Neurons are able to express long-lasting and activity-dependent modulations of their synapses. This plastic property supports memory and conveys an extraordinary adaptive value, because it allows an individual to learn from, and respond to, changes in the environment. Molecular and physiological changes at the cellular level as well as network interactions are required in order to encode a pattern of synaptic activity into a long-term memory. While the cellular mechanisms linking synaptic plasticity to memory have been intensively studied, those regulating network interactions have received less attention. Combining high-resolution fMRI and in vivo electrophysiology in rats, we have previously reported a functional remodelling of long-range hippocampal networks induced by long-term potentiation (LTP) of synaptic plasticity in the perforant pathway. Here, we present new results demonstrating an increased bilateral coupling in the hippocampus specifically supported by the mossy cell commissural/associational pathway in response to LTP. This fMRI-measured increase in bilateral connectivity is accompanied by potentiation of the corresponding polysynaptically evoked commissural potential in the contralateral dentate gyrus and depression of the inactive convergent commissural pathway to the ipsilateral dentate. We review these and previous findings in the broader context of memory consolidation.

1. Introduction

Brains are modular structures implementing highly distributed and efficient coding [1–4]. Sensory systems like vision, for instance, process multiple features of a complex scene in parallel, such as motion, colour and orientation among others [5] and integrate these features in one coherent, unitary perception [3,6]. Integration occurs at all levels in the hierarchy of the central nervous system, and memory is a prime example of the highest level integration. In an episode of our every-day life experience, highly processed multi-sensory information from all modalities is combined with emotions, motivation and previous experience and encoded in the form of memories for its long-term use as the basis for learning [7–10]. Given the central role of memory in cognition, unveiling the mechanisms supporting integration and storage of information is a major challenge for neuroscience. Research over recent years suggests that these processes depend on the interaction of molecular, cellular and systems-level mechanisms.

In this context, a global description of the functional connectivity between cortical and subcortical structures supporting memory is fundamental to understand integration in distributed brain networks. How and when does a brain structure interact with its many partners in the broader network of anatomically connected regions? In the hippocampus, a region well known to play a critical role in the acquisition of declarative memory [11,12], the local circuits are highly stereotypic and similar across different septo-temporal levels, while the extrahippocampal connectivity is heterogeneously distributed in this axis [13]. Several lines of evidence, mainly arriving from behavioural experiments in lesioned animals, indicate that, in parallel with the distribution of long-range connections, there exists a segregation of functions along the septo-temporal axis of the hippocampus [14,15]. This suggests that the broad...
function supported by a particular hippocampal level is given by its characteristic—anatomically defined—inputs and outputs and their dynamic properties, the latter being determined by context-dependent processes, such as short- and long-term synaptic plasticity and neuromodulation. The combination of both static and dynamic network descriptions is what we refer to here as functional connectivity.

Although conceptually appealing, a mechanistic link between the dynamic properties of synapses and those of activity propagation in the neuronal network in which they are embedded is not yet established. In this article, we review in a broader context some recent findings from our laboratory performing combined functional magnetic resonance imaging (fMRI)-electrophysiological experiments that suggest a functional reorganization of long-range hippocampal circuits controlled by synaptic plasticity and discuss their possible implication for the systems consolidation of memory. Furthermore, we present new results on the effects of synaptic plasticity on interhemispheric hippocampal communication.

(a) Cellular and systems consolidation of memories

In 1973, Bliss & Lømo [16] reported the first demonstration of a use-dependent strengthening of synaptic connections, a phenomenon known as long-term potentiation (LTP). They showed that neurons in the hippocampal formation can in fact undergo plastic changes in their synaptic inputs when stimulated repeatedly above a certain frequency threshold. This finding and others that followed were considered the experimental demonstration of Hebb’s postulate on synaptic strength and learning [17], and since then LTP has been widely accepted as the prevalent model of an experience-dependent modification of brain circuits [18,19]. This notion, recently referred to as cellular consolidation [20], has received support in experiments in which LTP induction by direct electric stimulation was replaced by learning processes, not only in the hippocampus [21–24] but also in cortical and subcortical areas such as the amygdala or the olfactory and sensorimotor cortices [25–31]. Similarly, impairment of hippocampal-dependent learning has been reported in experimental manipulations that either prevent LTP pharmacologically with NMDA (N-methyl-D-aspartate) receptor antagonists [32,33] or occlude synaptic plasticity by LTP overinduction [34].

