

Interactions between the Lateral Habenula and the Hippocampus: Implication for Spatial Memory Processes

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The lateral habenula (LHb) is an epithalamic structure connected with both the basal ganglia and the limbic system and that exerts a major influence on midbrain monoaminergic nuclei. The current view is that LHb receives and processes cortical information in order to select proper strategies in a variety of behavior. Recent evidence indicates that LHb might also be implicated in hippocampus-dependent memory processes. However, if and how LHb functionally interacts with the dorsal hippocampus (dHPC) is still unknown. We therefore performed simultaneous recordings within LHb and dHPC in both anesthetized and freely moving rats. We first showed that a subset of LHb cells were phase-locked to hippocampal theta oscillations. Furthermore, LHb generated spontaneous theta oscillatory activity, which was highly coherent with hippocampal theta oscillations. Using reversible LHb inactivation, we found that LHb might regulate dHPC theta oscillations. In addition, we showed that LHb silencing altered performance in a hippocampus-dependent spatial recognition task. Finally, increased coherence between LHb and dHPC was positively correlated to the memory performance in this test. Collectively, these results suggest that LHb functionally interacts with the hippocampus and is involved in hippocampus-dependent spatial information processing. *Neuropsychopharmacology* (2013) **38**, 2418–2426; doi:10.1038/npp.2013.142; published online 26 June 2013

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INTRODUCTION

The lateral habenula (LHb) is implicated in cortically driven functions such as the evaluation of motivational and reward values as well as the anticipation of motivational events (Bromberg-Martin *et al*, 2010; Matsumoto and Hikosaka, 2007, 2009), the selection of fear responses (Agetsuma *et al*, 2010; Okamoto *et al*, 2012), the integration of negative feedback information (Ullsperger and von Cramon, 2003), and attention (Lecourtier and Kelly, 2005). In addition, dysfunction of the habenula has been evidenced in psychiatric diseases, such as schizophrenia and depression (Hikosaka, 2010; Sartorius and Henn, 2007; Shepard *et al*, 2006). The current view is that LHb integrates information stemming from the basal ganglia and the limbic system to participate in the selection of appropriate behavioral strategies (Geisler and Trimble, 2008; Hikosaka, 2010).

More recently, a role in learning and memory has also been emphasized (Bromberg-Martin *et al*, 2010; Lecourtier

et al, 2004; Shumake *et al*, 2010). More precisely, lesion of the habenular complex induced spatial reference memory deficits in the water maze, during both acquisition and retention of the task (Lecourtier *et al*, 2004). These effects of habenular complex lesion mimic in multiple ways the results obtained after dorsal hippocampal (dHPC) lesion, indicating that LHb might functionally interact with hippocampal networks during spatial memory processes.

Information processing and storage by brain networks requires a high level of coordination between the multiple neuronal groups. One likely mechanism is through rhythmic activity of neuronal populations, which give rise to oscillations in local field potentials (Womelsdorf *et al*, 2007). Among these brain rhythms, the hippocampal theta oscillations have been described as a critical player in memory consolidation (Buzsaki, 2002). Disruption of theta rhythm results in spatial memory deficits (Winson, 1978) and restoration of theta-like rhythmicity restores learning capacities in rats (McNaughton *et al*, 2006). It is now well demonstrated that communication between brain areas involves phase synchronization (ie, coherence) of oscillations (Fries, 2005). Indeed, increases in theta coherence between the hippocampus and extra-hippocampal areas have been linked to memory performance in various tasks (Benchenane *et al*, 2010; Popa *et al*, 2010; Seidenbecher *et al*, 2003).

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Although evidence of interactions between LHb and dHPC is still missing, as there are no direct connections between the two structures, it is suggested by previous functional and electrophysiological studies. Using mapping of regional changes in brain metabolic activity, a functional correlation was shown to occur between LHb and dHPC during fear conditioning (Gonzalez-Pardo *et al*, 2012). Furthermore, electrical or iontophoretic activation of LHb modulates hippocampal pyramidal cell activity, mainly through a serotonergic relay residing in the median raphe nucleus (Zagami *et al*, 1995). Therefore, as functional coupling may occur between pairs of indirectly connected regions (Adachi *et al*, 2012; Bressler and Kelso, 2001; Leopold and Maier, 2012), LHb might be implicated in spatial memory processes through functional interactions with dHPC networks.

In the present study, using different experimental approaches ranging from *in vivo* electrophysiology to behavioral assessment of spatial memory processes, we show, for the first time, that LHb is functionally coupled with the dHPC and that this newly described cortico-subcortical network might have a key role in spatial memory processes.

MATERIALS AND METHODS

Animals and Surgery

A total of 38 male Long-Evans rats (250–350 g) were used in compliance with the regulations specified by the European Committee Council Directive (86/609/EEC; authorization n°67–215 for J-CC). They were housed individually in a quiet room, under a 12-h light/dark cycle (light on at 0700 hours) with *ad libitum* access to food and water.

