CHAPTER 5

PHASE-AMPLITUDE COUPLING IN RAT ORBITOFRONTAL CORTEX DISCRIMINATES BETWEEN CORRECT AND INCORRECT DECISIONS DURING ASSOCIATIVE STIMULUS-OUTCOME LEARNING

M. van Wingerden\textsuperscript{1,2}, R. van der Meij\textsuperscript{3}, E. Maris\textsuperscript{3}, T. Kalenscher\textsuperscript{2}, C.M.A. Pennartz\textsuperscript{1,4}

\textsuperscript{1}Center for Neuroscience, Faculty of Science, University of Amsterdam, Postal Box 94216, 1090 GE Amsterdam, the Netherlands
\textsuperscript{2}Institute of Experimental Psychology, Heinrich-Heine University Düsseldorf, Universitaetsstrasse 1, D-40225 Düsseldorf, Germany
\textsuperscript{3}Centre for Cognition; Donders Institute for Brain, Cognition and Behaviour Postal Box 9104; 6500 HE Nijmegen; The Netherlands
\textsuperscript{4}Cognitive Science Center Amsterdam, University of Amsterdam, Postal Box 94216, 1090 GE Amsterdam, the Netherlands

(submitted)
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ABSTRACT

Cross-frequency interactions in local field potentials (LFPs) are thought to support communication between brain structures. Here, we investigated theta (4-12 Hz) to gamma (30-100 Hz) phase-amplitude coupling in LFP recordings from rat orbitofrontal cortex. Across subsequent phases of an olfactory discrimination task, theta-to-gamma phase-amplitude coupling (PAC) was highest during the odor-sampling phase that preceded a go/no-go decision. This task-dependent modulation was not explained by oscillatory power. We found that PAC magnitude increased over trials for the subset of odor sampling periods that led to correct, but not incorrect decisions. Moreover, with learning, PAC became significantly larger for correct vs. incorrect trials. In conclusion, OFC theta-gamma PAC magnitude correlates with correct decision-making, which is based on the generation and utilization of stimulus-based outcome predictions.
INTRODUCTION
The orbitofrontal cortex (OFC) plays a key role in the adjustment of ongoing behavior, guided by stimuli in the external world and an animal’s current motivational state. It is thought to fulfill this role, at least in part, by representing the current relative values of single cues or cue configurations. In particular, OFC is suggested to update value representations when rules linking stimuli to rewarding or aversive outcomes are changed, as both humans and animals with orbitofrontal damage show impairments in integrating changes in the associative value of stimuli in their choice behavior (Mishkin, 1964; Rolls et al., 1994; Baxter et al., 2000; Schoenbaum et al., 2002; Bohn et al., 2003a; Chudasama et al., 2003; Fellows and Farah, 2003; Pennartz et al., 2011a). Moreover, OFC spike patterns reflect the relative value of cues in a context-dependent manner, both at the single unit and population level (Schoenbaum et al., 1998; Tremblay and Schultz, 1999; Hikosaka and Watanabe, 2000; Wallis and Miller, 2003; Padoa-Schioppa and Assad, 2006; van Duuren et al., 2008, 2009; Sul et al., 2010; Wallis and Kennerley, 2011). Although the OFC receives afferent connections from all sensory modalities, it has no direct output to primary motor structures (Cavada et al., 2000; Ferry et al., 2000; Öngür and Price, 2000; Schilman et al., 2008; Groenewegen and Uylings, 2010; Hoover and Vertes, 2011). To influence behavior, it is hypothesized that OFC transmits expected outcome information to cortical and subcortical structures such as the anterior cingulate (ACC), basolateral amygdala (BLA), striatum and autonomous brain stem centers, where it is used to modify stimulus-outcome, action-outcome or stimulus-action mappings, for instance by contributing to model-based reward predictions (e.g. stimulus-specific instead of cached value predictions) and prediction errors (Hare et al., 2008; Rolls et al., 2008; Schoenbaum et al., 2009, 2011; Takahashi et al., 2009; Pennartz et al., 2011b; Wallis and Kennerley, 2011; McDannald et al., 2012). In support of a cross-structural influence of OFC output, Stalnaker et al. (2007) showed that OFC lesion-induced impairment on a reversal task was alleviated by either lesioning or temporarily inactivating the ipsilateral BLA.