Important functional interactions between the hippocampus and other brain regions, however, are required in order to encode episodic information into long-term memories. Classic experiments by Penfield had already demonstrated that electrical stimulation of a number of structures in the medial temporal lobe (MTL) in humans elicited reports of multimodal imagery and mnemonic features, a phenomenon of memory reactivation also triggered by seizures in patients with temporal lobe epilepsy [35,36]. More direct evidence came from brain-damaged humans [11,37] and lesion studies in animals [38–40] which demonstrated that memory storage and retrieval engage a distributed network of reciprocal connections. MTL lesions impair the formation of new memories and often debilitate those acquired before the damage. The severity of this retrograde amnesia varies with the precise locus of temporal lobe injury, with hippocampal-restricted lesions mainly affecting new memory formation and sparing older ones [37]. Further implication of a distributed network of memory-related structures came from electrophysiological and fMRI studies showing, for instance, an important link between the MTL and the prefrontal cortex (PFC). Co-activation of the parahippocampal and the PFC in human fMRI experiments was found to predict subsequent memory performance [41,42], while in animals enhanced correlated firing and increased coupling in the theta frequency range between hippocampus and medial PFC were found when rats successfully used spatial memory [43], to mention one example.

A widely accepted view supports that the connections of the hippocampus with other MTL structures, and in turn their connections with more distributed areas, provide the former with an input representation of the environment. This spatio-temporally distributed, complex pattern of activity representing external events is processes to become a simplified, indexed new pattern of output activity that goes all the way back to the corresponding areas of the cortex. The connections within the hippocampal formation would be the ones undergoing the cellular consolidation process, setting and stabilizing in that way the engagement between the involved cortical areas [44]. The reactivation of this index, and the subsequent activation of the retrieved sites, would take place repeatedly over time making the specific memory stable and independent from the hippocampus. After this process, known as ‘systems consolidation’ [20,45,46], the PFC is believed to take over the role initially fulfilled by the hippocampus, indexing and binding information stored in distributed cortical networks to retrieve remote memories [47].

However, while the evidence supporting the link between synaptic plasticity in the hippocampus and memory encoding is robust, little is known about how experimentally measured regional synaptic modifications alter the activity of more global, widespread networks supporting system consolidation of memory.

(b) Synapse to network transformations: the use of combined fMRI–electrophysiology experiments

In an attempt to link the cellular and system levels of memory consolidation, that is to relate synaptic plasticity to widespread network dynamics, we simultaneously combined fMRI and in vivo electrophysiology in rats [48] and investigated the global functional consequences of LTP [49]. In these acute experiments, urethane-anæsthetized rats are implanted in the perforant pathway (PP) and dentate gyrus (DG) with MRI-compatible stimulating and recording electrodes, respectively. Blood oxygenation-level-dependent (BOLD) imaging allows us to record activity-related signals simultaneously over the entire brain, and in combination with microstimulation [48,50] represents a very powerful tool for the study of highly distributed networks. On the other hand, the simultaneously recorded electrophysiological signals let us unequivocally relate BOLD signals with the underlying neuronal activity [51]. In this way, functional connectivity in widely distributed brain networks as well as local synaptic potentials can be longitudinally followed in the same subject across, for instance, an experimental modification of synaptic strength.

It is important to note that the fMRI maps generated with this technique reflect a measure of functional connectivity between brain regions that is essentially different from that obtained from resting state fMRI, an imaging modality in which subjects are not stimulated by any external input or
Berger and colleagues have shown that at frequencies lower than 5 Hz, inputs to the hippocampus is frequency-dependent. Yeckel and colleagues had demonstrated that the relative strength of monosynaptic and multi-synaptic entorhinal anaesthetized animals had shown—perhaps not surprisingly—that electric stimulation of the medial perforant tract produces fMRI maps that spatially correlate with the characteristic pattern of terminal fields of its axons [53–56]. Activations were found in the DG, CA3 and CA1 regions, subiculum, entorhinal cortex and the septum [48]. The BOLD signal amplitude was a good predictor of the underlying electrophysiological responses as measured in combined fMRI–electrophysiology experiments in the DG (see below), very stable under urethane anaesthesia and robust across animals [48,49]. These characteristics conferred a quantitative value to the BOLD signal in long-term experiments as those required to investigate the systems-level consequences of LTP.