For single-cell recordings, seven rats were anesthetized with urethane (1.4 g/kg, *i.p.*) and placed in a stereotaxic frame. One extradural electrode located above the dHPC was used to record theta oscillations. The other, placed on the occipital crest, was used as a reference.

For freely moving recordings, 15 rats were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) and secured in a stereotaxic frame (Kopf, USA). Two tungsten microwire electrodes (50 μm ; impedance $\sim 1\text{M}^\circ$ at 1 KHz) were positioned in LHb (anteroposterior (AP), -3.5 ; medio-lateral (ML), ± 0.7 ; dorsoventral (DV), -4 from dura). Among those 15 rats, 10 were implanted with an additional electrode in the proximal stratum radiatum of region CA1 of the dHPC (AP, -3.8 ; ML, ± 2.7 ; DV, -2.3 from dura). Among those 10 rats, 4 were used to assess LHb-dHPC coherence during sleep-waking cycle and exploration of a known environment (see below) (LHb cells were recorded in 3 of those 4 rats) and 6 were used to assess LHb-dHPC coherence during the spatial recognition test (see below). Two screws were inserted in posterior and anterior portions of the skull as ground and reference, respectively. All electrodes and wires were fixed to the skull using Super-Bond (Sun Medical Co., Shiga, Japan). They were soldered to a six-pin circular connector (Plastic one, USA) that was secured to the skull using acrylic cement.

Inactivation of LHb during the spatial recognition test was done in 12 rats bilaterally implanted with guide cannulas 1 mm above LHb. Finally, four rats were implanted

with tungsten electrodes in the dHPC as described above, in addition to guide cannulas 1 mm above LHb in order to explore the effects of LHb inactivation on dHPC oscillations. Rats were allowed a 10-day post-operative rest before the experiments.

LHb Inactivation and Spatial Recognition Test

The test is adapted from Galani *et al* (1998). It was performed in an open field (OF; $45 \times 45 \times 30$ cm), including a spatial cue (black and white striped paper sheet, $29 \times 25\text{cm}^2$) on an inner side wall. A paper sheet comprising a mosaic of squares of equivalent sizes (4×4) was placed under the clear Plexiglas floor of the OF in order to assess locomotor activity by numbering the crossings of the different squares. The training phase comprised six 6-min sessions interspaced by 3-min home cage resting periods. Sessions 1–3 represented the habituation to the OF. Then, three objects were placed in the OF and remained at the same location during sessions 4–6 (habituation to the objects). For session 7 (spatial recognition), one object was placed in a previously empty location ('new location' object), a second object was substituted for the one which became 'new location' ('substituted' object), and the third object was left where it was before ('undisplaced' object) (see Figure 5a for a description of the OF and the protocol). LHb inactivation was performed after session 6 by an infusion of the GABA-A agonist muscimol (Musc), as previously described (Winter *et al*, 2011). We used Musc to selectively inhibit LHb, as GABA-A receptors are found in LHb, whereas the medial habenula does not present GABA-A receptor signaling (Wang *et al*, 2006). Musc ($n=6$ rats; $0.25\text{ }\mu\text{g}/\mu\text{l}$, $0.35\text{ }\mu\text{l}$ per side; Sigma) dissolved in artificial cerebrospinal fluid (aCSF; in mM: NaCl, 145; KCl, 2.7; MgCl_2 1.0; and CaCl_2 1.2) was infused in LHb over 1 min; the injection cannulas were left in place for an additional 30 s before being retracted. Control rats ($n=6$) were administered the same volume of aCSF at the same infusion rate. Session 7 was started 12 min post infusion. To assess spatial memory, the amount of time sniffing each object was compared for the two groups during session 7. In addition, a recognition index was calculated as follows: (('substituted' total sniffing time + 'new location' total sniffing time) / ('substituted' total sniffing time + 'new location' total sniffing time + 'undisplaced' total sniffing time)).

Electrophysiology

Single cell recordings. In the presence of spontaneously occurring hippocampal theta, single units were recorded in LHb using glass microelectrodes (10–20 $\text{M}\Omega$) filled with 0.5 M sodium acetate and 2.5% neurobiotin. The signal was amplified, filtered between 0.1 and 5 kHz and digitized at 15 kHz.

Freely moving recordings. Experiments began 10–14 days after surgery. Recordings were first carried out for 24 h across the spontaneous sleep-wake cycle. On the following day, rats were placed in the same OF used for electrophysiological recordings for 30 min per day for 3 days. LFPs were recorded on the third day to collect data regarding the 'exploration of a known environment' condition. Animals

were connected via a rotating commutator (Plastic one; USA) to individual animal bioamps (AD instruments, UK). LFPs were amplified ($1000\times$), filtered (0.1–500 Hz), and digitized at 1000 Hz. For cell recordings, the signal was filtered between 0.3 and 10 KHz and sampled at 20 KHz.