Efficient cross-structural communication is thought to require a mechanism by which the excitability of structures involved in the transfer of information is synchronized, which may at the same time allow Hebbian modification of synapses linking neurons in an associative network or assembly. Mass neural activity, as observed in extracellularly recorded local
field potentials (LFPs), exhibits oscillatory patterns in different frequency bands that covary with neuronal excitability and can become coherent with LFP rhythms in remote structures, especially in low-frequency bands (Varela et al., 2001; Buzsáki and Draguhn, 2004; Fries, 2005; Canolty et al., 2006, 2010; Womelsdorf et al., 2007; Schroeder and Lakatos, 2009; Canolty and Knight, 2010). Interactions between lower and higher oscillatory frequencies have been found in the form of phase-amplitude coupling (PAC) where the phase of slower oscillations, reflecting general excitability of networks, modulates the amplitude of high-frequent oscillations.

PAC has been described most extensively in humans (Canolty et al., 2006; Osipova et al., 2008; Cohen et al., 2009a; Maris et al., 2011), but has also been studied in rodents, especially in the hippocampus where the phase of theta oscillations (4-12 Hz) modulates gamma (30-100 Hz) oscillation amplitude (Bragin et al., 1995; Chrobak and Buzsáki, 1998; Lisman, 2005; Colgin et al., 2009). PAC may also occur across structures, such as the hippocampus and striatum, or hippocampus and neocortex (Sirota et al., 2008; Tort et al., 2008). Recent reports have related PAC in hippocampus to context-based rule retrieval (Tort et al., 2009) and, within neocortex, to working memory (Fujisawa and Buzsáki, 2011). However, it remains unclear whether and how PAC is involved in associative stimulus-reward learning, involving either cortical or subcortical structures.

We quantified PAC using weighted phaselocking factors (wPLFs, Maris et al., 2011; van der Meij et al., 2012) and found event-related PAC between theta and gamma frequencies in recordings from the orbitofrontal cortex (OFC) of rats engaged in a 2-odor associative stimulus-outcome learning task. wPLF magnitudes were largest during the cue-sampling period, in which the rat learned to discriminate between stimuli predicting positive versus negative outcome. PAC increased in a learning-dependent fashion and was significantly stronger when it preceded correct vs. incorrect decisions.

**EXPERIMENTAL PROCEDURES**

**Behavioral Task**

The general behavioral paradigm and recording methods have been reported earlier (van Wingerden et al., 2010a, 2010b). Briefly, we recorded LFPs and single unit data from 3 male Wistar rats engaged in an olfactory decision task.
making task (10 sessions in total, Fig. 5.1). Contingent on rules linking stimuli to outcomes, animals decided to make a ‘Go’-response (locomotion to fluid well, waiting and subsequent outcome delivery) or a ‘NoGo’-response (refraining from entering the fluid well for 5 sec. post-stimulus). Upon entering the fluid well, the delivery of outcome was delayed by 1000 ms to probe neuronal correlates of expected outcome in the absence of overall body movement. Novel odorants were used on each session. Go/NoGo trials were pseudorandomly ordered in blocks of five plus five trials. Acquisition of task rules was indexed by a behavioral criterion, measuring the proportion of correct decisions (Hits & Correct Rejections) over a 20-trial moving window.

Recording Equipment
Using a 64-channel Neuralynx system (Bozeman, MT), we recorded from an array of 11 tetrodes (Gray et al., 1995), with a reference electrode placed in the supplementary motor cortex dorsal to OFC. LFP signals were extracted from each tetrode by lowpass filtering the continuously sampled (at 1874 Hz) signal between 1 and 475 Hz. Events in the behavioral task were co-registered and time-stamped by the Cheetah system. Histological verification indicated that most recordings were made from ventral and lateral orbitofrontal (VO/LO) and agranular insular (AI) cortex with some spread into dorsolateral orbitofrontal (DLO) cortex (Paxinos and Watson, 2007).