In the first study [48], by systematically varying frequency and intensity of the stimulating current, we concluded that (1) a certain level of activity, in an approximately constant population of neurons, must be reached in order to start a detectable BOLD signal; (2) the activity threshold for BOLD elicitation can be reached by applying trains of pulses at relatively low frequencies (approx. 4 Hz); (3) once the threshold is surpassed, the BOLD signal (magnitude and extension of the activation) is linearly correlated with the stimulating current; and (4) at current intensities evoking a half-maximal population spike in the electrophysiology and frequencies between 10 and 20 Hz, the activity spreads through the ipsi- and contralateral hippocampal formations, whereas at frequencies lower than 5 Hz and higher than 20 Hz, the activity is restricted to the ipsilateral hippocampus. Interestingly, previous electrophysiological investigations of anaesthetized animals had demonstrated that the relative strength of monosynaptic and multi-synaptic entorhinal inputs to the hippocampus is frequency-dependent. Yeckel and Berger [57] have shown that at frequencies lower than 5 Hz or higher than 20 Hz the pyramidal neurons in the hippocampus are preferentially stimulated monosynaptically. By contrast, frequencies of 10–15 Hz greatly enhance polysynaptic excitation of pyramidal neurons through the intrinsic pathways. Our fMRI results were in perfect match with the above electrophysiological result and consistent with a frequency-dependent activity transfer between and within hippocampal formations. As an important added value, fMRI experiments offered a spatial representation of the consequences of such synaptic frequency filtering.

(c) Long-term potentiation induces a functional reorganization of long-range brain networks

Together with the information channels established by the specific axonal projections, sometimes referred to as the wiring diagram of the network, the plastic properties of synapses at different timescales is most likely the major determinant of activity propagation in the brain. While it is clear that synaptic strength determines the functional coupling between pre- and postsynaptic neurons, the polysynaptic impact of potentiating or depressing specific sets of synapses on the dynamic properties of wider networks is not yet understood. We used the combined electrophysiology–fMRI technique to investigate this issue in the hippocampus [49]. We first recorded activation maps in response to electric stimulation of the PP with test protocols not altering synaptic plasticity, and then induced LTP with a high-frequency stimulation of the PP with test protocols not altering synaptic plasticity. The magnitude of the BOLD signal in the tetanized DG was potentiated proportionally to the LTP control. The colour-coded scale represents positive and negative BOLD response in per cent change from baseline. The position of the tip of the stimulation electrode is marked by the black arrowhead. Adapted with permission from [49].

Figure 1. LTP of synaptic strength in the perforant pathway changes the hippocampal long-range functional connectivity. (a) Schematic of the experimental preparation. The placement of the stimulating (S) and recording electrodes is shown in a three-dimensional reconstruction of the rat brain. The hippocampal surface is displayed in red. The location of the MRI slices shown in (d) is also illustrated. (b) Evoked local field potentials (LFP) and (c) BOLD signal time course recorded in the hippocampus (HF), prefrontal cortex (PFC), nucleus accumbens (Acb), the anterior olfactory nucleus (AON) and the perirhinal cortex (PRh), during perforant pathway stimulation, before (black) and after (red) induction of LTP. Arrow points towards the EPSP and asterisks mark the population spike. (d) Functional maps thresholded (p < 0.001 uncorrected) and overlaid on horizontal FLASH anatomical scans, showing brain active areas during perforant path stimulation before (control) and after synaptic potentiation (LTP). The colour-coded scale represents positive and negative BOLD response in per cent change from baseline. The position of the tip of the stimulation electrode is marked by the black arrowhead. Adapted with permission from [49].
proper, the subiculum and entorhinal cortex. The result suggests an unanticipated increase in the bilateral coupling of both hippocampal formations as a consequence of unilateral synaptic potentiation (see below). Third, in a proportion of animals (67% of cases), we found that PP stimulation after LTP activates a number of extrahippocampal structures, such as the PFC, nucleus accumbens (Acb) and perirhinal cortex (PRh). All these effects were observed immediately after LTP induction and lasted for the duration of the experiment (2–3 h after potentiation).

The reported synaptically driven functional reorganization was not associated with global increases in excitability because only a subset of the numerous hippocampal outputs [13,59] were activated. Extrahippocampal activations were entirely contingent on LTP induction, as stimulation of the PP with saturating current intensities (up to 1.0 mA) in controls never produced activations in those regions [48,49]. The results were well supported by anatomical findings demonstrating important monosynaptic and bidirectional connectivity between the hippocampus and all identified extrahippocampal structures in our study [13]. Interestingly, the newly recruited structures by hippocampal activation after LTP have been repeatedly involved in memory processing over the years [11,13,41,43,60–64].