Recordings during the spatial recognition test. The same electrophysiological set up was used and recordings were performed on six rats during the spatial recognition test.

Data analysis. All the analyses were performed using custom-made scripts in Matlab (MathWorks, USA).

Sleep-wake cycle. Sleep stages were determined first using visual scoring and a hypnogram was created based upon the following characteristics: wake (W) was characterized by an activated low-amplitude field activity; and slow wave sleep (SWS) was clearly distinguished by high-voltage slow waves and spindles and a regular and pronounced theta rhythm signaled the onset of rapid eye movement sleep (REM or paradoxical sleep). To confirm this visual scoring, we used a recently described algorithm for behavioral state classification based on the analysis of LFPs (Supplementary Figure S1, (Gervasoni *et al*, 2004)).

Spectral analysis. Power spectra and spectral coherence were calculated using the Chronux signal processing toolbox (Bokil *et al*, 2010) with a time–frequency product of three and five tapers. Coherence values above the 95% confidence interval were considered significant. For time–frequency analysis, we used a 4-s window and moved across the data in 1-s increments. Power (integrated band power for theta; 6–9 Hz and gamma, 30–100 Hz) and coherence (mean coherence over the specified frequency bands) were calculated in 4-s bins over at least 4 periods of 2 min for each vigilance state.

The phase locking value (PLV) between LHb and dHPC was quantified using at least 2 min of continuous data and was computed as previously described (Gu *et al*, 2013). Briefly, we calculated the mean resultant length (CircStat, toolbox; (Berens, 2009) of the phase difference between the two LFPs. A value of 1 indicates perfect phase synchrony. To test the significance of the PLV, we generated a surrogate set of 200–400 PLVs by shuffling the data between 2 s and the length of the entire signal used for the original analysis. The original PLV was required to be greater than the mean + 2SD of this surrogate shuffled data set in order to be considered statistically significant.

During the spatial recognition test, power spectra and spectral coherence were assessed during each session.

Extracellular spiking analysis. Single units were sorted using the principal component analysis on Spike2 software (CED, UK) based on spike waveform. Phase locking to theta and gamma rhythm was then assessed using Matlab (Supplementary Materials and Methods).

Histology. Electrodes and guide cannulas placements were verified using cresyl violet staining on 40 μm -thick brain slices. Muscimol infusion sites were identified using microinjections of fluorescent muscimol (Invitrogen,

USA); 40 μm -thick slices were observed under a microscope at a wave length of 544 nm.

Statistical Analyses

All data are expressed as mean \pm SEM. Statistical analyses used Graphpad (Prism). Paired *t*-tests were used to compare two means. For multiple-group analyses, two-way ANOVA and Bonferroni's *post-hoc* test were used. For analyzing spatial recognition data, we used a Group \times Object ANOVA, followed by a Newman–Keuls *post-hoc* test. Activity measures during session 7 were compared using paired *t*-tests.

RESULTS

Spontaneous Activity in LHb

Recent data indicate that LHb might have a role in hippocampus-dependent memory processes (Lecourtier *et al*, 2004). However, evidence of functional interactions between the LHb and hippocampus is still missing. To determine whether LHb cell firing was modulated by ongoing hippocampal oscillatory activity, we performed single-cell extracellular recordings using glass micro-electrodes in urethane-anesthetized animals. A sample of 15 neurons was recorded in LHb ($n=7$ rats) during at least one complete brain state alternation (LIA and theta state; (Clement *et al*, 2008)). LHb neurons could be segregated into two sub-groups according to their firing rate during brain state alternations. Neurons from the first subgroup ($n=7$) were characterized by a tonic and regular discharge pattern but did not show any modification of their firing rate between the different brain states (11.3 ± 5.9 Hz during LIA vs 10.1 ± 5.6 Hz during theta, $t_6 = 1.878$; $p > 0.1$, paired *t*-test; Figure 1a). The second group ($n=8$) contained neurons exhibiting a significant tonic increase in their mean firing rate during theta states compared with LIA states (6.3 ± 1.8 Hz during LIA vs 11.6 ± 2.4 Hz during theta, $t_8 = 6.129$; $p < 0.001$, paired *t*-test; Figure 1b). They were therefore classified as theta-on cells (Colom and Bland, 1987). These results indicate that LHb contains a sub-population of neurons whose firing rate is closely related to hippocampal activity. However, under urethane anesthesia, the hippocampus only exhibits atropine-sensitive theta (or type 2 theta, (Kramis *et al*, 1975)). To further determine whether LHb cell firing was related to dHPC oscillatory activity in freely moving animals (with the presence of atropine-resistant hippocampal theta or type 1 theta), we were able to record cells within LHb during a complete sleep and waking cycle ($n=7$ cells in 3 rats) together with local field potential recordings in the dHPC in non-anesthetized animals. As anticipated with the results from the urethane-anesthetized animals, LHb cells were more active during theta oscillations (both during active wake and REM) than during slow-wave sleep (7.913 ± 3.19 Hz during Wake, 6.552 ± 3.18 Hz for SWS and 9.33 ± 3.5 Hz for REM, $F_{2,6} = 4.884$; $p < 0.05$, one-way ANOVA; Figure 2b). Among those cells, three exhibit a clear bimodal distribution in the inter-spike interval histogram (Figure 2d), with the peaks located at gamma (25 ms, 40 Hz) and theta (115 ms, 8.5 Hz) period. In addition, these cells were phase-locked to dHPC theta but not gamma oscillations (Figure 2e). Therefore, a

subset of LHb cells maintains a close phase relationship with dHPC theta oscillations.