Phase-amplitude coupling
To quantify phase-amplitude coupling (PAC) between different frequencies recorded from OFC LFPs, we created weighted phase-locking factors (wPLFs; van der Meij et al., 2012). A wPLF was computed by convolving the LFP signal in a 600 ms window with complex-valued wavelets having a length of 3 cycles for each frequency, multiplied with a Hanning taper of equal length. Only those frequencies for which the wavelet had an integer number of samples per cycle were obtained, yielding a frequency resolution of about 1 Hz for the frequencies up to 20 Hz and a resolution of about 4 Hz for the frequencies up to 100 Hz. Next, we entered the obtained complex-valued signal, incorporating phase and amplitude, from one electrode (ch1) and the amplitude-envelope from another electrode (ch2) into the wPLF function. The wPLF is itself complex-valued, and represents a normalized index of the consistency of the relation of amplitude from ch2 with the phase of ch1.
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over trials, and the preferred coupling phase, separately for all frequency combinations.

Selection of physiologically relevant phase-amplitude coupling
We were interested in the possible interaction of theta-band frequencies (6-10 Hz) and gamma-range rhythms (40-90 Hz). Therefore, we sorted the channels per session according to theta power, averaged over trials in the time window of interest, and separately, also according to averaged gamma power. We selected the four channels with highest theta and gamma power as phase or amplitude-providing channels per session, respectively. Subsequent wPLF magnitudes are only reported for between-channel pairs. For each session, we selected the frequency pair from the range of frequency combinations of interest ([6-12 Hz; theta] and [40-90 Hz; gamma]) with the highest wPLF magnitude, averaged over all channel combinations in a session.

Selection of optimal PAC window
Based on previous research, we hypothesized that theta-gamma PAC would be most prominent in the early odor sampling period, where increases in oscillatory power and spike-field synchronization in these frequency bands coexist (van Wingerden et al., 2010b). To determine an optimal window for detecting wPLF magnitude, we used a sliding window approach, calculating magnitudes per session in blocks of 600 ms. We next moved this analysis window from -1.4 to +1.4 seconds relative to odor onset, yielding 12 time windows centered on time points from -1.1 to +1.1 seconds stepped in 200 ms increments (Fig. 5.2A). We narrowed this period down to the time window that resulted in the highest wPLF magnitudes, averaged across sessions and for both odor exemplars (selected window: centered at +0.5 seconds after odor onset, spanning from +0.2 to +0.8 seconds after odor onset).

Comparisons between trial types
As a first step, whole session averages were constructed for wPLF magnitude, per trial type. These averages were pairwise compared across sessions between trial types using Wilcoxon’s Matched-Pairs Signed Rank test (WMPSR, Fig. 5.2B).

For the investigation of changes in wPLF magnitude within a session, we first created a time-series of magnitudes by averaging across a sliding
window block of 4 trials. Next, these smoothed averages were binned in quintiles of session length (i.e. first, second, third, fourth & last 20% of trials). Differences across trial types and quintiles were first assessed with a repeated measures ANOVA using trial type and quintile as within-subject factors. To make pairwise comparisons between trial types within a quintile, we performed a bootstrapped permutation test of Wilcoxon’s Matched-Pairs Signed Rank statistics. Basically, for N = 5000 repetitions, we randomly permuted the trial type label in a pairwise comparison for each session, yielding a mix of original and reversed labeling across sessions per bootstrap. For these permuted paired distributions, we calculated a WMPSR test, retaining the Z value (reflecting the test statistic using an approximation to the normal distribution). Finally, the Z value of the WMPSR test using the full original trial type labeling was compared against this permutation distribution (Fig. 5.3A).

Controlling for fluctuations in theta power in phase-providing channels
Because the wPLF magnitude parameter, and indeed any PAC measure, depends on accurate estimation of the phase of the phase-providing frequency, power differences in the phase-providing frequency between trials or conditions under comparison will affect the signal to noise ratio (SNR) of the estimated phase. Using the same quintile approach, we compared theta power between trial types and trial blocks within sessions, averaging the theta power over the four selected channels and across the selected frequencies (6-12 Hz, Fig. 5.3B,C).