(d) Considerations on the origin of fMRI signals

Functional MRI signals are mainly produced by the increase in cerebral blood flow (CBF) induced by vasoactive compounds released during neuronal activation, although brain blood volume does also contribute (for a review, see [65]). An important matter for neuroimaging is therefore to understand which aspects of neuronal work are reflected in increased CBF. Experiments simultaneously combining fMRI and electrophysiological recordings in the primary visual cortex of anaesthetized monkeys showed that the imaging signal evoked by visual stimulation maximally correlates with the local field potential (LFP), an aggregate measure of synaptic and active dendritic currents [51]. Although the correlation of the BOLD signal was only slightly higher towards LFP compared with spiking activity (multunit and single unit activity), the LFP signal was the only predictor of the haemodynamic response when long stimulation protocols that habituate spiking activity were used. Consistent with these findings were studies in the rat cerebellar cortex which convincingly showed that local CBF can indeed be dissociated from spiking activity while strongly correlated with LFPs [66–68].

Based on the above results, it is believed that neuroimaging signals reflect the local processing of incoming neuronal activity to a particular area, rather than the output message being sent in outgoing efferent neuronal activity. The demonstration that local synaptic plasticity modulates the amplitude of the BOLD signal in the LTP experiment [49] reinforces this view. Of note, in the hippocampus, the axial organization of the cellular elements, with a rather precise alignment of dendritic shafts and somas, minimizes the cancellation of current sources from the LFP generators and facilitates the neurophysiological interpretation of the electrically evoked field potentials, such as synaptic currents reflected in the EPSP and spiking activity in the population spike. Using this preparation, we were able to unequivocally show that the EPSP slope was a precise predictor of BOLD signal amplitude, better than either the population spike or the electrical current used for stimulation [48,49]. This result has recently been confirmed in experiments combining electrophysiological recordings with hippocampal CBF measurements based on Laser-Doppler flowmetry [69]. Overall, fMRI with BOLD contrast offers quantitative and reliable measures of local synaptic processing.

(e) Theoretical considerations of long-term potentiation-fMRI experiments for memory formation

The described results demonstrated that activity patterns previously thought to primarily induce synaptic plasticity locally in the ipsilateral DG, in fact altered subsequent activity across a wide network of interconnected brain regions. Both synaptic potentiation in the DG and network reorganization were blocked by MK801 suggesting that the same NMDA-dependent mechanism attributed to memory encoding in the hippocampus may also explain the kind of network interactions required for systems consolidation. This view departs from the classical model [12] in that hippocampal–neocortical interaction would not be a progressive and necessary consequence of cellular consolidation, but rather, triggered by an active gating mechanism operated by synaptic plasticity. These findings suggest a potential mechanism to efficiently route activity propagation and information channelling in parallel distributed neuronal networks. Whether this mechanism generalizes across brain regions will require further investigation. For the moment, it is interesting to note that visual perceptual learning [59] in humans modifies the synchrony of spontaneous BOLD signals between visual and frontal–parietal networks in a way that predicts the degree of learning [70,71].

Reorganization of hippocampal and PFC networks in humans has already been demonstrated in fMRI studies during memory recall [42], in line with lesion and imaging (2-deoxyglucose) data in rodents [72,73] and consistent with the idea of a hippocampal to neocortical shift in memory consolidation. A progressive decrease in hippocampal activity and increase of that in the medial PFC were observed at recall over a three-month period. The initial shift from hippocampal to PFC activation was detected as fast as 24 h after the initial encoding [42]. These results pointed towards a systems consolidation of memory faster than that initially considered in the classical consolidation theory [12,74], although alternative interpretations of PFC activation as a consequence of a more effortful recall could not be ruled out [75,76]. In the same experiment, slow-wave sleep (SWS) duration after encoding correlated with memory performance and hippocampal deactivation suggesting a causative link. During SWS, a replay of neuronal firing sequences associated with earlier learning occurs and is predominantly found during high-frequency oscillatory events in the hippocampus, the so-called ripples [76–78], and concomitantly in the medial PFC [79,80]. Disruption of ripple sequences during SWS interferes with memory consolidation [81]. It is important to note that neuronal activity during ripples is capable of inducing LTP at CA1 synapses [82], and therefore a similar network reorganization and information channelling as the one described in our fMRI-LTP experiments [49] may also help to explain the information transfer from the
hippocampus to the PFC during memory consolidation in rest/sleep periods. Our data also suggest, however, that hippocampal–PFC interactions may take place even earlier, during memory encoding.

