Regulation of locomotion by hippocampal theta oscillations revealed by optogenetic entrainment

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Abstract

Hippocampal theta oscillations (5-10 Hz, Jung and Kornmüller, 1938) occur during exploration and REM sleep (Buzsáki, 2002). The medial septum is an essential theta rhythm generator (Petsche *et al.*, 1962) and forwards ascending inputs to the hippocampus (Bland and Oddie, 2001). Among the neuronal populations within the medial septum GABAergic neurons exhibits theta rhythmic burst firing (Hangya *et al.*, 2009) and have been suggested to rhythmically disinhibit hippocampal pyramidal cells via innervation of hippocampal interneurons (Freund and Antal, 1988). During locomotion the frequency of theta oscillations is correlated with running speed (Vanderwolf, 1969). However, whether hippocampal theta oscillations regulate locomotor speed remained a long-standing question (Vanderwolf, 1969; Grastyan *et al.*, 1959), as electrical stimulation of ascending hippocampal afferent regions inevitably involves unspecific activation of brain circuitry involved in locomotor control (Green and Arduini, 1954). Only since recently distinct brain pathways can be manipulated with high spatial and temporal precision using optogenetics (Yizhar *et al.*, 2011b). In this study, we achieved entrainment of hippocampal theta oscillations by rhythmic optogenetic stimulation of medial septal GABAergic cell terminals in the hippocampus. When the frequency of theta oscillations was optogenetically controlled, it was no longer correlated with running speed. Hence, as indicated by earlier lesion and pharmacological studies (reviewed in Bland and Oddie, 2001), the association of theta frequency with running speed is mediated by ascending afferents. We next found that the entrainment of hippocampal theta oscillations, regardless of stimulation frequency within the theta range (6-12 Hz), mediated slower and more regular running speed. The optogenetic entrainment promoted a lower variability of theta amplitude. A correlation between theta amplitude variability and running speed was observed for optogenetically entrained as well as for spontaneous theta oscillations. Further analysis of the data (Bender *et al.*, 2015) indicated that lower amplitude variability is associated with a more stable output of pyramidal cells across theta cycles. Moreover, we revealed that the behavioural effect disappeared when the main hippocampal subcortical output, to the lateral septum (LS, Risold and Swanson, 1996), was inhibited: pharmaco- or optogenetic inhibition of this pathway prevented the decrease in speed or speed variability during entrainment of hippocampal theta oscillations. The LS is also innervated by the medial prefrontal cortex (mPFC, Carus-Cadavieco *et al.*, 2017), which guides goal-directed behaviour (Miller and Cohen, 2001). Cognitive processes are linked to gamma oscillations (30-90 Hz, Cardin *et al.*, 2009, Kim *et al.*, 2016), which allow binding of sensory, e.g. visual, informations into complex representations (Singer and Gray, 1995). We found that optogenetic stimulation of the mPFC to LS pathway at gamma frequencies did not influence running speed, but improved performance in a spatial food-rewarded learning task. In conclusion, our findings suggest that hippocampal theta oscillations can regulate locomotor speed via the LS and gamma oscillations within the mPFC to LS pathway support spatial goal-directed behaviour.

Zusammenfassung

Hippocampale Theta-Oszillationen (5-10 Hz, Jung und Kornmüller, 1938) treten während Fortbewegung und REM-Schlaf auf (Buzsáki, 2002). Das mediale Septum ist ein wichtiger Taktgeber des Theta-Rhythmus (Petsche *et al.*, 1962) und leitet Signale aus subkortikalen Gehirnregionen an den Hippocampus weiter (Bland und Oddie, 2001). Innerhalb der Zellpopulationen des medialen Septums weisen GABAerge Neurone theta-rhythmische Aktivität auf (Hangya *et al.*, 2009) und es wird angenommen, dass sie hippocampale Pyramidenzellen über Innervation von hippocampalen Interneuronen disinhibieren (Freund und Antal, 1988). Die Frequenz von Theta-Oszillationen korreliert mit Bewegungsgeschwindigkeit (Vanderwolf, 1969). Ob jedoch hippocampale Theta-Oszillationen die Bewegungsgeschwindigkeit regulieren blieb eine lange bestehende Frage (Vanderwolf, 1969; Grastyan *et al.*, 1959), da elektrische Stimulation von subkortikalen Gehirnregionen zwar hippocampale Theta-Oszillationen erzeugen kann, aber unweigerlich auch eine unspezifische Aktivierung von Motorregionen mit sich führt (Green und Arduini, 1954). Erst seit kurzem können spezifische neuronale Signalwege mit hoher zeitlicher und räumlicher Auflösung mittels optogenetischer Methoden zielgerichtet manipuliert werden (Yizhar *et al.*, 2011b). In dieser Studie erreichten wir Taktgebung von hippocampalen Theta-Oszillationen über optogenetische Stimulation von hippocampalen Axonterminalen der GABAergen Zellen des medialen Septums. Wenn die Frequenz der Theta-Oszillationen auf diese Weise durch die Stimulation bestimmt wurde, war sie nicht mehr mit der Bewegungsgeschwindigkeit korreliert. Dies deutet, in Übereinstimmung mit früheren Läsions- und pharmakologischen Studien (Bland und Oddie, 2001), darauf hin, dass subkortikale Regionen die hippocampale Frequenz der Theta-Oszillationen entsprechend der Bewegungsgeschwindigkeit laufend aktualisieren. Wir fanden weiter heraus, dass sich bei gleichbleibender Taktgebung der optogenetischen Stimulation im Frequenzbereich der Theta-Oszillationen die Bewegungsgeschwindigkeit verlangsamte. Die Taktgebung bedingte eine geringere Variabilität der Amplitude der Theta-Oszillationen. Eine Korrelation zwischen der Variabilität der Amplitude der Theta-Oszillationen und Bewegungsgeschwindigkeit wurde während Taktgebung und bei spontan auftretenden Theta-Oszillationen detektiert. Weitere Analyse der Daten (Bender *et al.*, 2015) ergab, dass die Amplitudenvariabilität mit der Aktivitätsvariabilität einzelner hippocampaler Neurone assoziiert ist. Die Geschwindigkeitsregulation durch den Hippocampus fand nur statt, wenn das laterale Septum, die subkortikale Hauptausgangsstation des Hippocampus (Risold und Swanson, 1996), nicht inhibiert wurde: bei pharmako- oder optogenetischer Inhibition dieses Signalweges führte die Taktgebung des Theta-Rhythmus nicht zu einer langsameren oder gleichmäßigeren Bewegungsgeschwindigkeit. Das laterale Septum wird auch vom medialen prefrontalen Kortex (mPFC) innerviert (Carus-Cadavieco *et al.*, 2017), welcher zielgerichtetes Verhalten steuert (Miller und Cohen, 2001). Kognitive Prozesse werden mit Gamma-Oszillationen (30-90 Hz, Cardin *et al.*, 2009, Kim *et al.*,

2016) assoziiert, welche sensorische, z.B. visuelle, Informationen zeitlich binden und somit komplexe Repräsentationen ermöglichen (Singer und Gray, 1995). Wir haben herausgefunden, dass optogenetische Stimulation des Signalweges vom mPFC zum lateralen Septum im Frequenzbereich der Gamma-Oszillationen die Bewegungsgeschwindigkeit nicht beeinflusst, und stattdessen die Leistung bei einer räumlichen Lernaufgabe mit Futterbelohnung verbessert.