Within-session changes in wPLF magnitude
To compare within-session changes in wPLF magnitude across sessions, we Z-scored these magnitudes per trial type (Hit, False Alarm, Correct Rejection; Misses rarely occurred and were not further analyzed, Fig. 5.4) to reflect relative changes. These Z-scored magnitudes were again binned in quintiles. Changes in Z-scored magnitudes across sessions were assessed by computing Pearson’s Correlation coefficients.
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RESULTS
We recorded LFP signals from the OFC of 3 rats engaged in a 2-odor discrimination associative learning task (Fig. 5.1A). In previous research we found increased spectral power and spike-field rhythmic synchronization in the theta (6-12 Hz) and gamma (40-90 Hz) frequency bands, compared to baseline, specifically during the sampling of outcome-predictive odors in this task (van Wingerden et al., 2010a, 2010b). Therefore, we asked whether a systematic relation between theta phase and gamma amplitude exists during odor sampling, and whether this relationship is modulated by associative learning, and correlates with decision-making performance.

Theta-gamma PAC occurs time-locked to odor onset
To quantify the dynamics of phase-amplitude coupling, we calculated between-channel phase-amplitude coupling (PAC) using weighted phase-locking factors (wPLFs; van der Meij et al., 2012) per session, in time windows of 600 ms time-locked to odor onset, separately for odor sampling periods using stimuli predicting an appetitive outcome (S+ odors predicting sucrose solution) or aversive outcome (S- odors predicting quinine solution). Fig. 5.1 shows the color-coded magnitude of the wPLF bispectrum for an example session, averaged over all trials and selected between-channel combinations. The upper row shows the bispectra for the periods of sampling odor predicting sucrose (S+, “positive odor”), while the lower row depicts the sampling periods related to the odor predicting negative outcome (S-, “negative odor”). Fig. 5.2A shows the time course of wPLF magnitude, averaged across sessions and relative to odor onset, for S+ trials (blue, sucrose outcome) and S- trials (red, quinine outcome). Both time courses peak around 500 ms into the odor-sampling period and decay to levels not significantly above pre-odor sampling baseline periods after odor offset. Hereafter we will use this time window, ranging from 200 to 800 ms after odor onset.

Channel selection and frequency pair selection for theta-gamma PAC
We defined frequencies of interest (FOI) to detect PAC between theta and gamma frequency bands based on our previous results. Briefly, we sorted channels on theta-band and gamma-band spectral power. Channel combinations between the channels with the highest theta and highest gamma...
power were used to calculate between-channel wPLFs (max. 16). Next, we selected the 2x2 frequency block in the theta (phase-providing) x gamma (amplitude providing) frequency bispectrum with the highest averaged wPLF magnitude as a FOIxFOI window per session. Further results are reported as wPLF magnitudes averaged over this FOIxFOI window.

**Theta-Gamma PAC is stronger for S+ compared to S- odor sampling**

We next analyzed whether differences in wPLF magnitude could be observed between trial types. When we compared S+ versus S- odor sampling
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periods within sessions, we found significantly higher wPLF magnitudes for S+ trials compared to all S- trials (Wilcoxon’s matched-pairs signed rank test, p<0.05, Fig. 5.2B). Furthermore, this result could be unpacked in a significant difference between Hit trials (S+ trials with a Go response) and False Alarm trials (S- trials with Go response, p < 0.05 WMPSR test), but not Correct Rejections (S- trials with a NoGo response; False Alarms vs. Correct Rejections: not significant).