Enhanced coupling of the hippocampus with the PFC, PRh and Acb was found in fMRI data immediately after LTP induction, paralleling the timing of synaptic strength potentiation in the DG [49]. What could be the relevance of this functional reorganization for the first stages of memory encoding? We believe that early network interactions triggered by synaptic plasticity may optimize the coordination of two memory buffers for systems consolidation, one in the hippocampus and another in PFC. In experiments using a hippocampal-dependent pair-associate task for rats, it was elegantly demonstrated that systems consolidation can occur very rapidly when information is assimilated in a previously stored ‘schema’ in the neocortex [83]. In these cases, encoding is hippocampus-dependent but quickly consolidates in neocortex, reflecting an influence of prior knowledge on the rate of consolidation [84]. Coordination between the hippocampus and the prelimbic regions of the medial PFC were required not only for retrieval but also during memory encoding [64]. In this view [85], parallel encoding in cortex and hippocampus would establish the relevant associative links (orchestrated by the hippocampus) between objects and events initially disconnected but already represented in the cortex. Additional support of hippocampal–PFC interaction during encoding comes from early human fMRI studies showing that activations in right PFC and bilateral parahippocampal cortex during memory encoding are predictive of how well a visual experience will be remembered [41].

The above results suggest a parallel encoding of memories working cooperatively. Interestingly, our fMRI results indicate that PFC–hippocampal interactions can be driven in a bottom-up manner by synaptic plasticity in the hippocampus. This interpretation contrasts with the ‘classical’ view of memory formation in that an early and hippocampal-dependent participation of the PFC would be required at encoding.

(f) Enhanced interhemispheric communication

An unanticipated consequence of LTP induction found in the above fMRI experiments was the large increase in the recruitment of contralateral hippocampal subfields (figures 1d and 2). In fact, the relative potentiation of fMRI signals was significantly higher in the contralateral formation (figure 2).
In line with this result, bilateral changes in the expression of neurotrophins and trk receptor mRNA were found as a consequence of unilateral LTP [86]. We interpreted these findings as an enhanced commissural communication based on unilateral strengthening of synaptic weights. This conclusion was supported by previous findings demonstrating the absence of LTP in the crossed monosynaptic entorhinal projection to the contralateral hippocampus [87], and therefore discarding a direct effect of tetanization. A polysynaptic spread of the stimulation protocol used to induce LTP (200 Hz) was similarly discarded based on the filtering of high frequencies reported in the hippocampus (see above) [48, 57]. As the low-frequency stimulation used as test stimulus is able to propagate transsynaptically, the polysynaptic activation of brain areas after LTP is most probably the result of potentiated synaptic currents elicited by a test stimulus delivered at a frequency that spreads multi-synaptically. Contralateral activation may therefore reflect increased commissural–associational communication.

Although some reports start to show an intriguing bilateral asymmetry in rat hippocampus [88–91], little is known about its functional relevance. Two commissural systems connect the hippocampus, the CA3 contralateral projection to CA1, CA2 and CA3, and the contralateral DG projection mainly formed by hilar mossy cells (MCs). The CA3 network is thought to operate as an autoassociative memory with capability to selectively retrieve a specific pattern of firing activity (from among several possible patterns) when provided with a partial environmental cue [92, 93]. The MC network, on the other hand, implements heteroassociative connections with granule cells (GCs) in more rostral and caudal positions relative to the MC soma location and with homotopic positions of the contralateral DG. Based on this divergent architecture, it has been suggested that the MC associative systems would be important in pattern separation and sequence learning (for a review, see [94]). In principle, the enhanced bilateral coupling observed after LTP induction in our fMRI experiments could be the result of either increased CA3 commissural output, or an increased hilar commissural pathway, or both. A different engagement of each of the commissural/associational loops will thus be most probably associated with different computational meanings. In the following paragraphs, we present new data on LTP-induced network reorganization focusing on the bilateral hippocampal crosstalk.

2. Methods

A total of 14 male Sprague-Dawley rats (250–350 g) were used in electrophysiology and fMRI studies. All experiments were approved by the local authorities (IN-CSIC) and were performed in accordance with Spanish (law 32/2007) and European regulations (EU directive 86/609, EU decree 2001-486).