Zusammenfassend weisen unsere Ergebnisse darauf hin, dass über das laterale Septum hippocampale Theta-Oszillationen die Bewegungsgeschwindigkeit regulieren und Gamma-Oszillationen des mPFC Signalweges zum lateralen Septum zielgerichtetes Verhalten im Raum unterstützen können.

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1. Literature Review

1.1. Brief overview of brain oscillations

Groups of strongly interconnected and active neurons form cell assemblies which are suggested to represent cognitive entities in the nervous system (Hebb, 1949). As these groups are highly interconnected, activation of a sufficient number of neurons can lead to activation of the entire assembly (reviewed in Buzsaki, 2010). Chaining of cell assemblies can provide the basis for the intrinsic flow of cognitive processes (reviewed in Harris, 2005). Groups of cell assemblies tend to engage in rhythmic synchronization, meaning that time windows of maximal and minimal excitability are synchronized. Input, which arrives during the excited state, will have a greater impact on the network state than input which arrives during a less excited state. Cell assemblies synchronize within one brain region and across brain regions. Rhythmic synchronization of neurons facilitates the communication across brain regions (Fries, 2015). When activity across brain regions is precisely coordinated, information is more efficiently transferred. Coherence of neural signals allows to set up highly specific patterns of effective neuronal coupling and enables flexible and context-dependent binding, selection of relevant information and efficient routing of signals through processing pathways (Singer and Gray, 1995; Engel and Singer, 2001). Within a certain time window the preferred frequency range and the synchrony across different brain regions depend on the task performed and the internal state of the animal. Network synchrony could arise from the coupling of neuronal oscillators, which possess intrinsically determined frequency preferences. Brain oscillations are suggested to support the organization of information flow within the brain, sensory processing, guidance of behaviours, such as generating rhythmic motor output, decision making and memory consolidation. Brain oscillations are preserved in all mammals as well as birds and reptiles (Shein-Idelson *et al.*, 2016; Buzsaki *et al.*, 2013) and have been suggested to be inherent to the brain architecture (Buzsaki and Freeman, 2015). Abnormal synchronization contributes to motor and psychiatric diseases such as Parkinson's disease, epilepsy, schizophrenia or dementia (reviewed in Schnitzler and Gross, 2005).

Brain oscillations recorded from the human scalp electroencephalogram (EEG) were first described by Berger, 1929. The EEG can capture cortical electrical activity in superficial cortical layers. To study electrical events in deeper brain locations, electrodes are inserted in the region of interest to record the local field potential (LFP). Any transmembrane current leading to intra- and extracellular voltage fluctuations in vicinity to the recording electrode contributes to the LFP (Buzsaki *et al.*, 2012). During synaptic activity the influx of cations from the extra- to intracellular milieu gives rise to a local extracellular sink, which is balanced by an extracellular source (opposing ion flux), also called passive or return current. The time course of a single action potential is shorter, the strongest field is generated within 2 ms. However, if many neurons discharge simultaneously, the currents can substantially contribute to the LFP. Further, spike afterhyperpolarisation, long-lasting (10-100ms)

calcium spikes, intrinsic currents, ion exchange through electrical synapses and neuron-glia interactions can contribute to the LFP (Buzsaki *et al.*, 2012). Characteristics of brain rhythms, the amplitude and frequency, depend on the proportional contribution of multiple sources and the properties of the brain tissue. The preferred frequency range depends on the patterns of connectivity, synaptic properties and anatomical aspects.

1.2. The hippocampal formation

1.2.1. Anatomy of the hippocampus

The hippocampus is part of the limbic system located in the temporal lobe in close proximity to the cerebral cortex. The hippocampal formation is composed of the hippocampus proper, areas CA1, CA2, CA3 (CA for Cornu Ammonis), the subiculum and dentate gyrus (DG, Fig. 1.1). The entorhinal cortex (EC) is assigned to the hippocampal formation. The hippocampus can be considered a cortical module. It consists of multiple layers. The laminar structure comprises stratum (str.) oriens (or.), str. pyramidale (pyr.), str. radiatum (rad.), str. lacunosum-moleculare (L-M) and str. granulosum (Ramón y Cajal, 1893; Lorente de Nó, 1934). The hippocampus, in contrast to the cortex, has the shape of a seahorse ("hippocampus" is latin for "seahorse") and runs along the septo-temporal axis in rodents, corresponding to the posterior-to-anterior axis in humans.

Across species the basic architecture, appearance and connectivity of the hippocampus is largely conserved, although commissural projections are sparse in humans (Gloor *et al.*, 1993). The connectivity scheme in the human hippocampus is expected to be similar as in the rodent hippocampus. The number of neurons in the hippocampus increased only 10-20 fold from rat to human, while the neocortex expanded by several orders of magnitude. The number of cell layers in stratum pyramidale increased (on average 5 cells in rodents and 30 cells in humans) and became more heterogeneous in humans (see Buzsaki, 2006).