The presence of theta frequency firing suggests that LHb might spontaneously generate theta oscillations. We therefore performed LFP recordings within LHb ($n=9$ rats) during natural sleep-waking cycle and exploration of a familiar environment. We found that LHb displayed marked theta oscillations during both exploration and rapid-eye movement sleep (REM; Figures 2f and g). The spectral power of LHb theta oscillations was not different between REM and exploration (18 ± 2.7 vs $15 \pm 2 \times 10^{-3} \text{ mV}^2$ for REM and exploration, respectively, $t_8 = 1.203$; $p > 0.05$; Figure 2h). In accordance with LHb cells discharge pattern, we also detected the presence of prominent gamma rhythms in LHb (Figure 2g, inset). Gamma power was significantly higher during exploration than during REM (4.52 ± 0.6 vs $6.3 \pm 0.8 \times 10^{-3} \text{ mV}^2$ for REM and exploration, respectively, $t_8 = 3.051$; $p < 0.05$, paired t -test; Figure 2h). Taken together, these data clearly indicate that LHb exhibits spontaneous oscillatory activity in the theta and gamma range that cannot be explained by volume conduction from the overlying hippocampus (Supplementary Figure S2). In addition, LHb activity maintains a close relationship with the dHPC.

Interactions Between LHb and dHPC

To further determine whether LHb and the dHPC were functionally related, we performed simultaneous LFP recordings in LHb and dHPC ($n=4$) during the natural sleep-wake cycle and during the exploration of a familiar environment.

LHb and dHPC LFPs exhibited strong and significant coherence at theta rhythm during both REM and exploration (theta band coherence: 0.92 ± 0.01 during REM vs 0.84 ± 0.03 during exploration; Figure 3b, left). To determine whether this theta coherence was related to dHPC theta power, we analyzed the phase locking value (PLV)

between LHb and dHPC LFPs. A comparable PLV was also observed (theta PLV: 0.91 ± 0.01 during REM vs 0.78 ± 0.06 during exploration), suggesting that high spectral coherence was mediated by high phase-phase synchrony and not power alone (Figure 3b, right). As LHb LFPs exhibit gamma oscillations, we also computed the coherence at gamma rhythm between LHb and dHPC (Figure 3b). We found that dHPC and LHb maintained a significant albeit low gamma coherence during both behavioral states (gamma band coherence: 0.45 ± 0.07 during REM vs 0.49 ± 0.1 during exploration).

In addition, we calculated the phase lag between LHb and dHPC theta oscillations during both REM sleep and exploration of a known environment (Figures 3c and d). In accordance with the coherence and PLV analysis, we showed that LHb theta oscillations maintain a highly stable phase relationship with dHPC theta activity.

Effect of LHb Inactivation on dHPC Theta Oscillations

As LHb and dHPC theta oscillations were highly phase-locked, we then assessed whether LHb might regulate dHPC oscillatory activity (Hangya *et al*, 2009). To this end, we recorded LFPs within dHPC following bilateral LHb inactivation in four rats. They consecutively received Musc and aCSF with a 24-h delay between each treatment. We computed dHPC theta and gamma power as well as theta peak frequency during active wake in the 10–20 min period following Musc ($0.25 \mu\text{g}/\mu\text{l}$, $0.35 \mu\text{l}$ per side) or aCSF infusion. We found that LHb inactivation led to a reduction in the predominant frequency of theta oscillations (7.11 ± 0.11 Hz following aCSF vs 6.29 ± 0.32 Hz following Musc, $t_3 = 3.756$; $p < 0.05$, paired t -test; Figure 4c middle). The amplitude of theta oscillations was not changed following Musc injection compared with aCSF ($t_3 = 1.71$; $p > 0.1$, paired t -test) despite a tendency to decrease ($0.26 \pm 0.05 \text{ mV}^2$ after aCSF vs $0.16 \pm 0.02 \text{ mV}^2$ after Musc; Figure 4c left). However, no effect was seen on hippocampal