(a) Electrode implantation, microestimulation and recording

For all experiments, electrophysiology-only or combined fMRI–electrophysiology experiments, the animals were anaesthetized with urethane (1.2–1.5 g kg\(^{-1}\), i.p.) and secured in a stereotaxic device. Stimulating electrodes were implanted using standard surgical and stereotaxic procedures, as described previously [48, 49]. A twisted platinum-iridium Teflon-coated bipolar electrode (200 mm diameter, 10–15 kW: A-M Systems, WA, USA) was positioned in the medial PP (from \(\lambda\) : 0 mm anteroposterior and 4.1–4.5 mm lateral, 2.5–3 mm ventral to the dural surface) for orthodromic stimulation of the DG and the hippocampus proper [95]. Charge balanced biphasic 0.1 ms duration pulses were delivered with a constant current source and a pulse generator (STG2004, Multichannel Systems, Reutlingen, Germany). Electrodes were implanted bilaterally in electrophysiology-only experiments.

(i) Electrophysiology-only recordings

Electrodes were silicon electrode arrays (single shank, 100 μm spacing, 32 channels; Neuronexus Technologies) implanted bilaterally placed at 3.5 mm caudal and 2.5 mm lateral from bregma to record across different CA1 and DG levels in the dorsal hippocampus of both hemispheres.

(ii) Combined fMRI–electrophysiology recordings

Glass-micropipette electrodes were guided to the hilus of the DG using the typical profile of evoked potentials [96]. Stimulating and recording electrodes were secured to the skull with dental cement and plastic screws and the animal was transferred to the scanner and fixed in a custom-made MRI-compatible stereotaxic device.

(iii) Stimulation protocols

LTP was induced in all experiments as previously described [49]. In short, LTP was induced by high-frequency stimulation of the PP with episodes of six trains of pulses (each train delivered at 200 Hz and lasting 40 ms, with four pulses per train and trains delivered every 10 s) repeated three times with pauses of 2 min between episodes. Test stimulation for electrophysiological recording consisted of single pulses at different current intensities (input–output curves) delivered bilaterally, before, immediately after and 3 h after LTP induction. For fMRI recordings, a block design was used that consisted of 10 periods of 4 s stimulation epochs at 5–10 Hz and current intensities evoking half-maximal population spikes in the DG, each followed by a resting epoch of 26 s (10 min in total), which was repeated five times per animal. Functional maps were acquired before and 3 h after LTP induction.

After filtering (0.1 Hz–3 kHz) and amplification, the electrophysiological signals were digitized (20 kHz acquisition rate) and stored in a personal computer for offline processing with MatLab and Spike2. The population spike in the hilus of the DG was measured as the amplitude from the precedent positive crest and the negative peak, and the EPSPs were measured as the maximal slope of the falling potentials recorded in the molecular layer.

(b) MR imaging

For MRI experiments, urethane-anaesthetized animals were placed in a custom-made animal holder with adjustable bite and ear bars and positioned on the magnet bed. Their temperature, heart rate, oxygen saturation (SpO2) and breathing rate were monitored throughout the session, and the experiments were carried out in a horizontal 7 T scanner with a 30 cm diameter bore (BioSpec 70/ 30V; Bruker Medical, Ettlingen, Germany). Data were acquired during stimulation of the PP with test stimuli (see above) before and after LTP induction. Acquisition was performed in 15 coronal slices using a GE-EPI sequence applying the following parameters: FOV, 25 × 25 mm; slice thickness, 1 mm; matrix, 96 × 96; segments, 1; FA, 60°; TE, 15 ms; TR, 2000 ms. T2 weighted anatomical images were collected using a rapid acquisition relaxation enhanced sequence (RARE): FOV, 25 × 25 mm; 15 slices; slice thickness, 1 mm; matrix, 192 × 192; T1eff, 56 ms; TR, 2 s; RARE factor, 8. A 1H rat brain receive-only phase array coil with integrated combiner and preamplifier, and no tune/no match, was employed in combination with the actively detuned transmit-only resonator (Bruker BioSpin MRI GmbH, Germany).

