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# HOME-CAGE ODORS SPATIAL CUES ELICIT THETA PHASE/GAMMA AMPLITUDE COUPLING BETWEEN OLFACTORY BULB AND DORSAL HIPPOCAMPUS

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**Abstract—The brain oscillations may play a critical role in synchronizing neuronal assemblies in order to establish appropriate sensory-motor integration. In fact, studies have demonstrated phase-amplitude coupling of distinct oscillatory rhythms during cognitive processes. Here we investigated whether olfacto-hippocampal coupling occurs when mice are detecting familiar odors located in a spatially restricted area of a new context. The spatial olfactory task (SOT) was designed to expose mice to a new environment in which only one quadrant (target) contains odors provided by its own home-cage bedding. As predicted, mice showed a significant higher exploration preference to the target quadrant; which was impaired by olfactory epithelium lesion ($ZnSO_4$). Furthermore, mice were able to discriminate odors from a different cage and avoided the quadrant with predator odor 2,4,5-trimethylthiazoline (TMT), reinforcing the specificity of the SOT. The local field potential (LFP) analysis of non-lesioned mice revealed higher gamma activity (35–100 Hz) in the main olfactory bulb (MOB) and a significant theta phase/gamma amplitude coupling between MOB and dorsal hippocampus, only during exploration of home-cage odors (i.e. in the target quadrant). Our results suggest that exploration of familiar odors in a new context involves dynamic coupling between the olfactory bulb and dorsal hippocampus.** 

**Key words: familiar odor, TMT, olfactory bulb, hippocampus, cross-frequency coupling.**

## INTRODUCTION

Each animal has its own pool of volatile and non-volatile odors, combining to form an individual-specific ‘‘olfactory signature” (Carr et al., 1976; Sawyer et al., 1984; Matochik, 1988; Popik et al., 1991; Ferguson et al., 2002). Included in these social odors are urine, feces or other components, which may contain natural odors and pheromones. It is by processing these stimuli that rodents categorize conspecifics as familiar or unfamiliar, form hierarchic relations, perceive predators in the environment and evaluate potential sexual partners (Doty, 1986; Brennan, 2004; Keverne, 2004; Restrepo et al., 2004; Kavaliers et al., 2005). Odors are primarily translated in the olfactory epithelium and subsequently processed in the main olfactory bulb (MOB) (Popik et al., 1991; Bluthe and Dantzer, 1992). The MOB mitral cells project to several areas in the olfactory system, such as the piriform cortex, which is known to be involved in the storage and retrieval of memory traces (Barkai and Saar, 2001; Haberly, 2001). Furthermore, MOB projections are only two synapses away from connecting to the hippocampal dentate gyrus (Vanderwolf, 1992). Equally, the hippocampus (HIP) also sends projections back into the granule cell layer of the MOB: (a) directly from ventral hippocampus CA1 neurons, and (b) indirectly through the entorhinal cortex (van Groen and Wyss, 1990; Gulyas et al., 1998).

Odor processing in rodents is paramount to exploratory behavior used to search for food, conspecifics and potential sexual partners, as well as to avoid predators. Essentially, close functional cross talking between areas involved in both spatial memory and odor processing must exist for successfully triggering appropriate behavior which, in turn, brings evolutionary advantages. In fact, recent theories propose that individual odor-pattern recognition and appropriate sensory-motor integration rely extensively on bidirectional interactions between primary olfactory areas and hippocampal regions (Squire and Alvarez, 1995; Buzsaki, 1996; Nadel and Moscovitch, 1997; Eichenbaum, 2000; Wiltgen et al., 2004). Such interaction imposes temporal and/or phase constraints between areas, resulting in neural discharge synchronization;

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*Abbreviations:* CFC, cross-frequency coupling; dHIP, dorsal hippocampus; HIP, hippocampus; LFP, local field potential; MOB, main olfactory bulb; PFA, paraformaldehyde; PLV, phase locking value; SOT, spatial olfactory task; TMT, 2,4,5-trimethylthiazoline; vHIP, ventral hippocampus.

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which in turn leads to network plasticity and learning (Singer, 1993; Schaefer et al., 2006). In fact, it has been shown that the synchronization between the MOB and the ventral and dorsal parts of the hippocampus are related to the learning of new artificial odors (Martin et al., 2007). However, it is still unknown how these circuits are involved in the recognition of olfactory social information.

Local field potentials (LFPs) present many regular periodic patterns that may be divided into several oscillatory bands (Mitra et al., 2008). These bands have been shown to have straight correlation to specific behavioral states and/or sensory input (; Steriade and Hobson, 1976; Basar, 1980; Belitski et al., 2008). In fact, when recorded from different sites, such oscillators may dynamically couple (i.e. coherence, phase-amplitude modulation) during appropriate sensory-behavior integration (Varela et al., 2001; Fries, 2005, 2015; Senkowski et al., 2008; Tort et al., 2008); which has been proposed to play a paramount role in the emergence of neuronal assemblies (Buzsaki and Draguhn, 2004).