Figure 1.1
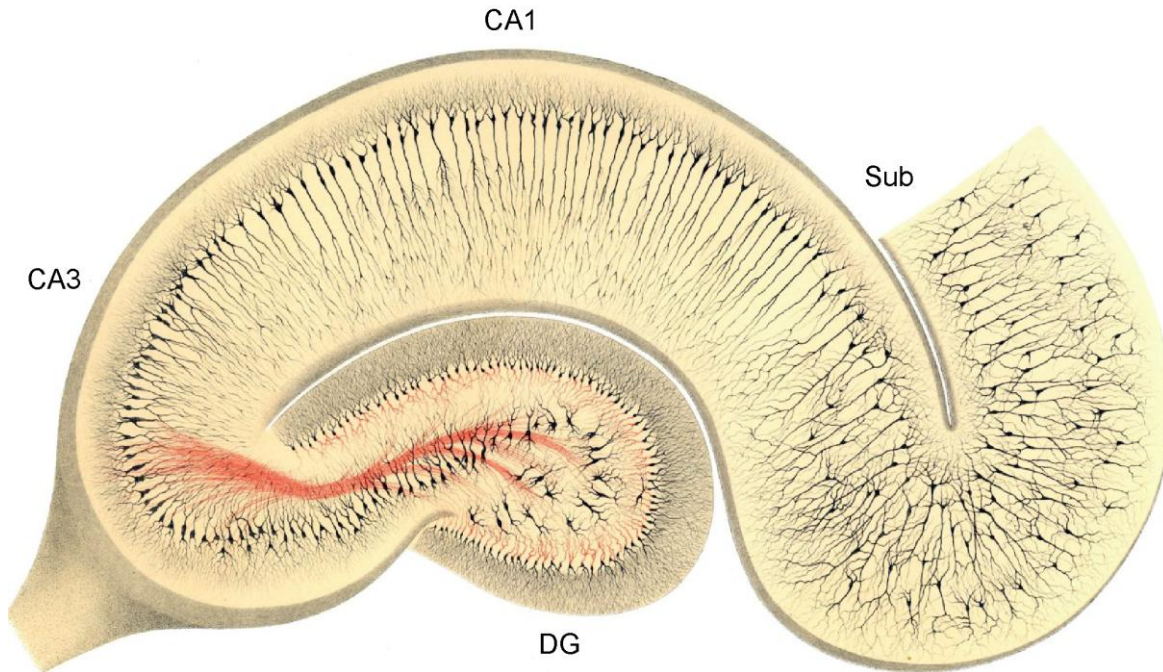
Figure 1.1. The hippocampus with areas CA1 and CA3, DG (dentate gyrus) and Sub (subiculum). Depicted are the principal cells. The highly organized structure of the hippocampus facilitates summation of extracellular currents. Modified drawing by C. Golgi (1894).

1.2.2. Hippocampal pyramidal cells

Pyramidal cell somata are located in stratum pyramidale. One pyramidal cell can be part of several assemblies which may contribute to different representations (Buzsaki, 2010). Due to high interconnectivity, activation of members of a cell assembly mediates activation of the whole assembly and recreation of stored patterns. Assemblies of hippocampal place cells, for instance, which are activated simultaneously when the animal enters a specific location in the environment, are distributed throughout the hippocampus (O'Keefe, 1978). Hence, the hippocampal architecture supports its function as an autoassociator.

CA1 pyramidal cells are largely organized in parallel. Cell density is approximately 450.000 per mm^3 in the mouse dorsal hippocampus (Jinno and Kosaka, 2010), about three times higher than at the ventral level. CA1 pyramidal cell collaterals travel parallel to the alveus in str. oriens and remain in this layer (Ramón y Cajal, 1893; Tamamaki and Nojyo, 1990; Lorente de Nó, 1934), but recurrent excitatory connections are sparse compared to the CA3 network (Deuchars and Thomson, 1996). CA1 pyramidal cells contact each other typically via a single synapse (Deuchars and Thomson, 1996). They mainly input onto local interneurons, the subiculum and extrahippocampal brain structures (Cenquizca and Swanson, 2006; Freund and Buzsaki, 1996). Dendrites of CA1 pyramidal cells spread in the stratum oriens where inputs from septal fibres and commissural fibres arrive. CA1 pyramidal cells receive excitation from distinct afferent pathways, which target the proximal and distal domains of the dendrites. The main source of excitation of the CA1 pyramidal cells stems from CA3 pyramidal cells longitudinally widespread associational projections termed Schaffer collaterals and arrives in str.

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radiatum and str. oriens (Schaffer, 1892). Via this pathway ipsilateral feed-forward CA3-CA1 excitation is mediated (Szirmai *et al.*, 2012).

The CA3 pyramidal cells build a strongly recurrent collateral system. Cell density is approximately 170.000 per mm^3 in the mouse dorsal and ventral hippocampus (Jinno and Kosaka, 2010). One CA3 pyramidal can share 20-60 thousand synapses within the ipsilateral rat hippocampus (Sik *et al.*, 1993; Li *et al.*, 1994). The CA3 pyramidal cells are strongly interconnected via a longitudinally projecting recurrent associational system and form a CA1-bound large three-dimensional space in the hippocampus. Neighbouring CA3 cells project to neighbouring CA1 cells (Brivanlou *et al.*, 2004). The CA2 cells also have extensive collaterals and compose with CA3 a strongly recursive network.

Commissural projections in the hippocampus are mostly formed by CA3 as well as hilar mossy cells (Blackstad, 1956; Gottlieb and Cowan, 1973; Swanson *et al.*, 1978; Li *et al.*, 1994). CA3 pyramidal cells project to all subregions of the contralateral hippocampus (Blackstad, 1956; Fricke and Cowan, 1978), where they target interneurons as well as pyramidal cells. Commissural pathways are mainly excitatory, but also inhibitory projections were detected (Ribak *et al.*, 1986). CA1 pyramidal cells receive excitation from contralateral CA3 via commissural projections (Shinohara *et al.*, 2008). CA1 neurons project to the contralateral CA1 at the septal hippocampal pole, but CA1 commissural projections are weaker than CA3 commissural projections (Van Groen and Wyss, 1988; van Groen and Wyss, 1990). The subiculum does not receive or send commissural signals directly.

1.2.3. Hippocampal interneurons

Interneurons orchestrate the activity of hippocampal pyramidal cells. Most, if not all, hippocampal interneurons are GABAergic (Freund and Buzsaki, 1996). So far, over twenty different interneuron classes have been described in the CA1 hippocampal region (Klausberger and Somogyi, 2008). Among others, basket cells, axo-axonic cells, bistratified cells, O-LM cells, Schaffer collateral associated cells, perforant path associated cells and hippocampo-septal cells can be distinguished (reviewed in Somogyi and Klausberger, 2005). Interneurons can be differentiated according to morphology, especially regarding their target cell type, expression of neuropeptides (somatostatin (SOM), cholecystokinin, vasoactive intestinal peptide, and neuropeptide-Y) and calcium binding proteins (parvalbumin (PV), calretinin and calbindin), as well as functional characteristic, such as the action potential phenotype. PV is a marker for fast-spiking GABAergic cells (Morris *et al.*, 1999). SOM-immunoreactive cells in CA1 can be detected in str. oriens and correspond to O-LM cells (Forro *et al.*, 2015; Freund and Buzsaki, 1996). PV-immunoreactive cells in CA1 can be detected in str. pyramidale (>50 %), str. oriens (30-40 %) and str. radiatum (34 %, reviewed in Freund and Buzsaki, 1996). They account for half of the interneurons in close proximity to pyramidal cell somata. PV$^+$ cells are basket cells, bistratified cells and axo-axonic cells, but not all of those cells contain PV. One basket cell was estimated to contact more than 1500 pyramidal cells and 60 other PV$^+$ interneurons, one bistratified cell to contact 2500 pyramidal cells and one axo-axonic cell to contact 1000 pyramidal cells in the rat

(Sik *et al.*, 1995). It was estimated that 30-40 PV+ interneurons converge onto a single pyramidal cell (Sik *et al.*, 1995).