Functional MRI data were analysed offline using our own software developed in Matlab, which included Statistical Parametric
Mapping package (SPM8, www.fil.ion.ucl.ac.uk/spm), Analysis of Functional Neuroimages (AFNI, http://afni.nimh.nih.gov/afni) and FSL Software (FMRIB, http://www.fmrib.ox.ac.uk/fsl/). For electric-stimulation fMRI, after linear detrending, temporal (0.015–0.2 Hz) and spatial filtering (3 × 3 Gaussian kernel of 1.5 sigma) of voxel time series, a general linear model or cross-correlation analysis was applied with a simple boxcar model shifted forward in time, typically by 2 s, or a boxcar convolved with a gamma probability density function (Matlab). Functional maps were generated from voxels that had a significant component for the model and they were clustered together in space. Similar results were obtained with the different analytical methods. For functional connectivity between bilateral hippocampal structures, images are brain extracted, co-registered and intensity normalized. Afterwards, temporal data were corrected by applying detrending, global regression and time filtering (0.002–0.1 Hz). BOLD time courses of hippocampal voxels, extracted using a rat atlas registered to the functional images [97], were used to compute the bilateral cross-correlation coefficients.

3. Results and discussion

In combined electrophysiology–fMRI experiments, we performed high-resolution echo-planar imaging (EPI) acquisitions in coronal orientation to facilitate the identification of hippocampal subfields in functional images. Electric stimulation of the PP with 4 s trains at 5 Hz and current intensities producing half-maximal population spike amplitude produced the expected activation of all ipsilateral hippocampal subfields. No contralateral propagation of activity was found in the group analysis with this mild stimulation protocol (figure 2b). Three hours after LTP induction the BOLD signal amplitude was increased (figure 2e) and the polysynaptic recruitment of contralateral hippocampal territories largely enhanced (figure 2f), in agreement with previous findings. Activation maps in coronal orientation showed activity propagation mainly concentrated in the septal portion of the contralateral hippocampus. To further investigate the spatial distribution of the enhanced bilateral coupling, we analysed interhemispheric communication by computing the voxel-wise cross-correlation of BOLD time series between all voxels in homotopic hippocampal regions, before and after LTP induction. As clearly illustrated for group analysis (figure 2e) and individual examples (figure 2f–g), ipsilateral LTP selectively enhanced the correlation between regions of the DG activated by a test stimulus applied to the ipsilateral PP. This result suggests that the LTP-triggered increase in bilateral coupling is in fact supported by the MC associations/commissural pathway with no (or limited) contribution of the CA3 network. Previous electrophysiological investigations demonstrated that the crossed entorhinal monosynaptic input to the contralateral DG is not potentiated by LTP protocols inducing a robust facilitation in the ipsilateral GC, while the convergent monosynaptic input from the contralateral entorhinal cortex is depressed [87]. To investigate the synaptic underpinnings of the above fMRI finding, we therefore focused on the polysynaptically evoked field potentials recorded across the ipsilateral (RI) and contralateral (R2) DG in response to the activation of the conditioned (tetanized, S1) or unconditioned (contralateral, S2) PPs (see Methods).

Electric stimulation of the medial PP produced the well-known profile of evoked potentials across the ipsilateral DG [55,98], with a negative-going monosynaptic EPSP (approx. 2 ms delay) recorded in the middle third of the molecular layer corresponding to the current sink and a positive potential in the soma and polymorphic layers interrupted at suprathreshold intensities by a fast population spike with maximal amplitude in the centre of the hilus (figure 3a). Stimulation of the contralateral PP generates a polysynaptic EPSP (approx. 9–10 ms delay) with a current sink in the inner third of the molecular layer (figure 3b), in good agreement with previous reports [99,100] and the known anatomy of the commissural projection of the MCs [54,101,102]. Using this preparation, we followed the electrophysiological consequences of unilateral LTP induction as a measure of commissural communication. As shown in figure 3c, LTP induction produced in the ipsilateral side (S1 → R1) the expected long-lasting potentiation of the monosynaptic EPSP without affecting the unconditioned pathway (S2 → R2) (figure 3d). Interestingly, it also induced a long-lasting potentiation of the polysynaptic EPSP in the contralateral inner molecular layer of the dentate (S1 → R2) (figure 3e). This result demonstrates, in good agreement with the fMRI findings (figure 2), that LTP enhances the MC commissural output. Concomitantly, with this potentiation we found a long-term depression of the ipsilateral EPSP evoked by activation of the convergent contralateral (unconditioned) PP (S2 → R1), which we interpret as heterosynaptic depression in a pathway inactive during tetanization (figure 3f). These results together with those reported by Levi & Steward [87] on monosynaptic bilateral entorhino–hippocampal connections are summarized in figure 3g. Although initial studies did not find changes in polysynaptic associative responses after PP LTP [103], more recent electrophysiological studies in mice clearly show LTP of the ipsilateral trisynaptic associative pathway (PP → GC → MC → GC) in response to PP tetanization [104]. Furthermore, the authors also demonstrate plastic associations between ipsilateral entorhinal and MC inputs into GCs [104].

