements. This was the case whenever we compared phase-locking between trial types (Fig. 2.2B-C, 3A-B, 4C), different task periods (Fig. 2.2D) and different cell groups (Fig. 2.4A-B). Only in the time-resolved estimate of phase-locking (Fig. 2.2A), we used a lower criterion of N=40 spikes per cell because here the sliding time windows (500ms) were half the size of regular LFP windows. We further reduced the statistical variance of the spike-LFP phase-locking estimate by means of a bootstrapping procedure. For every repetition, we drew a fixed number of spikes without replacement from all spikes in the sample. For each sample drawn, we determined the spike-LFP phase-locking value. Subsequently, we averaged these spike-LFP phase-locking values across all bootstrapped samples (N = 5000), producing an unbiased phase-locking
value. Nonetheless, the statistical significance of spike-LFP phase-locking can still be assessed across the entire sample of spikes, using the Rayleigh test \((P < 0.001)\).

**Time-resolved power spectra & timing of theta oscillation onset**

For every tetrode, we isolated LFP data segments in 500 ms windows (setting the Rayleigh frequency resolution at 2 Hz) centered on the time point of interest, separated by time steps of 10 ms (Fig. 2.3C-D, at 6 Hz). For figure 1C, we used a window of 800 ms, yielding a frequency resolution of 1.25 Hz. Using equation 1, the LFP power of the \(i\)th segment was determined as \( |X_i(f)| \)

\[
|X_i(f)| = \sum_{t} w(t) x_i(t) e^{-2t/f} \pi
\]

(4)

To obtain the baseline corrected LFP power \(P_i(f)\), we first determined the LFP power in the intertrial-interval in the same way as in the task period. We averaged the baseline LFP power across time segments and trials. We then defined the baseline corrected trial LFP power by dividing the trial LFP power by the baseline LFP power. Since the number of trials before and after reversal differs between sessions, the averaged power modulation shown in figure 2.3C-D was only calculated for trials with \(N \geq 5\) contributing sessions. To study the dynamics of theta oscillation onset around the odor-outcome reversal (Fig. 2.3E), we calculated a center of mass (COM) index for the waiting period as

\[
COM = \frac{\sum_{t} \left( P_i(f) \tau_i \right)}{\sum_{i} P_i(f)}
\]

(5)

where \(N\) is the number of data segments between -2 and +2 seconds relative to reward delivery, and \(\tau_i\) indicates the center of the LFP segment and ranges from -2 sec to +2 sec. To determine the trial-by-trial timing of the onset of the theta oscillation on Hit trials (Fig. 2.3F), we determined the first time point from -2 to +2 seconds relative to reward delivery where the calculated theta power was greater than 50% of the maximum theta power in that segment (threshold crossing). We pooled these values as a function of trial number relative to reversal across sessions and performed a regression analysis.
Fig. 2.2 Modulation of spike-field phase-locking during reward expectation and delivery

(A) Time-course of average (across all cells) phase-locking centered on reward delivery. Phase-locking was calculated in 500 ms symmetric windows centered on each time point. Solid line: reward delivery; dashed line: start of waiting period. (B) Average spike-field phase-locking spectra during waiting period, separated for sucrose, quinine delivery, and during (unrewarded) intertrial-interval fluid-pokes. Shaded regions indicate 95% bootstrapped confidence intervals on the mean. (C) Same as in (B), but now during fluid delivery period. (D) Bar plot of phase-locking values at 6 Hz (mean ± SEM) for different task periods. M, movement; $D_s$, sucrose delivery; $D_Q$, quinine delivery; $W_s$, waiting period before sucrose; $W_Q$, waiting period before quinine; ITI, Intertrial interval. Numbers indicate percentages of cells significantly phase-locked (Rayleigh’s test $p<0.05$). Comparisons: $***$: $p<0.001$; $**$: $p<0.01$; $*$: $p<0.05$, Wilcoxon’s matched-pairs signed rank test.
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of theta onset versus trial number on the first 15 Hit trials after reversal. To control for unequal trial numbers in the post-reversal phase we created a randomization distribution of correlation coefficients for this regression by shuffling trials from all sessions with >4 post-reversal Hit trials. We then compared the observed correlation coefficient against the randomization distribution of correlation coefficients to obtain the probability of finding that result. This statistical procedure ensured that any bias due to unequal post-reversal trial numbers was removed.

Multiple Regression Analysis
To investigate the contribution of a type of behavioral correlate in firing rate to phase-locking values during a specific period, we coded the presence or absence of such a correlate per cell in a binary fashion. Next, we constructed a design matrix with firing-rate correlate presence as dummy independent variables to explain phase-locking values as a dependent variable, separately for each trial period. This resulted in a matrix of beta-weights (correlation-coefficients) and associated p-values (Fig. 2.4A-B). As a control predictor, rat identity was entered, which yielded no additional explanatory power.