1.2.4. Extrinsic connections, the hippocampus-EC loop

Input to the hippocampus arrives from cortical as well as subcortical structures, such as the raphe nucleus, hypothalamus, supramammillary bodies and from the medial septum (MS, described below, reviewed in Bland and Oddie, 2001). Synapses of extrahippocampal fibres in the hippocampus proper make about 10 % of synapses.

The entorhinal cortex conveys information from the neocortex via the perforant path to the DG, CA3 and subiculum. In turn, CA1 hippocampal neurons and the subiculum, but not CA3 pyramidal cells, project to the entorhinal cortex (Naber *et al.*, 2001). The reciprocity of hippocampal and entorhinal cortex inputs allow reverberating circuits to be established through this loop. The entorhinal cortex is, apart from the lateral septum, the main hippocampal output region.

Output of the hippocampus is sent to other cortical and subcortical structures (van Groen and Wyss, 1990). The primary subcortical output structure is the lateral septum (described below, Risold and Swanson, 1996).

1.2.5. Hippocampal network oscillations

During slow-wave-sleep and quiet wakefulness hippocampal discharge is not synchronized and the LFP is characterized by large irregular activity (LIA, an irregular waveform with a broadband frequency range of 0.5–25 Hz, Leung *et al.*, 1982), occasionally interrupted by ultrafast ripple oscillations (140-200 Hz), which derive from highly synchronized activity of pyramidal cells and promote long-term-potentiation (Buzsaki, 1986). During exploration and rapid-eye movement (REM) sleep, the hippocampal network expresses continuous rhythmic activity at theta frequencies (5-12 Hz, 1–2 mV, Jung and Kornmüller, 1938) coupled with gamma (30-120 Hz) oscillations (reviewed in Colgin, 2016). Then, time windows of highest excitability of neuronal assemblies in the hippocampus become synchronized and occur repeatedly every 100-200 ms. The highly organized laminar structure of the hippocampus facilitates the summation of extracellular currents, which contribute to the current generation of the theta rhythm (Fig. 1.1., see Buzsaki, 2006).

1.3. Hippocampal theta oscillations

1.3.1. Theta oscillations in the hippocampus

Theta frequency is coherent across all hippocampal layers (Bullock *et al.*, 1990) and across hemispheres (Buzsaki *et al.*, 2003; Kocsis *et al.*, 1994). Instead, coherence of gamma oscillations across hemispheres can be low (Sabolek *et al.*, 2009). Theta frequency is approximately constant along the hippocampal septo-temporal axis (Patel *et al.*, 2012; Lubenov and Siapas, 2009).

Theta phase is synchronous in homotypic regions across hippocampal hemispheres, but changes with depth (Buzsaki *et al.*, 2003; Kocsis *et al.*, 1994). The phase reversal is detected at the level just below the pyramidal layer (Petsche and Stumpf, 1960; Green, 1960).

Theta amplitude is coherent between the septal and intermediate hippocampus, whereas the coherence is low between the septal and ventral hippocampus (Sabolek *et al.*, 2009; Maurer *et al.*, 2005; Hinman *et al.*, 2011; Patel *et al.*, 2012). Ventral hippocampal theta oscillations are generally less stable, have lower power and are often absent in the presence of dorsal hippocampal theta (Royer *et al.*, 2010). Theta amplitude differs between layers, it is highest in str. lacunosum-moleculare in CA1, where theta is most regular (Green, 1960; Buzsaki, 2002).

Theta waves travel predominantly along the septo-temporal hippocampal axis (Lubenov and Siapas, 2009; Patel *et al.*, 2012). Also neuronal spiking is organized as a travelling wave: the mean phase of firing systematically advances across the grid of the wave propagation (Patel *et al.*, 2012). Travelling theta waves have been later reported in the human hippocampus and the propagation speed was found to depend on theta frequency (Zhang and Jacobs, 2015).

1.3.2. Theta oscillations across brain structures

The entire limbic system and the cortex display theta oscillations (Colom *et al.*, 1988; Vertes *et al.*, 2001; Kocsis and Vertes, 1994; Bullock *et al.*, 1990; Siapas *et al.*, 2005; Seidenbecher *et al.*, 2003). During certain tasks specific brain regions are entrained together with the hippocampus at theta frequencies. A portion of cortical neurons fire phase-locked to hippocampal theta (Siapas *et al.*, 2005, Sirota *et al.*, 2008). The entorhinal cortex comprises "theta-off" cells and tonic as well as rhythmic "theta-on" cells. Many entorhinal cortex cells discharge phase-locked to hippocampal theta (Alonso and Llinas, 1989; Mizuseki *et al.*, 2009) and show a wide distribution of preferred firing phases (Dickson *et al.*, 1995). The coactivation of pyramidal cells in hippocampus and entorhinal cortex is lowest during REM sleep and exploration (Mizuseki and Buzsaki, 2014). The directionality of communication between the hippocampus and the cortex during theta is not yet completely understood and may dynamically shift depending on the task the animal performs at that moment.

The amygdalo-hippocampal circuit, for instance, is coupled by theta oscillations during fear conditioning (Seidenbecher *et al.*, 2003). Here, the fear-related information conveyed by the amygdala

might be associated with the environmental context provided by spatial inputs arriving to the hippocampus (Seidenbecher *et al.*, 2003).

Hippocampal theta also correlates with the activity of neurons in sensory brain regions. For instance, auditory and visual neurons show state-dependent phase-locking to hippocampal theta oscillations (Gambini *et al.*, 2002; Pedemonte *et al.*, 1996; Velluti and Pedemonte, 2002). Furthermore, hippocampal theta is coupled with a cortical respiration-locked rhythm (Biskamp *et al.*, 2017). Olfactory bulb oscillations are correlated with hippocampal theta during olfactory discrimination tasks (Kay, 2005). The integration of respiratory rate by hippocampal theta oscillations is suggested to be mediated via the medial septum (Tsanov *et al.*, 2014).