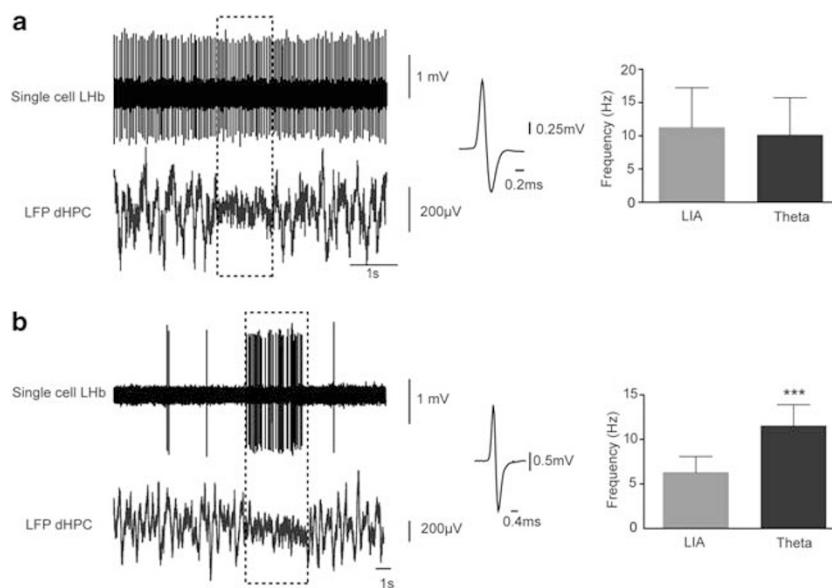


Figure 1 LHb neuronal firing in urethane anesthetized animals. (a) Raw data (left) and mean firing rate (right) of unrelated LHb cells. These cells are active during both LIA and theta states (dashed rectangle). (b) Raw data (left) and mean firing rate (right) of theta-on cells in LHb. Note the selective increase in firing rate during theta state (dashed rectangle; *** $p < 0.001$).

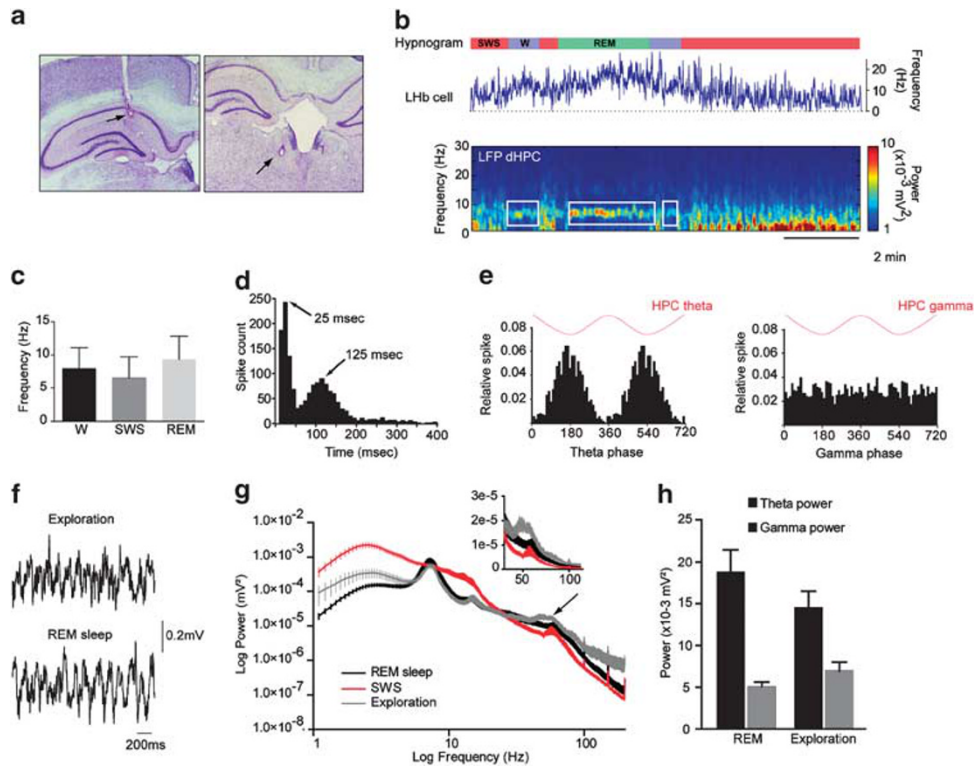


Figure 2 Discharge and oscillatory activity of LHb neurons across sleep–wake states. (a) Location of the recording electrode within LHb and dHPC (arrows). (b) Discharge of LHb neurons across sleep–wake states. Note the selective increase in frequency during W and REM (theta state, highlighted in the dHPC spectrogram). (c) Mean firing rate of LHb neurons across sleep–wake cycle. (d) Inter-spike interval histogram of one LHb neuron illustrating the bimodal distribution with the peaks located at gamma (25 ms, 40 Hz) and theta period (115 ms, 8.5 Hz). (e) Cells in LHb exhibits phase relationship with dHPC theta oscillations but no gamma oscillations. (f) Raw LFP trace recorded in LHb during REM and exploration. (g) LHb mean power spectrum ($n = 9$ rats) during REM, slow wave sleep and exploration of a familiar environment. Note the peak at theta frequency during REM and exploration, and the presence of sustained gamma activity (inset). (h) LHb mean theta and gamma power during REM and exploration of a familiar environment.

gamma oscillation (Figure 4c right). Collectively, these results suggest that LHb might specifically regulate hippocampal theta oscillations.