RESULTS
We used tetrode arrays (Gray et al., 1995) to record single unit activity and local field potentials (LFP) from the OFC in 17 sessions from 3 male Wistar rats. Before each session, the tetrodes were moved individually to optimize the number of cells recorded simultaneously. Histological verification of the tetrode endpoints and recording tracks showed that all recordings were performed between 3.2 and 4.2 mm anterior of bregma and confined to the ventral and lateral aspects of the OFC (Fig. 2.5C-E). Rats performed a 2-odor discrimination task in which they were required, in each new session, to associate one novel odor (S+) to a positive trial outcome (sucrose solution) and a second novel odor (S-) to a negative outcome (quinine solution). All recordings were confirmed as having been made from OFC, as shown in the example in figure 2.5E. This yielded a total of 525 well-isolated single units (15-54 units per session, median: 29) in conjunction with LFP signals recorded simultaneously. In all analyses where pairs of single units and LFPs were used, the unit was recorded on a different tetrode than the LFP to avoid a possible frequency bias when the LFP around a spike was filtered.
Fig. 2.3 Learning-related changes in phase-locking and LFP

(A) Spike-field phase-locking spectra for pre-reversal phase, separated in early (cyan) and late (magenta) trials. (B) Same as (A), but now for reversal phase. (C) Trial-by-trial modulation of theta power (6 Hz) relative to reversal. Pseudocolor scale indicates theta power modulation compared to baseline, averaged over contributing sessions (see Experimental Procedures). Before reversal, odor 1 predicts sucrose ($O_1$-S). After reversal odor 1 predicts quinine ($O_1$-Q). Dashed white line, start of waiting period; solid white line, fluid delivery; ACQ, acquisition; REV, reversal. (D) Same as (C), but now synchronized on fluid delivery following odor 2. (E) Hit/False alarm trials show opposite patterns for theta power Center-of-Mass (COM, see text). Dashed lines indicate moment of fluid delivery. Red and green lines indicate mean shifts. (F) Trial-by-trial timing of theta onset on Hit trials in relation to reversal. Green line indicates mean first time point in time window where instant theta power reached >50% of maximum theta power on that trial. Shading indicates standard deviation.
Following odor sampling, rats either made a Go decision, consisting of a locomotor response towards a fluid well followed by a nose poke into the well and an immobile waiting period of 1 second before reinforcer delivery, or a No-Go decision, refraining from these responses (Fig. 2.1A-B). Rats required 86 ± 6 (mean ± SEM) trials to reach the criterion of 85% correct go/no-go decisions over the last 20 trials, making on average 16 ± 2 False Alarm responses (i.e., erroneous Go responses, Fig 2.1B). When the criterion was met, rats were exposed to a reversal schedule. Now, the odor previously associated with sucrose was coupled to quinine and vice versa. After reversal, rats quickly adjusted their behavior to the new odor-outcome contingencies, performing above chance level after on average 30 ± 4 trials (Binomial test, p<0.05). This provides evidence that the rats also learned the reversed odor-outcome contingencies. On Hit trials, both before and after reversal, the mean reaction time (measured from odor offset to reward delivery) was negatively correlated with increasing trial number (both p < 0.05, Spearman’s rank correlation). Licking behavior was analyzed separately (see below).

**Analysis of Oscillatory Activity**

We observed increments in theta power (4-12 Hz) in the LFPs during the waiting period of each trial, when the rat anticipated reward, and during reward consumption, but not during movement (Fig. 2.1B-C). The characteristics of these oscillations are different from the well-known theta rhythm recorded from hippocampus, which has been linked to locomotion and spatial and episodic memory formation (O'Keefe and Recce, 1993; Skaggs et al., 1996; Buzsáki, 2005). The LFP reflects the activity of a large number of neurons and could be confounded by volume conduction of signals from other brain areas. In addition to the tetrode recordings, we performed recordings in two additional animals in the same behavioral paradigm using linear silicon probes with electrodes spanning the dorso-ventral axis of the OFC (Fig. 2.5C-D). Current-source density analysis of the recorded LFPs showed the presence of a sink-source pair in the OFC, indicating that at least part of the recorded theta oscillatory activity is locally generated (Fig. 2.5A-B). Next, we examined whether theta-band rhythmicity was also visible in the firing patterns of single neurons recorded from the OFC. We determined the phase of each spike relative to the LFP components for frequencies ranging from 3
Fig. 2.4 Relationship between behavioral firing-rate correlates and spike-field phase-locking for different trial periods

(A) Regression coefficients for the 4 categories of possible behavioral correlates. Only the waiting period correlate is a significant positive predictor of phase-locking values during the Waiting period (tested at 6Hz). Shaded regions indicate SEM. (B) as in (A), but now for the Fluid Delivery period (C) Phase-locking spectra separate for trials in which the outcome decoded from the neuron’s firing rate matched (green) or failed to match (red) the actual outcome. Shaded regions indicate 95% bootstrapped confidence intervals on the mean. Significant difference at 6 Hz (P< 0.01, Wilcoxon’s matched pairs signed rank test)
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Fig. 2.5 Current source density (CSD) analysis and histology

(A) Modulation of theta power (bandpass filtered 4-12 Hz) by recording site on a linear silicon probe. Time is synchronized on reward delivery. Pseudocolors show z-scored modulation of theta power compared to inter-trial interval theta power, averaged over N=31 reward deliveries. White traces show LFP traces (filtered 1-475 Hz) from two sites, corresponding to maxima from CSD plot. Red lines show alignment of peaks and troughs between example traces. (B) Current source density analysis of a representative reward delivery event. Sources and sinks are depicted in pseudocolor (arbitrary units).
to 30 Hz. The normalized sum of these single cell phase-vectors has a mean angle and a resultant length between 0 and 1. The resultant length of the vector reflects how consistently a cell fires to a phase of the theta oscillation, with a spike-field phase-locking value of 0 indicating no consistency in phases, and a value of 1 indicating full consistency of spike phases (for a detailed explanation, see Materials & Methods). In a majority of OFC units (51%), spikes were significantly phase-locked to theta oscillations, with a maximum strength of locking at 6 Hz (Fig. 2.1D).