1.3.3. Theta-gamma coupling

Cross-frequency coupling describes the interaction between oscillations with different frequency bands (Jensen and Colgin, 2007). In rodents, as well as in humans, during locomotion as well as during REM sleep, gamma oscillations are modulated by theta oscillations (Bragin *et al.*, 1995; Canolty *et al.*, 2006; Belluscio *et al.*, 2012; Colgin, 2015; Korotkova *et al.*, 2010; Wulff *et al.*, 2009). First of all, power of gamma is increased -amplitude is largest - during theta compared to non-theta states (Bragin *et al.*, 1995; Csicsvari *et al.*, 2003). Second, instantaneous changes in gamma amplitude depend on theta phase (Bragin *et al.*, 1995; Colgin *et al.*, 2009; Penttonen *et al.*, 1998). Third, theta and gamma frequency are positively correlated (Bragin *et al.*, 1995; Belluscio *et al.*, 2012). Fourth, gamma phase is coupled to theta phase (Belluscio *et al.*, 2012; Zheng *et al.*, 2016). Further, while slower gamma oscillations are coupled to the descending theta phase, higher frequency gamma oscillations are coupled to the theta peak (Colgin *et al.*, 2009). The exact mechanism of theta-gamma coupling is not fully understood. According to one scenario, excitation at a particular theta phase triggers the onset of gamma by inducing gamma-frequency bursting of interneurons (Colgin, 2016). Experimental and modelling data suggest that in the hippocampus inhibitory inputs onto PV^{+} cells, which may arise from intrahippocampal interneurons and/or inhibitory pacemaker neurons in the medial septum, are essential for proper theta-gamma coupling (Wulff *et al.*, 2009). Also glutamatergic drive to PV[+] interneurons is required for theta-gamma coupling (Korotkova *et al.*, 2010). Coupled gamma and theta oscillations may support a temporal organization of event sequences within a theta cycle and allow a more powerful output of the system since cells fire more closely in time (Lisman and Jensen, 2013).

1.3.4. Hippocampal theta oscillations in humans

The human hippocampus displays theta oscillations during virtual and actual movement (Bohbot *et al.*, 2017) as well as during REM sleep (Cantero *et al.*, 2003). First studies in patients who were performing virtual navigation tasks demonstrated a slow frequency oscillation (1-4 Hz) in the hippocampus (Ekstrom *et al.*, 2005; Arnolds *et al.*, 1980; Jacobs, 2014). It was sometimes doubted that this oscillatory pattern resembled theta activity, as the frequency was lower as of theta oscillations in

rodents and it is quite untypical that frequency bands of brain oscillations scale inversely with brain size. A recent study reported higher frequency theta (7-9 Hz) in human hippocampus during real-world movement, hence movement with motor, vestibular and proprioceptive feedback (Bohbot *et al.*, 2017). This oscillation was absent during immobile periods.

1.4. Mechanisms supporting hippocampal theta rhythm

Intrinsic theta oscillations (5 Hz) were reported in the isolated rat hippocampus (Goutagny *et al.*, 2009). Which physiological mechanisms facilitate the theta state in the hippocampus? Possible mechanisms including resonance properties of neurons, rebound spiking and network orchestration via interneurons, are briefly summarized in the following.

1.4.1. Hippocampal pyramidal cells resonate at theta frequencies

Resonant properties at theta frequencies have been reported for hippocampal pyramidal cells (Stark *et al.*, 2013; Pike *et al.*, 2000) and some interneurons subtypes, for instance O-LM cells (Maccaferri and McBain, 1996; Pike *et al.*, 2000). Resonance occurs due to an amplifying mechanism within a certain frequency band and is established for the theta band, for instance, via the action of HCN (hyperpolarization-activated cyclic nucleotide–gated) channels, which are widely expressed throughout the brain (Koch *et al.*, 2004; Robinson and Siegelbaum, 2003). These voltage-gated cation channels, expressed in the distal dendrites of hippocampal pyramidal cells (Magee, 1998), function as band-pass filters. They facilitate conduction of inputs at theta frequencies to the somata (Narayanan and Johnston, 2007; Vaidya and Johnston, 2013). That way they may reduce response variability due to a variable timing of the incoming synaptic inputs (Vaidya and Johnston, 2013). Accordingly, hippocampal pyramidal cell stimulation at theta frequencies is more efficient in driving hippocampal network activity than at lower frequencies (Andersen and Lomo, 1967). But also upon sufficiently strong intracellular depolarization the resonant properties can lead to self-sustained oscillations of membrane potential. Pharmacological blockade of HCN channels abolishes theta resonance (Stark *et al.*, 2013).

The hyperpolarization-activated (I_h) current plays an important role in rebound spiking of hippocampal interneurons (Lien *et al.*, 2002) and pyramidal cells (Ascoli *et al.*, 2010). Rebound spiking is systematically abolished by the blockage or reduction of the I_h current in CA1 pyramidal cells (Ascoli *et al.*, 2010). Rebound spiking can occur in response to perisomatic GABAergic inhibition (Buhl *et al.*, 1994). Postinhibitory rebound spiking helps to sustain spontaneous persistent activity and oscillatory dynamics, such as central pattern generation or sustained reverberation (Bottjer, 2005) and produces theta-synchronized firing across multiple pyramidal cells in the hippocampus (Cobb *et al.*, 1995). Theta rhythmic activation of hippocampal PV+ cells can elicit rebound spiking and theta-rhythmic firing in hippocampal pyramidal cells *in vivo* (Stark *et al.*, 2013). In contrast, when PV+ cells are activated at random intervals, theta spiking resonance is not evoked (Stark *et al.*, 2013).

Various types of hippocampal neurons feature gap junctions evident by anatomical, molecular and physiological techniques (e.g. Schmitz *et al.*, 2001). Gap junctions enable various types of high frequency oscillations (e.g. Draguhn *et al.*, 1998, Buhl *et al.*, 2003) and, furthermore, can be potentially involved in theta oscillations, yet the evidence for that presently is not sufficient (Bland *et al.*, 2003; Buhl *et al.*, 2003).

1.4.2. A network of hippocampal interneurons controls the firing of the principal cells

A functional network of interneurons is essential for the generation of network oscillations. When interneurons are not functional, the brain exhibits seizures and higher brain functions fail (Marin, 2012).

Hippocampal interneurons play an essential role in timing the activity of pyramidal cells (Freund and Buzsaki, 1996; Buzsaki and Chrobak, 1995; Cobb *et al.*, 1995). Apart from intrinsic properties the interplay between the magnitude of dendritic excitation and rhythmic inhibition by interneurons is suggested to be responsible for the theta phase assignment of pyramidal cells' spikes (Harris *et al.*, 2002; Hu *et al.*, 2014).

In the hippocampus nearly all interneurons discharge rhythmically during theta, but their phase relationship differs (Klausberger *et al.*, 2003). Axo-axonic cells, basket cells and bistratified cells mediate perisomatic inhibition and discharge preferably during the theta peak, when discharge of pyramidal cells is minimal (Klausberger *et al.*, 2003). O-LM interneurons discharge in phase with pyramidal cells (Klausberger *et al.*, 2003).