Effect of LHb Inactivation on Recognition Memory

We further assessed the potential role of LHb in hippocampus-dependent behavior by exploring the consequences of LHb inhibition during a spatial recognition test requiring intact hippocampal functioning (Figure 5a; Galani *et al*, 1998). Rats were treated with Musc ($n = 6$ rats) or aCSF ($n = 6$ rats) within LHb before session 7. We found that LHb inhibition led to marked memory deficits as the ANOVA showed a significant Group \times Object interaction ($F_{2,20} = 11.43$; $p < 0.001$; Figure 5b). *Post-hoc* analyses showed that during session 7 aCSF-treated rats spent significantly more time sniffing the ‘new location’ object ($p < 0.01$ and $p < 0.01$ as compared with the ‘undisplaced’ and ‘substituted’ objects, respectively), whereas Musc-treated rats spent an equivalent sniffing time on the three objects (Figure 5b). Analysis of the recognition index showed a significant difference between the two groups ($t_{10} = 5.00$; $p < 0.001$; Figure 5d); whereas aCSF-treated rats performed significantly above chance level (ie, 0.66; $p < 0.05$), Musc-treated rats did not. These results are unlikely to reflect a bias due to object characteristics or Musc-induced lack of motivation or exploratory behavior (see Supplementary Figure S3).

Finally, we aimed to determine whether LHb–dHPC coherence might predict memory performance. To this end, we recorded LFPs within LHb and dHPC during the spatial recognition test ($n = 6$ rats). As expected, all rats detected the spatial change of the objects and performed significantly above chance level (mean recognition index = 0.7817 ± 0.037 ; $t_5 = 7.559$, $p < 0.001$). In addition, LHb–dHPC coherence during session 7 was linearly correlated with performance ($F_{1,4} = 10.63$, $p < 0.05$, Figure 5d). Furthermore, two distinct sub-groups could be identified based on memory performance. Interestingly, rats performing significantly better also exhibit significantly higher LHb–dHPC theta coherence during the test (Supplementary Figure S4). Altogether, our data indicate that LHb–dHPC coherence might have a key role in hippocampus-dependent spatial memory processing.

DISCUSSION

The major findings of the present study are that LHb cell firing is related to hippocampal theta oscillations and that LHb and dHPC might be functionally coupled through theta oscillatory activity. Furthermore, LHb influences dHPC theta oscillations, and LHb inactivation alters performance in a hippocampus-dependent spatial recognition task. Finally, LHb–dHPC coherence is directly related to memory performance. Together, these findings clearly indicate that

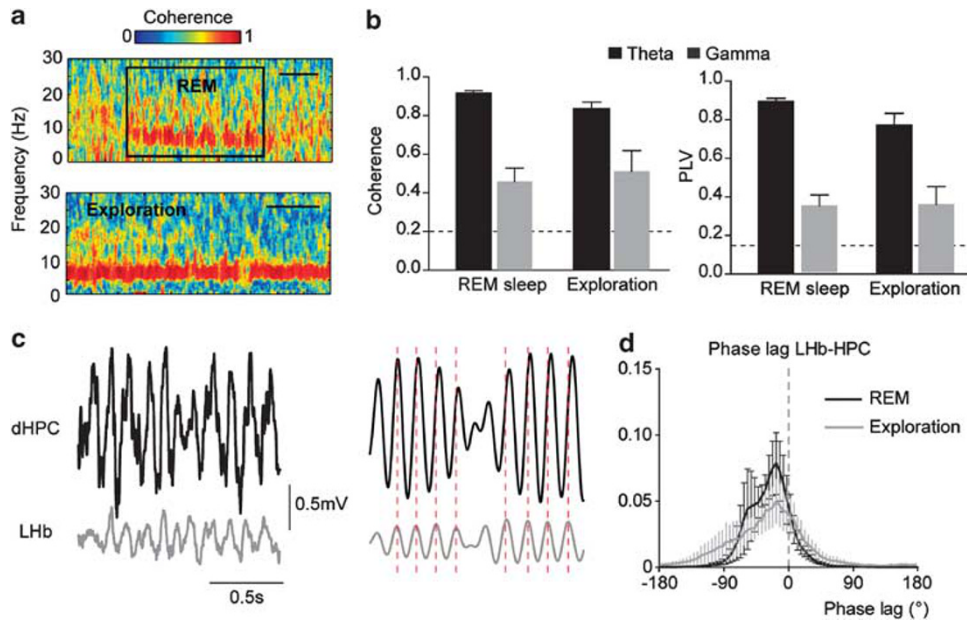


Figure 3 Interactions between LHb and dHPC. (a) Cohereogram illustrating theta coherence dynamics between LHb and dHPC during REM (upper panel) and exploration of a familiar environment. Bar = 1 min. (b) Mean theta and gamma coherence (left) or PLV (right) between LHb and dHPC. Note the similar pattern between the two analyses. (c) Raw (left) and theta filtered (right) traces simultaneously acquired in dHPC and LHb. (d) Mean phase-lag between LHb and dHPC. Note that LHb theta oscillations present a clear phase-advanced relative to dHPC theta rhythm.