**Theta-band phase-locking in relation to behavioral task periods**

Based on the prominent theta-band activity during the waiting period, we hypothesized that OFC theta oscillations may contribute to processing of information related to outcome expectancy. To address this issue, we investigated theta-band phase-locking during this period as a function of time (Fig. 2.2A). We calculated time-frequency representations of phase-locking using symmetric segments of 500 ms centered on each time point, in steps of 10 ms. We found that the strength of spike-field phase-locking steeply rose during the waiting period, with a peak at 6 Hz, and rapidly collapsed at reward delivery (Fig. 2.2A). To contrast phase-locking across different event-related time windows, we defined the following task periods in each trial: the odor sampling period (0.5 s window starting at odor onset), the movement period (a variable-length window from odor offset until 0.2 s before fluid well entry), the waiting period (a 0.8 s window from 0.2 s after fluid well entry until fluid delivery) and the fluid delivery period (a 2 s window starting at fluid delivery). For each cell, a task-period specific spike-LFP phase-locking spectrum was determined using the sample of spikes across all applicable trials within the task period under consideration. To remove the spike-number related bias when comparing between different windows, we applied the bootstrapping

(continued from page 44)
Black traces depict filtered LFP (4-12 Hz) from 16 recording sites. (C) Brain section with part of the silicon probe in place. Purple box outlines the area from which (A) and (B) were obtained. In black, raw traces of part of the trial depicted in (B) show that sinks and sources are located within OFC. Black scale bar: 1s. (D) Corresponding figure from standardized rat brain atlas. OFC depicted by VO and LO. (E) Brain section from rat implanted with tetrodes. Two tetrode tracks are visible. Purple boxes mark tetrode endpoint lesions.
procedure using 50 spikes per cell for each condition. Subsequently, we averaged the spike-LFP phase-locking spectrum across all units considered for a given task period.

This analysis confirmed that the strongest theta-band phase-locking occurred during the waiting period (Fig. 2.2B-D). Importantly, the increment in phase-locking during the waiting period was specific to the expectation of a positive outcome: phase-locking was significantly higher during the waiting period before sucrose compared to the waiting period before quinine and intertrial interval fluid-well visits (Fig. 2.2B). It was also significantly higher than phase-locking during both the sucrose and quinine delivery periods (Fig. 2.2C; all comparisons: Wilcoxon’s matched pairs signed rank (WMPSR) test between values at 6 Hz, unless noted otherwise, Fig. 2.2D). These observations confirm that theta-band phase-locking does not occur merely as a consequence of immobility or a general state of outcome expectancy, but relates specifically to expectancy of reward due to a learned association. Second, the enhanced phase-locking during reward anticipation does not appear to be a trivial consequence of enhanced theta power, because the reward delivery period was marked by elevated theta power but a low degree of phase-locking when all cells were considered (Fig. 2.1C, 2.2C).

To investigate whether theta-band rhythmicity is also present in the local spiking activity per se, we used the high-pass filtered (600-6000 Hz) multi-unit spike train instead of the LFP to calculate phase-locking of units (see supplementary material). Again, we found spectrally specific phase-locking during the waiting period that was significantly higher in the waiting period before sucrose delivery, compared to the waiting period before quinine delivery ($P < 0.01$ WMPSR, Fig. 2.7A). In addition, there was significantly more theta-band rhythmicity in the spiking output of the OFC during the waiting period before sucrose compared to both the waiting period before quinine and the intertrial interval ($P < 0.05$, one-tailed $t$-test, Fig. 2.7B-D). Both results confirm the presence of outcome-dependent theta-band rhythmicity in OFC spiking activity during the anticipatory period.

Spectral analysis of the oscillatory activity recorded during different trial periods indicated that besides the prominent theta-band (peak at 6 Hz) activity found during the waiting and reward delivery periods (Fig. 2.1C), another, weaker type of theta-band activity was present during the odor sampling period, peaking at 9 Hz. Because the focus of this paper is on...
Figure 2.6 Classification of neurons according to behavioral correlates of firing rate

Peri-event time histograms of units from four different classes. Panels show rasterplots (bottom) and average firing rate (top) in 100 ms bins for the same unit, split for Hit (left) and False Alarm (right) trials, aligned to the event in the top left corner. Blue and magenta lines signify mean firing rate during the inter-trial interval period and time of reversal, respectively. Red line indicates bins with firing rates significantly different from inter-trial interval (p<0.01 Wilcoxon's matched-pairs signed-rank test).
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reward expectancy outside stimulus periods, theta-band phase-locking during the odor sampling period will not be further discussed below.