PV+ interneurons play a major role in orchestrating network dynamics, as research within the last decades revealed (reviewed in Hu *et al.*, 2014). Their dendrites are only weakly excitable, allowing them to integrate many inputs. Their axons are highly excitable and therefore can propagate signals with high temporal precision to many target cells. At the presynapse calcium channels and release sensors are tightly coupled, which additionally increases the efficacy and speed of the signal transmission. In the hippocampus intra- and/or extrahippocampal inhibitory input onto PV+ cells is essential for theta oscillations (Wulff *et al.*, 2009; Stark *et al.*, 2013; Cobb *et al.*, 1995; Amilhon *et al.*, 2015). Among hippocampal PV+ interneurons axo-axonic cells were attributed a key role in timing inhibition of pyramidal cells. The axon initial segment is exclusively innervated by axo-axonic cells (Somogyi *et al.*, 2014), while all other compartments of hippocampal pyramidal cells receive GABAergic input from multiple interneuron types with distinct firing dynamics (Royer *et al.*, 2012). Furthermore, during theta they are most active when pyramidal cells are most silent, whereas during high frequency ripple oscillations, when many pyramidal cells fire synchronously, axo-axonic cells are inhibited.

SOM+ cells, instead, were considered to modulate external inputs (Amilhon *et al.*, 2015). O-LM cells, for instance, gate the information flow in CA1 arriving from CA3 and the entorhinal cortex (Leao *et al.*, 2012). While O-LM cells show intrinsic theta rhythmic activity (Maccaferri and McBain, 1996),

reciprocal connectivity with fast-spiking cells is required for synchronization of network activity (Rotstein *et al.*, 2005). Their mutual inhibition may explain why O-LM and fast-spiking cells fire out of phase during theta.

Hippocampal pyramidal cells project to local interneurons via a single synapse (Gulyas *et al.*, 1993) which is particularly efficient (Csicsvari *et al.*, 1998). Interneurons then again provide feedback inhibition to several hundreds to thousands of pyramidal cells (Sik *et al.*, 1995; Cobb *et al.*, 1995). These local recurrent dynamics are proposed to play a fundamental role in local theta generation (Leung, 1998), but the underlying mechanisms are still not completely understood.

1.4.3. Theta rhythm generators and current generators

"Current generator" refers to the transmembrane currents which give rise to the magnitude of the recorded field. Current source density analysis revealed a current source in str. pyramidale coupled to a sink in str. L-M (Kamondi *et al.*, 1998). According to one model, the theta dipole across the CA1 layer arises from inhibitory inputs to the soma and proximal dendrites of the pyramidal cell, on the one hand, and excitatory inputs to the distal apical dendrites, on the other hand (model of Leung, 1998, Buzsaki, 2002). Theta currents at distal dendrites from pyramidal cells arise from at least three mechanisms: sinks mediated through the NMDA receptors, sinks due to rhythmic, voltage dependent calcium spikes in distal dendrites of strongly excited neurons, sources mediated by O-LM cells which are innervated by CA1 pyramidal cells and inhibit less activated pyramidal cells (winner takes all, Buzsaki, 2002).

"Rhythm generator" refers to a mechanistic function responsible for the emergence and control of the oscillation pattern and frequency. According to Buzsaki (2002), several theta rhythm-generating mechanisms and numerous theta current dipoles exist. Fluctuations of theta power, coherence and phase in a layer-specific manner support this concept (Montgomery *et al.*, 2009). The hippocampal CA3 recurrent collateral system can act as a theta rhythm generator (Kocsis *et al.*, 1999). Local hippocampal theta rhythm generators may also exist in humans (Mormann *et al.*, 2008).

The medial septum is considered the major theta rhythm generator (Petsche *et al.*, 1962). Theta oscillations in the hippocampus and entorhinal cortex are phase locked and both are abolished by medial septum lesions (Rawlins and Olton, 1982; Mitchell *et al.*, 1982). Inputs from the entorhinal cortex arrive at distal apical dendrites pyramidal cells via the perforant path. After removal of the entorhinal cortex or upon ablation of NMDA receptors of PV^{+} cells within the hippocampus, the current source in the pyramidal layer remains, however, the sink-source in distal dendrites disappears (Kamondi *et al.*, 1998; Korotkova *et al.*, 2010).

1.5. Theta rhythm regulation by ascending afferents is mediated via the medial septum

1.5.1. The ascending brain stem hippocampus synchronizing pathway

The ascending brainstem hippocampus synchronizing pathway (Bland and Oddie, 2001) relays proprioceptive signals. This pathway originates in the brain stem reticular formation (RF) and ascends to the posterior hypothalamus (PH) and supramammillary nucleus (SUM, Vertes and Kocsis, 1997; Bland and Oddie, 2001). The SUM translates activity levels into rhythmic firing (Kirk *et al.*, 1996) and forwards signals to the medial septum (Vertes and McKenna, 2000). Synchronous burst activity within the medial septum is enhanced by inputs from the brainstem that results in tonic afferent excitation (Brazhnik and Fox, 1997). Electrical stimulation of these regions elicits theta activity in the medial septum and hippocampus (reviewed in Bland and Oddie, 2001). Frequency of theta rhythmic electrical stimulation in the medial septum corresponds to elicited hippocampal theta frequency (Colom et al., 1987, Gray and Ball 1970, Bland et al, 2006). Instead, the intensity of RF or PH electrical stimulation correlates with hippocampal theta frequency (Bland *et al.*, 2006a; McNaughton *et al.*, 2007). When stimulated simultaneously, the frequency of hippocampal theta matches the stimulation frequency of medial septum stimulation and not the stimulation intensity delivered to the PH (Scarlett and Bland, 1997). Electrical stimulation of the RF (Grillner and Shik, 1973; James *et al.*, 1977), hypothalamus (Green and Arduini, 1954; Whishaw and Nikkel, 1975; Sinnamon *et al.*, 1999) and SUM (Sinnamon, 1984) elicits locomotor activity. A portion of medial septum cells display speed-related activity (Fuhrmann *et al.*, 2015; Zhou *et al.*, 1999; King *et al.*, 1998). Optogenetic stimulation of glutamatergic cells in the medial septum elicits locomotor activity while unspecific electrical stimulation does not (Bland *et al.*, 2006a; Fuhrmann *et al.*, 2015; James *et al.*, 1977; Kramis and Routtenberg, 1977).

Opposing influences on theta, on the other hand, are mediated by the median raphe, among other brain structures (Vertes, 1981). Electrical stimulation of the median raphe desynchronizes hippocampal LFP (Vertes, 1981) mediated through direct median raphe to hippocampus projections or via projections via the medial septum (Vertes and Martin, 1988). Median raphe electrical stimulation disrupts rhythmic theta discharge in the medial septum, while suppression of the serotonergic median raphe cells activates medial septum rhythmic firing and elicits theta (Kinney *et al.*, 1996). The median raphe can be seen as a functional antagonist to the reticular formation (Kitchigina *et al.*, 1999).