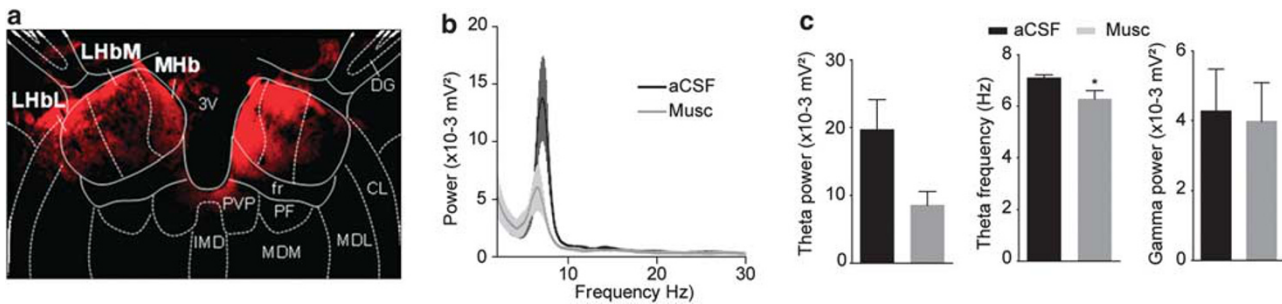


Figure 4 Reversible inactivation of LHb induces hippocampal theta alterations. (a) Representative photograph of the diffusion of fluorescent Muscimol within LHb. (b) Power spectrum analysis of dHPC oscillatory activity following aCSF or Musc infusion within LHb. (c) LHb inactivation through Musc infusion ($n = 4$ rats) induces decrease in hippocampal theta power and frequency ($*p < 0.05$) but had no effect on hippocampal gamma power.

LHb is part of a cortical/subcortical network involved in spatial information processing.

It is well known that spatial memory processes require intact hippocampal functioning (see (Frankland and Bontempi, 2005) for review). Therefore, the fact that habenular complex lesion induced marked spatial memory impairment (Lecourtier *et al*, 2004) indicates that LHb and dHPC might interact at some point during encoding and/or retrieval of a spatial memory. However, how these two structures are functionally interacting, despite the lack of direct physical connection, remains to be established. One suggested mechanism for such interaction is through neuronal synchronization by brain oscillations (Womelsdorf *et al*, 2007). By combining multiple experimental approaches, we showed that LHb and dHPC might form a functionally connected network structured through theta oscillatory activity. The observed strong theta coherence between LHb and dHPC suggests that these regions are functionally connected and are likely to

exchange relevant information related to a particular behavioral context.

To our knowledge, it is the first time that oscillatory activity has been described within LHb in freely moving animals. One issue could reside in the fact that recorded LFPs can arise from volume conduction of distant signals (Kajikawa and Schroeder, 2011). However, the fact that cellular activity within LHb is phase-locked to hippocampal theta oscillations and that theta rhythm is still present in LHb after local referencing (Supplementary Figure S2) suggests that LHb activity is theta modulated. LHb theta oscillations are likely generated through the influence of an external pacemaker.

Given the important projection from the medial septum/diagonal band of Broca complex (MS/DBB; (Tomimoto *et al*, 1987), it is likely that LHb activity is, in part, modulated by septal afferences, which also represent a strong dHPC theta modulator (Bland *et al*, 1996; Goutagny *et al*, 2008). However, it is unlikely that LHb only represents