**Strength of Theta-band Phase-locking Correlates with Learning**

We further investigated the relationship between theta-band phase-locking and flexible learning. Because neural representations of reward expectancy are thought to be updated when an animal acquires or adjusts odor-reward associations (Rolls et al., 1996; Schoenbaum et al., 1998; Padoa-Schioppa and Assad, 2006), we hypothesized that theta-band rhythmicity is relevant for modification of these representations. To determine changes in spike-LFP phase-locking value across different stages of sessions in which odor-reward representations were modified, as expressed by changes in the behavior of the animal, we divided each session into four quadrants: the first and second half of the trials during the acquisition of odor-reward contingencies (pre-reversal period, Fig. 2.3A), and the first and second half of the trials after the reversal of odor-reward contingencies (post-reversal period, Fig. 2.3B).

In support of our hypothesis, we observed a significant increase in phase-locking during the waiting period from the first to second half of the pre-reversal period ($P < 0.001$, Fig. 2.3A). During the post-reversal period, we first observed a significantly lower value of theta-band phase-locking during the waiting period on early trials compared to the second half of acquisition ($P < 0.001$). As the animal acquired the reversed odor-outcome contingencies, theta-band phase-locking under the sucrose rewarded condition returned to pre-reversal levels. During the second half of the reversal period, theta-band phase-locking in this period was significantly higher compared to the first half ($P < 0.001$, Fig. 2.3B). These findings demonstrate that theta-band phase-locking during the waiting period does not reflect a fixed appetitive/aversive outcome dichotomy, but is related to the rat’s current outcome expectancy.

**Trial-by-trial timing of LFP theta power modulation**

Generally, spike-field phase-locking methods cannot be used on a trial-by-trial level because the number of spikes per trial is too low. To study the learning-related changes in theta-band oscillatory activity on a trial-by-trial basis, we instead examined the evolution of LFP theta power across trials in relation to fluid delivery. Typically, on acquisition Hit trials, the onset of the theta power increment was situated in the waiting period (Fig. 2.3C, upper).
On Hit trials immediately following reversal, reward delivery is unexpected and the onset of the theta power increment appeared in the reward delivery phase (Fig. 2.3D, lower), even though the behavioral sequence leading up to sucrose delivery is very similar. As the rat acquired the reversed odor-reinforcer contingencies, the onset of the theta power increment gradually returned to the waiting period (Fig. 2.3D, lower). For False Alarm trials, an opposite pattern was observed. During acquisition, the theta power increment was generally much weaker (Fig. 2.3D, upper). On early reversal
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False Alarm trials, the onset of the theta power increment was situated in the anticipatory period (Fig. 2.4C, lower), resembling acquisition Hit trials. Because these False Alarm trials followed sampling of the previously positive odor, the elevated theta power may reflect the not yet updated outcome expectancy pattern. On subsequent False Alarm trials, theta power gradually subsided, in line with the continued coupling of negative outcome to this odor. To quantify these observations across sessions, we calculated the center-of-mass (COM, where ‘mass’ is theta power) timing relative to fluid delivery, averaged across acquisition trials versus early reversal trials and separately for Hit and False Alarm trials (Fig. 2.3E, green and red). Indeed, the relation between COM timing before and after reversal for Hits and False Alarms is opposite in almost all sessions. On Hit trials, from acquisition (Fig. 2.3C, upper) to early reversal (post-reversal Hit trial 1-5, Fig. 2.3D, lower) the COM shifted from 0.072 ± 0.033 sec to 0.670 ± 0.033 sec (P < 0.001), while on False Alarm trials, the COM shifted in the other direction from 0.022 ± 0.075 sec to -0.106 ± 0.042 sec (P < 0.001). To specify this shift on a trial-by-trial basis, we defined the onset of the theta oscillation as the first moment when theta power crossed a threshold set at 50% of the maximum theta power on that trial. Regression analysis across sessions showed that, in the first 15 Hit trials post-reversal, theta onset shifted systematically to earlier moments with increasing trial number (slope: -0.066 sec/trial, p<0.05, Fig. 2.3F). These results confirm that theta-band oscillatory activity mirrors the dynamic updating of outcome expectancies during learning, although behaviors closely associated with expectancy also need to be taken into account (see below).

Phase-locking by Neuronal Groups with Different Task-related Firing Patterns

So far, we have pooled all neurons irrespective of their firing-rate selectivity for particular task phases and behavioral events. However, OFC cells are known to exhibit a wide range of firing-rate modulations in relation to task events and a subset of OFC cells shows firing-rate sensitivity to actual and predicted reward (Schoenbaum et al., 1998; Wallis and Miller, 2003; Padoa-Schioppa and Assad, 2006; van Duuren et al., 2008, 2009). Because strong theta-band phase-locking during the waiting period becomes a reliable predictor of reward with learning, we predict that this property is predominantly expressed by those cells that show outcome selectivity in
Figure 2.8 Frequency of licking and spike-field phase-locking per period

(A) Mean spike-field phase-locking values (+S.E.M.) and mean licking frequency (+S.E.M.) for sucrose waiting periods (green) and sucrose consumption periods (orange) are plotted. These values show a double dissociation. (B) Like (A), but now quinine waiting periods (red) and intertrial interval visits to the fluid well (blue) are plotted.