1.5.2. The medial septum as the nodal point of the ascending brain stem synchronizing pathway

The medial septum is considered the nodal point of the ascending brain stem synchronizing pathway. It is located centrally in the brain, in a strategic position to orchestrate the limbic system. The medial septum is highly developed in mammals, especially in humans (Andy and Stephan, 1968). The septum and hippocampus are strongly interconnected. Indeed, they are often referred to as one system, the "septohippocampal system". The medial septum was suggested to translate increasing activation

levels of subcortical regions into theta rhythmic signals which are relayed to the hippocampus (reviewed in Bland and Oddie, 2001).

Early studies revealed a role of the medial septum in hippocampal theta regulation, as lesions of the medial septum abolished hippocampal theta (Petsche *et al.*, 1962). Many studies followed, which confirmed the central role of the medial septum in theta generation. Lesions or inactivation of the medial septum disrupted theta not only in the hippocampus, but also in the medial EC (Jeffery *et al.*, 1995). Septal lesions in awake animals decreased the activity of hippocampal interneurons (Mizumori *et al.*, 1989). Cellular activity of medial septum "theta-on" cells preceded hippocampal LFP theta by 500 ms, while hippocampal cells adjusted firing rates coincident with onset of local LFP theta, supporting the view that septohippocampal projections initiate theta (Toth *et al.*, 1997; Bland *et al.*, 1999). HCN-expressing medial septal cells fire rhythmically at theta frequencies and are phase-locked to hippocampal theta (Hangya *et al.*, 2009). The expression of hippocampal theta depends on the proportion of the medial septum cells involved in the rhythmic process, and its frequency within the theta-range is determined by the frequencies of theta-bursts in the medial septum (Vinogradova, 1995). Medial septum pacemaker cells, PV- and/or HCN-immunoreactive cells, lead hippocampal theta, which was revealed via the Z-shift method: they were maximally phase coupled to a later period (approximately 80 ms) of hippocampal LFP (Z-shift>0, Hangya *et al.*, 2009). PV-immunonegative medial septal cells were less strongly phase coupled to hippocampal theta, the Z-value was lower and the median delay was not significantly different from zero. HCN-immunonegative medial septal cells were maximally phase coupled to an earlier period (approximately 80 ms) of hippocampal theta (Z-shift<0)

1.5.3. The medial septum to hippocampus pathway

Extensive projections are sent from the medial septum to the hippocampus proper, as well as to the subiculum, mainly via fornix and fimbria (Swanson and Cowan, 1979, Fig. 1.2a). The medial septum and entorhinal cortex are reciprocally connected (Alonso and Kohler, 1984). Much fewer projections are sent to other cortical areas (Unal *et al.*, 2015). Medial septum cells, mostly non-cholinergic, send descending projections to the PH, RF, the lateral hypothalamus (LH) or the SUM (Kalen and Wiklund, 1989; Borhegyi and Freund, 1998).

Glutamatergic cells comprise about 25 % of medial septum cells (Colom *et al.*, 2005). These project to hippocampal areas CA1, CA3 and the dentate gyrus (Colom *et al.*, 2005). There, they excite pyramidal cells as well as interneurons (Sotty *et al.*, 2003; Manseau *et al.*, 2005; Huh *et al.*, 2010). These projections, however, are sparse (Robinson *et al.*, 2016).

Cholinergic cells comprise about 30-50 % of medial septum cells (Kiss *et al.*, 1990). Cholinergic projections to the hippocampus were first documented by Frotscher and Leranth, 1985. They project to all hippocampal regions (Milner *et al.*, 1983; Amaral and Kurz, 1985) and target hippocampal layers evenly in the mouse, although with higher density in str. oriens, str. pyramidale and at the border

between str. radiatum and str. L-M (Aznavour *et al.*, 2002). Cholinergic medial septum cells innervate preferentially dendrites of hippocampal pyramidal cells (Wainer *et al.*, 1984). Accordingly, acetylcholine concentrations are highest in proximity to str. pyramidale (Zhang *et al.*, 2010). But also dendrites and cell bodies of interneurons are innervated (Leranth and Frotscher, 1987; Cobb and Davies, 2005).

GABAergic medial septum cells comprise about 30 % of medial septum cells and somata are located in the medial parts of the medial septum (Kiss *et al.*, 1990). The GABAergic medial septum to hippocampus pathway was first described by Misgeld and Frotscher, 1986. GABAergic medial septum cells terminate preferentially in str. oriens and str. radiatum of all hippocampal fields (Amaral and Kurz, 1985; Milner *et al.*, 1983). Importantly, GABAergic medial septum cells innervate exclusively hippocampal interneurons, but not pyramidal cells. This finding was first reported by Freund and Antal (1988, Fig. 1.2b) in the rat and later confirmed by Unal *et al.* (2015) in the mouse. They have been proven to synapse onto a variety of interneurons in the hippocampus, including PV+ and SOM+ cells (Freund and Antal, 1988; Takacs *et al.*, 2008; Yamano and Luiten, 1989; Gulyas *et al.*, 1990). The exclusive projection to interneurons is unique for the hippocampal projections: while GABAergic medial septum cells project preferentially to interneurons of other brain structures they also target a limited number of pyramidal cells, for instance, in the medial EC (Gonzalez-Sulser *et al.*, 2014) and other cortical areas such as the retrosplenial cortex (Unal *et al.*, 2015), or in the amygdala (McDonald *et al.*, 2011).

Figure 1.2

a
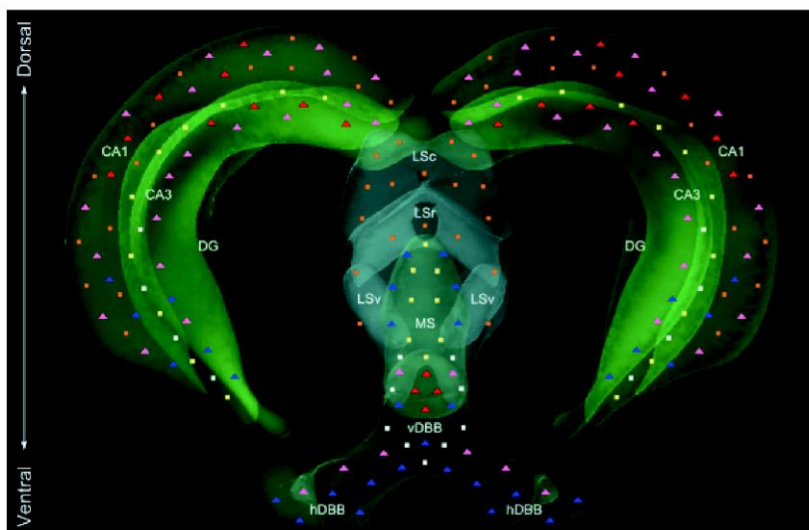
b
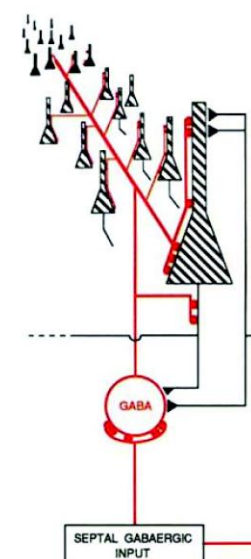
Figure 1.2. The septohippocampal formation. a) Three-dimensional representation of the septohippocampal complex including the medial septum (MS) with the vertical and horizontal diagonal band of broca (vDBB and hDBB), caudal (cLS), rostral (rLS) and ventral (vLS) lateral septum in blue and hippocampal areas CA1, CA3 and DG in green. Adapted from Teles-Grilo Ruivo and Mellor, 2013. **b)** GABAergic cells in the medial septum innervate exclusively hippocampal interneurons and therefore are suggested to release hippocampal pyramidal cells rhythmically from inhibition. Adapted from Freund and Antal, 1988.