PAC becomes stronger with learning for correct, but not incorrect trial types

Next, we analyzed if wPLF magnitudes are sensitive to learning within sessions. To compare within-session changes in wPLF magnitude between sessions comprising unequal trial counts, we divided all trials for each trial type in quintiles per session, and averaged the wPLF magnitude over the trials within a quintile. Next, we averaged these quintile magnitudes over sessions, and compared trial types. A repeated measures ANOVA with trial type and quintile as within-subject factors yielded significant main effects for both trial type ($F_{2,18} = 5.05; p < 0.05; \text{partial eta-squared } 0.36$) and quintile ($F_{1.73,15.56} = 7.06$ (Greenhouse-Geisser corrected); $p < 0.01; \text{partial eta-squared } 0.44$) while the interaction effect just missed significance ($F_{8,72} = 2.06; p = 0.051; \text{partial eta-squared } 0.19$). Simple within-subject contrasts for trial type revealed that Hit trials differed significantly from False Alarm Trials, but not Correct Rejections ($p < 0.05, p = 0.19 \text{ pairwise comparison [LSD corrected]}$), while there was a trend for False Alarm Trials and Correct Rejections to differ ($p = 0.052$). Simple within-subject contrasts for quintile revealed that Q4 and Q5 differed significantly from Q1 ($p < 0.05, p < 0.01 \text{ pairwise comparison [LSD corrected]}$), while there was a trend for False Alarm Trials and Correct Rejections to differ ($p = 0.052$). For the interaction term, simple contrasts showed a significant difference for Hit vs. FA trials in Q5 compared to Q1 ($p < 0.05 \text{ pairwise comparison [LSD corrected]}$). To confirm this latter finding using non-parametric statistics, we performed a bootstrapped permutation test of pairwise Wilcoxon’s matched-pairs signed rank statistics between trial types in quintile 5. This analysis showed that direct comparisons between trial types were significant in the last quintile (Hits larger than FA, $p < 0.01$, Hits larger than CREJ, CREJ larger than FA; both $p<0.05$ bootstrapped permutation WMPSR, Fig. 5.3A).
Because the wPLF magnitude calculation depends, in part, on an accurate estimation of the phase of theta frequency, fluctuations in theta power could exert a systematic influence on PAC measures. However, we found that larger wPLF magnitudes did not correlate with larger theta power. Fig. 5.3B shows that theta power did not vary systematically between trial types in any quintile (WMPSR, n.s.).

To exclude spurious effects in the analysis of Fig. 5.3B based on differences in LFP power level between sessions, we Z-scored theta power per session and examined the change in Z-values, averaged across sessions, over quintiles. We observed a negative trend in normalized theta power, suggesting that the increase in wPLF magnitude across quintiles is not due to better phase estimation in LFP epochs with higher theta power (Fig. 5.3C).
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Fig. 5.3A shows a positive trend in wPLF magnitude for some trial types (Hits, Correct Rejections); however, differences in wPLF magnitude between sessions could potentially have influenced this result. Therefore, we proceeded to analyze changes in normalized (Z-scored) wPLF magnitudes within sessions, to assess whether PAC correlated with learning. To analyze within-session changes in wPLF magnitude per trial type across sessions, we z-scored wPLF magnitudes per session, separately for each trial type. These z-scored magnitudes, pooled across sessions, showed a linear increase over quintiles for Hits and CREJ but not FA trials (Pearson correlation coefficients: 0.69 (Hits, p<0.001), 0.48 (CREJ, p<0.001), 0.12 (FA, n.s.); Fig. 5.4). In conclusion, we found that PAC became stronger across learning trials, but only for correct (Hits and Correct Rejections) and not incorrect decisions.

DISCUSSION

We have shown here that phase-amplitude coupling (PAC) between theta (6-12) and gamma (40-90 Hz) activity exists between LFPs recorded from rat OFC. Previously, we identified rhythmic mass activity and spike-field synchronization in theta and gamma bands in the context of this behavioral task (van Wingerden et al., 2010a, 2010b), but the relationship between these rhythms remained unknown. In our olfactory discrimination task, where rats learn the association between a stimulus (S+) predicting positive outcome and a stimulus (S-) predicting negative outcome, theta-gamma PAC appeared time-locked to odor sampling (Fig. 5.1).