In the present study, we developed a new spatial olfactory task (SOT) in order to test the hypothesis that olfacto-hippocampal coupling occurs during exploration of familiar odors in a new context. The SOT was conducted on a cage divided in virtual quadrants, evenly distributed with bedding, but having only one of the quadrants containing bedding from the animal's home cage (target quadrant), without providing any other form of sensory cues (i.e. behavioral response abolished by transient-lesion specific to olfactory epithelium). Before the SOT, animals were allowed to habituate in the same environment with all quadrants having new cage bedding. Simultaneous video-LFP recordings from the MOB and hippocampus allowed the extraction of electrographic parameters at the exact time intervals animals were exploring different quadrants.

## EXPERIMENTAL PROCEDURES

### Subjects

Adult (8–12-week-old) Swiss male mice were purchased from the Animal Facility of the Universidade Federal de Minas Gerais (Brazil). Animals were maintained in groups of 3–5 per cage, under a 12-h light/12-h dark cycle in a climate-controlled environment with humidity of 55 ± 10% and temperature of 22 ± 2 °C (Alesco, Brazil). Filtered water and food were available *ad libitum*. All experiments were performed during the light phase of the cycle. The Ethical Committee for the Use of Animals (CEUA) in the Universidade Federal de Minas Gerais (protocol 42/2014) approved all experiments. CEUA directives are in compliance to NIH guidelines for care and use of research animals.

### Spatial odor task (SOT)

The task was performed in an open field (50 × 50 × 40 cm) located inside a Faraday cage evenly illuminated, though without any orientation clues. The box's floor was lined with 3 cm high of bedding and virtually divided into four quadrants: Target (TG), Right Adjacent (RA), Left Adjacent (LA) and Opposite (OP). The task consisted of two phases: habituation and test. During habituation, animals freely explored the open field during 10 min. Subsequently, the mouse was removed from the apparatus, while 100 $cm^3$ of new bedding (details explained below) was hidden underneath the clean bedding of a specific quadrant inside the box. Immediately after, the test session was initiated with the return of the animal to the box. The mouse was allowed to freely explore the open field for a period of 10 min. The total time of exploration in each quadrant was measured during both habituation and test phases. Exploratory behavior consisted of sniffing, digging and rearing. Between individual animal trials, the open field was cleaned and the bedding was changed; additionally, the quadrants and the relative position of the box in the room were randomized.

The experimental protocols listed next differ solely on the nature of the stimulus used to bait the TG during the test session. The control animals (Fig. 1A, C) used new clean bedding as stimulus. The familiar odors group (Figs. 1B, D and 2A) used bedding from the animal's own home cage (adapted from Arbuckle et al., 2015). In a different batch of animals, the TG was bait with a predator odor (Fig. 2D, E), well known to induce fear-related behavior in mice (reviewed by Fendt et al., 2005). The 2,4,5-trimethylthiazoline (TMT) was freshly prepared and diluted to 1% using deionized water (Hacquemand et al., 2010). The 100cm3 of new bedding was mixed with 1 mL of the 1% TMT solution and introduced into the TG immediately before the test session.

In addition, to evaluate whether mice would be able to discriminate familiar from new odors, we modified the original version of SOT by baiting TG and OP with two distinct sources of odors. The TG was baited with the familiar odor from the animal's home-cage, while the OP received bedding taken from a new Swiss adult male mice cage. The discrimination procedure then followed the same protocol used for previously described SOT.

All sessions were filmed and offline analysis was done using freeware computer rat-tracking software (X-Plo-Rat®; Laboratório de Comportamento Exploratório USP-RP Brazil). Exploratory behavior was analyzed by repeated measure 2-way ANOVA (Quadrant and Time as independent factors) followed by Bonferroni's *post hoc* test.

### Chemically induced anosmia

Intranasal administration of zinc sulfate was used as a chemically induced anosmia model. Animals were anesthetized with 5% isoflurane and then administered with 100 μL in each nostril of a solution containing 5% zinc sulfate (Anosmic group, $n = 6$) or saline (Control group, $n = 6$), according to previously published methodology (Harding et al., 1978; Burd, 1993). Between each nostril administration, animals were allowed for a 2-h recovery period (McBride et al., 2003). The mice were submitted to SOT at both 3 days (during anosmic period) and 4 weeks (after sensory recovering) after the procedure. Exploratory behaviors were analyzed by repeated