Table 2.1 Distribution of behavioral firing-rate correlates across the recorded population of cells

<table>
<thead>
<tr>
<th>Category</th>
<th>&amp; Odor Sampling</th>
<th>&amp; Movement Period</th>
<th>&amp; Waiting Period</th>
<th>&amp; Fluid Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor Sampling</td>
<td>43 (26) / 0.08</td>
<td>14 (6) / 0.03</td>
<td>9 (7) / 0.02</td>
<td>10 (8) / 0.02</td>
</tr>
<tr>
<td>Movement Period</td>
<td>14 (4) / 0.03</td>
<td>36 (12) / 0.07</td>
<td>9 (5) / 0.02</td>
<td>4 (0) / 0.01</td>
</tr>
<tr>
<td>Waiting Period</td>
<td>9 (2) / 0.02</td>
<td>9 (6) / 0.02</td>
<td>24 (12) / 0.05</td>
<td>9 (6) / 0.02</td>
</tr>
<tr>
<td>Fluid Delivery</td>
<td>10 (9) / 0.02</td>
<td>4 (2) / 0.01</td>
<td>9 (5) / 0.02</td>
<td>28 (20) / 0.06</td>
</tr>
</tbody>
</table>

The numbers in brackets in the leftmost column indicate total number of units with a behavioral correlate in that category (including double & triple correlates), thus amounting to more than 256 correlates. Numbers in parentheses indicate number of cells in correlate category that significantly discriminate between odors or outcomes. OD: odor sampling; MV: movement period; WT: waiting period; FD: fluid delivery.

Distribution of 256 significant task-related correlates from a sample of 201 out of 525 units. Rows show categories of task-related correlates, columns specify single and double correlates in absolute numbers and fractions of total cell number.
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their firing rates. To investigate this, we classified cells according to the task periods in which they showed firing-rate responses that were significantly different from baseline in four basic groups (Table 1 and Fig. 2.6). Often, cells exhibited firing rate correlates to more than one task period. To investigate the relationship between behavioral firing-rate correlates and phase-locking, we performed a multiple linear regression analysis to predict phase-locking values from the presence of one or more correlates per cell.

Of the four possible behavioral firing-rate correlates, only the presence of a firing-rate correlate during the waiting period for a given cell (W-cell) was a strong and positive predictor of theta-band phase-locking during the waiting period at 6 Hz (beta-weight regression coefficient \( \pm \) SE = 0.038 \( \pm \) 0.019 , \( p<0.05 \) two-sided t-test, Fig. 2.4A). Though it might seem obvious that the group of cells with a firing-rate correlate during the waiting period should also show the highest phase-locking value, this is not a trivial finding since for each cell, a fixed number of spikes was used to calculate phase-locking values, eliminating a bias towards higher phase-locking values due to high firing rate. Strikingly, when we restricted this analysis to the reward delivery period (Fig. 2.4B), only the W-cell category was positively correlated with phase-locking values during reward consumption (beta-weight \( \pm \) SE = 0.041 \( \pm \) 0.007, \( p<0.001 \)). These findings suggest that the increase in theta-band phase-locking is dynamically expressed in anticipation of and during actual delivery of reward by a select group of neurons that also express expected outcome information through differential firing rates.

**Trial-by-trial phase-locking per unit predicts outcome**

As phase-locking values cannot be meaningfully defined per trial, we could not determine if phase-locking values were a better predictor of upcoming outcome than single-cell firing rates. However, we could select the group of trials in which a single cell's firing rate suggested one outcome, when actually the other outcome was coming up (mismatch trials) and contrast the phase-locking values for this group with the values for the group of trials in which the firing-rate of a single cell correctly predicted the upcoming outcome (match trials). In this contrast, we included those cells that significantly predicted the upcoming outcome (using a Bonferroni correction for the total number of cells, see methods). To decode the predicted outcome per
cell per trial, we determined the mean firing-rate response in the waiting period for both quinine and sucrose delivery. Next, we used a leave-one-out decoding algorithm to predict for a single-cell trial response during the waiting period whether the actual fluid delivered was sucrose or quinine. This was determined by calculating the Euclidean distance of the single-cell’s firing rate on that trial to the mean firing rate in the training set of quinine and sucrose trials (the training set was defined as the set of trials remaining after removal of the single trial under study). Next, we pooled the phase-locking values per condition (match/mismatch) over cells (Fig. 2.4C). We hypothesized that the phase-locking value would be lower on mismatch trials than on match trials. As expected, we observed a higher phase-locking value (0.22 ± 0.02) for decoded trials with a correct prediction compared to decoded trials with an incorrect prediction (0.18 ± 0.01, P < 0.01, Fig. 2.4C). Thus, when the firing rates of single neurons correctly reflect the upcoming outcome, these cells are more strongly locked to the ongoing theta oscillation, possibly enhancing their effect on target cells. Conversely, when the outcome prediction associated with a unit’s firing pattern fails to match the upcoming outcome, the unit’s spikes on that trial are less phase-locked.