When the time-window exhibiting maximal wPLF magnitudes (for both S+ and S- trials, 0.2-0.8 s after odor onset, Fig. 5.2A) was analyzed on a whole-session basis, we found that wPLF magnitude was greater for S+ than S- trials (Fig. 5.2B). More specifically, for Hit trials, (S+ trials with a Go response) wPLF magnitude was greater than on S- trials with False Alarm responses, but not Correct Rejection responses (Fig. 5.2B). wPLF magnitudes also changed within sessions: in the last, but not the first quintile, wPLF magnitudes were significantly higher for Hit trials compared to both False Alarm trials (FA, Fig. 5.3A) and Correct Rejections (CREJ). Moreover, Z-scored wPLF magnitudes increased significantly within sessions for Hits and CREJ trials, but not FA trials (Fig. 5.4). Importantly, these within-sessions changes did not correlate with raw or z-scored theta power (Fig. 5.3B-C).

To our knowledge, this is the first report that relates theta-gamma PAC
recorded in rodents to stimulus-outcome learning in a temporally discrete manner. Recent reports have shown PAC in relation to context retrieval in the hippocampus (Tort et al., 2009) and working memory processes in the medial prefrontal cortex (mPFC; Fujisawa and Buzsáki, 2011). Tort et al. (2008, 2009) described PAC within the striatum and hippocampus, also between striato-hippocampal channel pairs. In their data, within-hippocampal CA3 layer theta-gamma PAC was modulated by learning, and persisted with overtraining. Whereas PAC in our data was strongest in the absence
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of whole-body movement, Tort et al., using an item-in-context learning paradigm, found that PAC was maximal during active spatial exploration in an open field maze, which they related to context-based retrieval of a task rule. This hippocampal PAC, however, differs from the OFC PAC described here in that it declined sharply upon stimulus sampling (odorant sniffing). Fujisawa and Buzsáki (2011) showed that the phase of a 4 Hz slow oscillation modulated the amplitude of gamma-band frequencies during the working memory component of a T-maze task, within the mPFC. Our data differ from this study in showing theta-gamma PAC in the absence of locomotion; in addition, we show that our measure of PAC strength, weighted phase locking factor (wPLF) magnitude (van der Meij et al., 2012), not only increased with task acquisition (cf. Tort et al., 2009), but also discriminated between correct and incorrect decisions. False Alarm trials showed significantly lower wPLF magnitudes across sessions, and these error trials lacked the within-session increase observed in correct trial types (Hits & Correct Rejections). We therefore hypothesize that orbitofrontal theta-gamma PAC in this task is instrumental in supporting stimulus evaluation, the generation of outcome expectancies and subsequent decision-making processes.

In support of this hypothesis, we previously showed that particular subsets of OFC single units phase-lock preferentially to different frequency bands. For instance, odor-responsive cells, phase-lock to theta-band rhythms. In contrast, locomotion-related cells, conveying information on action-outcome associations, phase-lock preferentially to gamma-band rhythms, but only during the odor-sampling period (van Wingerden et al., 2010b).

The modulation of gamma power by theta phase in this behavioral period may be instrumental in forging links between units that are part of assemblies across different brain areas, representing stimulus-outcome and action-outcome associations through Hebbian, spike-timing dependent plasticity (Bi and Poo, 1998; Cassenaer and Laurent, 2007). Furthermore, synchronized firing patterns conveying reward-related information within OFC may be essential for efficient modification of stimulus-outcome and action-outcome associations in target areas (Hare et al., 2008; Rolls et al., 2008; Schoenbaum et al., 2009, 2011; Takahashi et al., 2009; Pennartz et al., 2011a; Wallis and Kennerley, 2011). The “nesting” of OFC gamma activity in theta cycles may be beneficial to convey this type of information to
remote target areas because remote structures may become synchronized especially by low frequencies such as theta-band (Schroeder and Lakatos, 2009; Benchenane et al., 2010; Womelsdorf et al., 2010; Fujisawa and Buzsáki, 2011). The superimposed OFC gamma rhythm, reflecting local processing (Fries, 2009; van Wingerden et al., 2010b) could then promote precise spike timing within assemblies, possibly enhancing cross-structural STDP-dependent associative processes and retrieval of stimulus-outcome mappings. A critical test for our hypothesis would be the selective disruption of PAC or its components, for example using optogenetic control of spike timing or gamma synchronization (cf. Cardin et al., 2009).