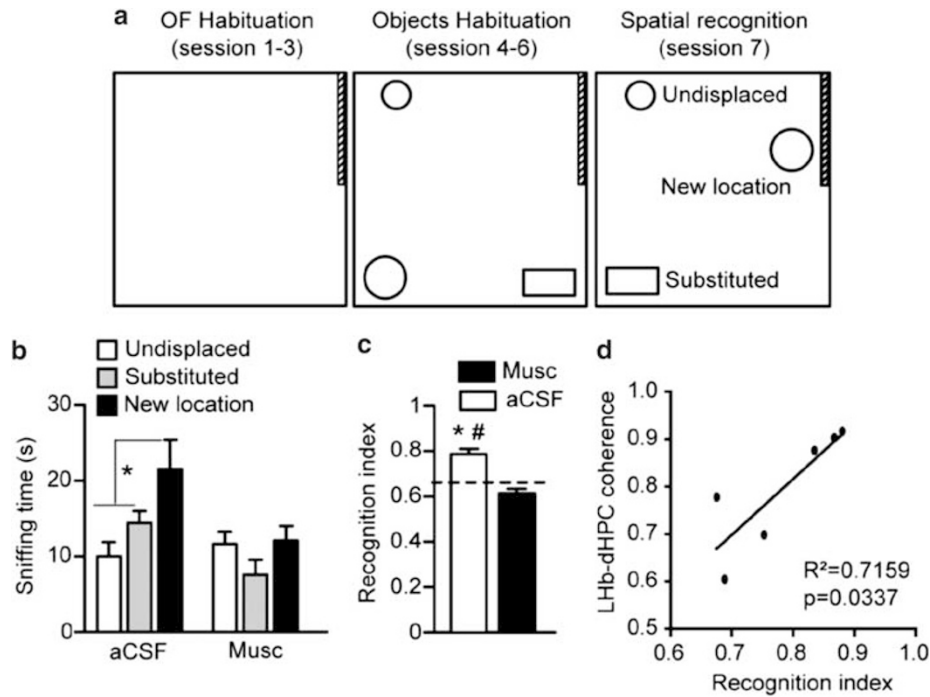


Figure 5 Reversible inactivation of LHb induces spatial recognition deficits. (a) Representation of the spatial recognition test used and adapted from Galani *et al* (1998). (b) During session 7, whereas control rats spent significantly more time sniffing the 'new location' object ($*p < 0.01$ vs 'substituted' and 'undisplaced'), Musc-treated rats spent an equivalent amount of time sniffing the three objects. (c) The recognition index showed that Musc-treated rats performed significantly worse than control rats during session 7 ($*p < 0.01$ vs Musc-treated rats; $\#p < 0.05$ vs chance level, ie, 0.66). (d) In addition, when LHb and dHPC field potentials are simultaneously recorded during the test, their coherence during session 7 is linearly correlated with performance.

a relay station of information arising from the MS/DBB complex and transmitted to the dHPC. One hypothesis is that LHb is processing different information stemming from not only the septal area, but also the basal ganglia, as already shown (Hong and Hikosaka, 2008), or from prefrontal cortical areas (see Geisler and Trimble, 2008).

In addition, the fact that selective LHb inactivation reduced the frequency of dHPC theta oscillation is particularly important, as, to date, only the supramammillary (SuM) nucleus was known to modulate hippocampal theta frequency (Pan and McNaughton, 2004). Anatomically, although there are no direct connections between LHb and dHPC, LHb projects to SuM. Therefore, it is possible that LHb influences SuM in order to modulate dHPC theta frequency. Such tuning might also occur through a modulation of dopamine release. Indeed, a hyperdopaminergic state, which has been shown to occur following LHb inactivation (Lecourtier *et al*, 2008), also induces a reduction in dHPC theta frequency (Dzirasa *et al*, 2009).

Furthermore, in our study, LHb inactivation led to marked deficits in a hippocampus-dependent spatial recognition task (Galani *et al*, 1998). This object-based task requires animals to focus their attention in order to collect information about the configuration of their environment. This information is then memorized and subsequently used to detect spatial changes during a test session taking place shortly after the last habituation session. We found that, during the test session, the level of coherence between LHb and dHPC at theta frequencies was positively correlated with the level of performance of the rats. This suggests that

LHb belongs to the network implicated in the processing of contextual information. However, the exact mechanisms responsible for the memory deficits observed after LHb inactivation still need to be clarified. One can hypothesize that the musc-induced memory deficits might arise from alterations, within dHPC, of key monoaminergic transmissions modulated by LHb, including dopamine which has a key role in dHPC-dependent short-term memory (Lisman *et al*, 2011). Also, LHb inactivation is likely to result in altered monoaminergic transmission within the prefrontal cortex (PFC) and consequently disturb PFC-dependent processes such as short-term memory and attention (Kesner and Churchwell, 2011; Robbins and Arnsten, 2009). As said above, and postulated by others (Geisler and Trimble, 2008), LHb possibly receives information from the basal ganglia, the septal area and PFC regions in order to select the proper strategy. Those information might be related not only to spatial configuration but also to goal-directed motor planning, attention, reaction to stress, and behavioral flexibility (see also Hikosaka, 2010).

In conclusion, this study shows that LHb belongs to the cortical-subcortical network implicated in the regulation of dHPC theta rhythm and spatial memory processes. Given the increasing evidence that LHb is involved in cortically driven behaviors, this study strongly suggests that LHb is in an ideal position not only to integrate and transmit cortical information to monoaminergic areas of the midbrain but also to generate a feedback to cortical structures in order to provide fine tuning of neural synchrony. Finally, our data suggest that LHb dysfunction in pathologies such as depression (Sartorius and Henn, 2007) participates in the

genesis of deficits of cortically mediated functions through the alterations of cortical networks.

FUNDING AND DISCLOSURE

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