**Theta-band oscillatory activity in relation to licking behavior**

Thus far, theta-band phase locking has been examined in the context of reward expectancy. In rats, expectancy can be tightly coupled to direct motor consequences such as anticipatory licking (Neafsey et al., 1986; Gutierrez et al., 2006, 2010). We found no systematic relationship between individual lick responses and theta activity in LFP traces (i.e., individual licks were not systematically associated with theta oscillations). However, based on the observation by Gutierrez et al. (2006) that OFC spiking activity may correlate with clusters of lick responses, we checked whether the frequency of licking was associated with theta-band phase locking under the respective trial conditions (Fig. 2.8). The intensity of licking was higher during the sucrose delivery period than during the sucrose waiting period, yet phase-locking was selectively strong for the waiting but not the delivery period (cf. Fig. 2.2D). Moreover, licking activity was significantly more frequent during the quinine-anticipation period as compared to visits to the fluid well during intertrial intervals, yet the phase-locking was weaker during this anticipation period (cf. Fig. 2.2D). Although these findings do not preclude any relationship
between theta-band activity and licking (Gutierrez et al., 2010), they do suggest that theta-band phase locking is unlikely to be linked directly to the vigor or intensity of rhythmic motor activity expressed as licking.

**DISCUSSION**

To summarize, our results indicate that OFC neurons engage in strong theta-band phase-locking in anticipation of reward, mirroring the animal’s current expectation of positive outcome as measured by behavioral Go responses. Specifically, cells whose firing rate was selectively modulated in anticipation of reward showed the strongest phase-locking to the ongoing LFP theta oscillation during reward anticipation and delivery periods. When, on reversal of odor-outcome contingencies, established outcome expectancies were violated, theta-band phase-locking declined (Fig. 2.3B) and the onset of theta oscillations following the previously unrewarded odor now appeared in the reward delivery period, coinciding with delivery of unexpected reward (Fig. 2.3D,F). As the animals acquired the reversed odor-outcome contingencies, the onset of theta oscillations shifted back over trials to earlier moments in the waiting period (Fig. 2.3D, lower).

Although the exact anatomical origin of LFP theta oscillations recorded from the OFC is not known, the present data suggest that theta oscillations are, at least in part, locally generated, while other brain areas may also contribute. First, theta-band rhythm was not detectable during locomotion towards the reward site, in contrast to what is expected for a hippocampal source (Fig. 2.1C). Second, we found a strong alignment of theta-band spike phases per cell (Fig. 2.1D) as well as between single cell mean phases (Fig. 2.1E), suggesting that rhythmic spiking and synaptic activity at the population level contribute to the recorded LFP theta oscillations. Third, the results of the current source density analysis indicate the presence of a sink-source couple within the OFC, suggesting a local source of theta oscillations (Fig. 2.5A-C), although not precluding other, more remote sources. Finally, when we substituted the multi-unit spiking activity for the LFP as a reference to calculate phase-locking and when we calculated the frequency components of the autocorrelograms of composite multi-unit spike trains (cMUAs), spectrally specific differences in theta-band oscillatory activity between behavioral conditions remained present (Fig. 2.7). Multi-unit activity originates from a much smaller tissue volume than the LFP (Mitzdorf, 1987; Logothetis,
in addition to correlating with reward expectancy, the question arises whether theta-band phase-locking is also related to licking behavior (Gutierrez et al., 2006, 2010). Unlike licking, which occurred across both waiting and delivery periods, theta-band phase-locking was selectively enhanced only during the sucrose anticipation period (Fig. 2.2D). Theta-band phase-locking and the frequency of lick responses were dissociated between sucrose anticipation and consumption periods (Fig. 2.8A). Moreover, during the quinine anticipation period in False Alarm trials the frequency of lick responses was higher than in intertrial intervals, whereas phase-locking was weaker (Fig. 2.8B), again indicating a dissociation. Thus, phase-locking does not appear to correlate primarily to licking behavior, but a more global linkage certainly may exist because reward expectancy can be considered a primary drive to initiate licking. Synchronized firing across OFC, amygdala and agranular insular cortex may arise in conjunction with licking-related activity (Gutierrez et al., 2010), which however does not contradict a concurrent role in reward expectancy coding. Such an interleaving of rhythmic movement and information coding has been noted before in hippocampus, somatosensory and olfactory structures, where theta activity correlates with locomotion, whisking and respiratory rhythms, respectively (Vanderwolf, 1969; Lisman, 2005; O’Keefe and Burgess, 2005; Kleinfeld et al., 2006; Verhagen et al., 2007).

The present findings may have significant implications for our understanding of neural systems mechanisms for processing and acquiring reward information in the brain. Analysis of reward processing has thus far focused on firing-rate modulation by prediction or delivery of reward (Schultz et al., 1997; Schoenbaum et al., 1998; van Duuren et al., 2008), and thus the temporal structuring of reward-related firing patterns remained unknown. Phase-locking of OFC firing, as described here, may facilitate the temporal alignment of OFC output with firing in target areas, such as the amygdala, striatum and neocortical sensory-associational areas (Carmichael and Price, 1995b; Schilman et al., 2008), and enhance the impact of this synchronized output during selective phases of the oscillation (Salinas and Sejnowski, 2003), therefore spike-MUA phase-locking and cMUA autocorrelation are measures local to the OFC. Thus, our results demonstrate that during reward expectation, the spiking output of the OFC not only shows theta-band rhythmicity, but also phase-locks to locally recorded neural mass activity.
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2000; Engel et al., 2001). This impact may well depend on phase consistency between theta oscillations in OFC and in target areas themselves (Chrobak and Buzsáki, 1998; Hyman et al., 2003; Womelsdorf et al., 2007; Schroeder and Lakatos, 2009). Theta-band phase-locking may thus facilitate the communication between areas involved in reward-dependent learning.