Overall the results illustrate a hilar commissural system with synapses able to potentiate and depress in response to PP activity, and therefore well suited to associate or eliminate (update) items in a hippocampal memory trace, respectively. The highly divergent connectivity pattern of MCs spreading longitudinally in the ipsilateral DG and contralaterally to not only homotopic but also heterotopic territories [105] indicates a main role in the redistribution and integration of information among GCs at different septo-temporal levels. The reported enhancement of activity propagation in the MC-commissural system, but not in the CA3 network, triggered by LTP most probably reflects the bilateral coordination of both MC associative loops. The relevance of this finding for memory processing is not yet clear, but we suggest, in line with existing theories on MC function [106,107], an increased specificity in the recollection of bilaterally stored activity patterns by increasing s parsification and integration in a bilaterally coordinated MC associative network. The mechanism supporting the commissural selectivity (MC versus CA3) is not known but may suggest, as for the specificity in the extrahippocampal recruited regions, that LTP opens specific information channels within the hippocampus.

(a) Concluding remarks

Functional MRI investigations of classical LTP experiments [16] have unveiled a number of additional and, to a certain extent, unexpected effects of local strengthening of synaptic currents in the PP input to the hippocampus. This LTP-induced network reorganization was demonstrated to enhance the functional coupling between the hippocampus and neocortical...
structures, such as the PFC or the PRh, and subcortical nucleus, for example the accumbens [49]. We have interpreted these findings in the context of memory processing and have suggested that the reported NMDA receptor-dependent and fast increase in hippocampal–PFC communication may represent a mechanism to coordinate two memory buffers operating in parallel, even during memory encoding, as suggested by the fast consolidation of new information in pre-existing cortical schemas [64]. We have also shown that functional activation in the contralateral hippocampus is

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**Figure 3.** Electrophysiological commissural changes induced by LTP of the perforant pathway. (a) Current source density (CSD) analysis with evoked potentials overlaid in black. A representative example of the activity pattern recorded in the ipsilateral hippocampus across stratum radiatum (most dorsal recordings) and dentate gyrus (ventral) under stimulation of the ipsilateral perforant path (S1 → R1) is shown. The location of the different strata is illustrated by cell drawings on the left. The cartoons displayed in the top of all panels indicate the relevant positions of stimulating and recording electrodes for each particular case. (b) CSD and evoked potentials recorded in the same electrode as in (a) under contralateral perforant path stimulation (S2 → R1). Note the shift of the EPSP and associated current sink to more inner territories of the granule cell dendrite. (c–f) EPSP slopes as a function of stimulus intensity recorded before (blue), immediately after (red) and 3 h after (green) LTP induction, for all stimulation-recording pairs; S1 → R1 (c), S2 → R2 (d), S1 → R2 (e) and S2 → R1 (f). Insets show representative evoked potentials. (g) Scheme summarizing the effects of perforant LTP on the polysynaptic commissural activity propagation found in this study, together with the monosynaptic effects in the ipsilateral and crossed entorhinal projection described by Levy & Steward [87]. The pathways originating from the conditioned ipsilateral pathway are in black, the ones from the convergent (unconditioned) contralateral pathway are in grey. LTP is induced in S1. Symbols represent the direction of the recorded change on each pathway after LTP induction: ‘+’ potentiation, ‘−’ depression and ‘0’ unaltered. Statistical analysis was performed by a two-way ANOVA followed by Bonferroni post-hoc test (*p < 0.05, **p < 0.01, ***p < 0.001). Horizontal calibration bars represent 5 ms for all panels; vertical bars represent (a) 10 mV, (b) 2 mV, (c,d) 5 mV, (e) 1 mV, (f) 0.5 mV.
largely increased by ipsilateral LTP induction [49]. In the present experiments using high-resolution fMRI, we demonstrate that the increased bilateral coupling in the hippocampus is specifically supported by the MC commissural pathway. This enhanced MC commissural output may represent bilateral coordination of associational networks with a role in pattern separation and sequence learning [94].

References


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