Recently, Schoenbaum et al. (2007) suggested that outcome-expectancy representations in OFC may serve to update and modulate learned associations stored in amygdala and related structures by comparing expected to actual outcomes. We observed a sustained theta-band activity during reward expectancy and delivery, expressed by elevated theta power and phase-locking specific to outcome-selective cells. Previously, neural activity in amygdala and mPFC was shown to synchronize in the theta band (Pape et al., 1998; Siapas et al., 2005; Paz et al., 2008). We hypothesize that the synaptic updating process in OFC and related circuits underlying modification of stored associations may rely on spike-timing dependent plasticity (STDP; Levy and Steward, 1983; Cassenaer and Laurent, 2007) during coherent theta-band activity, which could be boosted by repetitive licking (Gutierrez et al., 2006). Under this hypothesis, the firing patterns phase-locked to OFC theta oscillations, convey a synchronized reward expectancy signal to target areas, which may or may not be continued into the period of the actual outcome, with significant consequences for learning. For example, when the outcome fails to meet the predicted signal (as in reversal learning), theta oscillatory activity in the delivery period collapses (Fig. 2.3C). Although this collapse was observed for theta-band LFP power, it can be safely assumed that the synchronization in spiking output is reduced at the same time, which may lead to a net weakening of synapses between OFC and target cells, consistent with STDP principles (Abbott and Nelson, 2000; Hayashi and Igarashi, 2009). Predictive synchronized firing after sampling of the other odor, previously coupled to quinine but now to reward, is regenerated at the moment of unpredicted reward (Fig. 2.3D,F), which may selectively strengthen another set of synapses onto target cells. During early reversal, the onset of oscillatory activity shifted to progressively earlier moments in the trial, in broad agreement with models of Temporal Difference (TD) learning (Schultz et al., 1997; Sutton and Barto, 1998). This class of Reinforcement Learning (RL) models posits the existence of a signal representing the error in reward prediction, as in classic conditioning theory.
This signal shifts backwards in time across learning trials towards the earliest reward-predicting event available to the subject. Despite this global similarity, our findings add two novel elements. First, the theta rhythm may implement a timing or ‘clocking’ system that provides temporal structure to value signals. Such a timing system may fill in an important gap in RL models that have been underconstrained as to how time steps may be neurally implemented (cf. Schultz et al., 1997). Second, in contrast to the transient nature of error signals, theta-band phase-locked activity conveys value signals in a sustained manner as they come to precede reward delivery. Thus, this type of value signal may complement an early and transient reward prediction error signal emitted by mesencephalic dopamine neurons after learning (Schultz et al., 1997). As compared to the quantities encoded in TD-learning and other RL models, the theta oscillatory activity in OFC resembles a Value function V(t), reflecting current reward predictions through time (cf. Pennartz, 1997; Niv and Schoenbaum, 2008). Because the theta oscillatory signal in OFC is expressed as a neural mass signal, it may exert vast and overriding effects on activity in its target structures. Although this hypothesis awaits further investigation, the current findings provide strong support for a role of the OFC in the temporal coordination of signaling expected and actual rewards, partly in conjunction with its role in rhythmic licking.

Future experiments may test the causal role of OFC theta oscillatory activity in the acquisition and reversal of learned associations, for instance by local infusions of substances that were shown to alter oscillations in other brain structures (e.g. GABA-B and muscarinic receptor antagonists [Leung and Shen, 2007] as well as blockers of Iₜ [Glasgow and Chapman, 2008]). Second, further experiments may examine whether information on negative outcome predictions is also temporally structured by way of oscillatory activity, and how positive predictions about non-ingestible rewards are coded. Aversion- or punishment-related information may be coded outside the region of OFC currently studied, for instance in anterior cingulate cortex (Johansen et al., 2001) or non-dopaminergic cell groups of the VTA (Ungless et al., 2004). Another possibility explaining the lack of theta-band phase-locking during Go responses prior to quinine is that False-alarm responses are generated by habit systems and in the absence of a clear negative expectancy, which if present would lead the animal to refrain from making a response that will be
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punished. Classical conditioning studies with an aversive outcome may help resolve this matter.

SUPPLEMENTAL MATERIAL

Silicon probe recordings & Current-source density analysis

For the experiments described in figure 2.5, we used two additional Wistar rats in the same weight and age group as the rats in the main experiment. Rat were trained on the same task, and implanted with a modified microdrive holding a 32-site linear silicon probe (NeuroNexusTech, Ann Arbor, MI, USA) with 1250μm² circular sites spaced 100 μm. We calculated the fluctuation of theta power per site as a function of time around reward delivery by calculating the baseline-corrected power of the LFP, filtered between 4-12 Hz, in 500 ms time windows in time steps of 50 ms. Next, we averaged z-scored power modulations, determined relative to baseline theta power during the inter-trial interval, across the number of reward delivery events to obtain an average time-site representation of theta power for a given event (Fig. 2.5A).

One-dimensional current source density (CSD) of the 4-12 Hz band-pass filtered LFP traces was calculated per event (Fig. 2.5B), using the formula

\[ CSD(z) = \frac{2\phi(z) - \phi(z + \Delta z) - \phi(z - \Delta z)}{(\Delta z)^2} \sigma(z) \]

(1)

where \( CSD(z) \) is the current source density at depth \( z \), in arbitrary units, \( \phi(z) \) is the recorded LFP at depth \( z \), \( \Delta z \) is the depth interval (200 microns, because only every other site was used to construct the CSD) and \( \sigma(z) \) is the conductivity in the \( z \)-direction, assumed constant.

Histology

After the final recording session, current (25 μA for 10 seconds) was passed through one lead per tetrode to mark the endpoint of the tetrode with a small lesion. The animals were deeply anesthetized with Nembutal (sodium pentobarbital, 60 mg/ml, 0.9 ml i.p.; Ceva Sante Animale, Maassluis, the Netherlands) and transcardially perfused with a 0.9% NaCl solution, followed by a 4% paraformaldehyde solution (pH 7.4 phosphate buffered). Following immersion fixation, coronal sections of 40 um were cut using a vibratome and stained with Cresyl Violet to reconstruct tetrode tracks and localize the
Histological verification of the tetrode endpoints and recording tracks showed that all recordings were performed between 3.2 and 4.2 mm anterior of bregma and confined to the ventral and lateral aspects of the OFC (fig. 2.5C-E).

Classification of neurons according to behavioral correlates of firing rate

Behavioral correlates of firing-rate changes were assessed by constructing peri-event time histograms (PETHs) synchronized on task events (i.e., on- and offset of odor sampling, fluid well entry, reward delivery). Firing rates in time bins (100 ms) around the event of interest were each compared against firing rates in 5 control bins from the ITI on a trial-by-trial basis to exclude within-session drift of firing rate as confounding factor and tested for significance using the non-parametric Wilcoxon’s matched-pairs signed-rank (WMPSR) test (p<0.01). Binned firing rates were considered significantly modulated in relation to the task event only if the test bin of interest differed significantly from all 5 baseline bins (van Duuren et al., 2007a). Cells were classified into four categories based on the task period in which they exhibited significant firing-rate changes (for odor sampling: change in relation to odor onset and during odor delivery; movement: change after odor offset but before fluid poke onset; waiting: change after fluid poke onset but before outcome; outcome: change following application of sucrose or quinine solution, Fig. 2.1). See table 1 for the distribution of neurons across categories and examples of units in each category.

Theta-band phase-locking and oscillatory spiking activity of OFC multi-unit activity: Spike-MUA phase-locking

To investigate whether theta-band rhythmicity is also present in the local spiking activity per se, we used the high-pass filtered (600-6000 Hz) multi-unit spike train (as a point process) instead of the LFP to form spike-MUA pairs and calculate phase-locking of units. Artifacts were deleted from the multi-unit activity by a template-matching algorithm incorporated in the MClust software and, as in spike-LFP analyses, we only used the multi-unit activity from the electrodes on which our unit was not recorded. Spike-phaselocking was now calculated using equations (1-3), except that the signal \( x_i(t) \) now represents the multi-unit spiking activity instead of the LFP. Again, we found spectrally specific phase-locking during the waiting period that was significantly higher
in the waiting period before sucrose delivery, compared to the waiting period before quinine delivery ($P < 0.01$ WMPSR, fig S3A). As predicted from earlier literature (Zeitler et al., 2006), the obtained spike-MUA phase-locking values were much lower than the spike-LFP phase-locking values. This is because we measure population phase from a very sparse sample (only the spiking output of the neurons around the electrode) of the actual population activity.

Lumped time-resolved autocorrelograms
We combined the spike trains of isolated single units from each tetrode in composite multi-unit signals (cMUAs) to assess the rhythmicity of the locally recorded spikes. To this effect, we computed autocorrelograms of the spike trains of these cMUAs restricted to a behavioral period by entering only spikes recorded in that period as the triggers for the autocorrelogram. The analysis was restricted to those cMUAs constructed from at least 2 isolated single units, with $N > 600$ spikes in each behavioral period that was considered (sucrose waiting period, quinine waiting period, inter-trial interval), yielding $N = 47$ eligible cMUAs. The autocorrelograms were constructed using bins of 10 ms, extending 500 ms in either direction of time lag zero (see Fig. 2.7B). For comparison, the autocorrelograms were normalized to an area of 1. Next, we computed power spectra of the Fourier-transformed autocorrelograms for each cMUA and compared these across behavioral conditions (Fig. 2.7C). A group analysis revealed significantly higher theta-band rhythmicity in the waiting period before sucrose compared to both the waiting period before quinine and the inter-trial interval (Fig. 2.7D).

Frequency of licks and spike-field phase-locking are not positively correlated
When the animals entered the fluid well, these entries were registered by photobeam interruptions and stored on a computer dedicated to behavioral data acquisition. Licking responses were detected by a separate photobeam situated within the fluid well.