1.5.4. Physiological properties of medial septum neuronal populations and theta rhythm regulation

Glutamatergic medial septum cells display a wide range of firing patterns, such as fast-, cluster-, burst- and slow-firing and also show spontaneous rhythmic firing at theta frequencies (Sotty *et al.*, 2003; Simon *et al.*, 2006; Huh *et al.*, 2010). Stimulation of glutamatergic cells within the medial septum can elicit theta oscillations in the hippocampus (Fuhrmann *et al.*, 2015). Stimulation of only their projections to the hippocampus, which are bundled in the fornix, does not elicit hippocampal theta (Robinson *et al.*, 2016). Theta generation via glutamatergic medial septum cells is suggested to be mediated via activation of local medial septum cholinergic or GABAergic neurons (Robinson *et al.*, 2016).

Cholinergic medial septum to hippocampus projections were initially suggested essential for hippocampal theta generation (Stewart and Fox, 1990), which was later questioned (Vandecasteele *et al.*, 2014; Simon *et al.*, 2006). They exhibit slow (around 5 Hz) and non-rhythmic activity *in vitro* (Griffith and Matthews, 1986; Markram and Segal, 1990a). Overall, firing properties of medial septal cholinergic cells *in vivo* are not yet conclusively characterized. In contrast to medial septum GABA cells, cholinergic medial septum cells do not exhibit I_h current and endogenous pacemaker properties (Varga *et al.*, 2008; Sotty *et al.*, 2003). Acetylcholine release in the hippocampus mediated by the medial septum activates various muscarinic receptors and nicotinic receptors (reviewed in Tsanov, 2015). Thereby it depolarizes the membrane potential of hippocampal interneurons and pyramidal cells and enhances hippocampal pyramidal cell responses to excitatory afferent inputs (Tsanov, 2015). The increase in membrane potential can drive hippocampal theta as well as gamma dynamics (Traub *et al.*, 1992; Fisahn *et al.*, 1998), probably by activation of voltage-dependent cation currents (Hoffman *et al.*, 1997). Hippocampal cells furthermore express KCNQ (voltage-gated potassium) channels which regulate their excitability (Fidzinski *et al.*, 2015) and are inhibited by acetylcholine (Fidzinski *et al.*, 2015; Wang *et al.*, 1998). Cholinergic medial septum cells are suggested to be the source of the atropine-sensitive component of the theta oscillation (Kramis *et al.*, 1975).

GABAergic medial septum cells are suggested to provide the theta rhythmic drive to the hippocampus. Freund and Antals' model (1988), according to which medial septum GABAergic interneurons disinhibit hippocampal pyramidal cells during distinct time windows was confirmed by further studies (Toth *et al.*, 1997; Vertes and Kocsis, 1997). Further evidence is summarized in the following.

First, GABAergic medial septum cells have pacemaker properties, which are probably mediated by HCN channels (Morris *et al.*, 2004). Hence, they can discharge spikes at theta frequencies upon depolarization, even without rhythmic input drive. Medial septum GABA cells exhibit fast signaling by burst firing at theta frequencies (Hangya *et al.*, 2009; Morris *et al.*, 1999; Borhegyi *et al.*, 2004).

Second, GABAergic medial septum cell firing is coordinated with hippocampal activity. Medial septum GABA cells recruit hippocampal ensembles with high temporal precision (Dannenberg *et al.*, 2015). Their bursts are coupled to hippocampal theta oscillations (Hangya *et al.*, 2009; Varga *et al.*,

2008; Borhegyi *et al.*, 2004; Simon *et al.*, 2006), locked to the through (178°) or the peak (330°) of the theta cycle (Borhegyi *et al.*, 2004). Rhythmic inhibition of hippocampal PV+ interneurons is important for hippocampal theta generation (Wulff *et al.*, 2009).

Third, manipulation of GABAergic medial septum cells affects hippocampal theta rhythm. For instance, if GABA release in the medial septum is abolished by injections of kainic acid in medial septum, theta amplitude is reduced by a much greater extent than if cholinergic cells are lesioned by 192 lgG-saporin medial septum injection (Yoder and Pang, 2005). Intra-MS infusions of Gabazine increases theta frequency (Chee *et al.*, 2015). Optogenetic inhibition of medial septal GABAergic cells reduces theta power during REM sleep (Boyce *et al.*, 2016).

Further evidence for the prominent involvement of GABAergic medial septum cells in hippcampal theta oscillations is given by the findings that medial septal GABA cells projecting to the hippocampus are most active during locomotion and upon sensory inputs (Kaifosh *et al.*, 2013) and that during development emergence of hippocampal theta bursts coincides with emergence of PV+ GABA cells in the medial septum and the hippocampus (Bender *et al.*, 1996)

The precise orchestration of hippocampal cell activity by medial septal cells is complex and not yet fully understood. Medial septum GABA cells innervate diverse populations of hippocampal interneurons (Gulyas *et al.*, 1990) that discharge on different phases of the hippocampal theta rhythm and release GABA onto distinct postsynaptic domains of the pyramidal cells (Somogyi *et al.*, 2014). Synchronous firing of medial septal GABAergic cells might be ensured via recurrent collateral interactions.

1.5.5. The hippocampus-septal feedback loop

Backprojections from the hippocampus to the medial septum are exclusively GABAergic and run along the fimbria (Sik *et al.*, 1994; Dragoi *et al.*, 1999; Gulyas *et al.*, 2003; Toth *et al.*, 1993; Alonso and Kohler, 1982). The fibre bundle follows further the fornix, which runs bellow the corpus callosum, and finally targets subcortical regions, including the medial septum. Here, medially located fibres arise from more temporal hippocampal levels. Hippocampal GABAergic cells innervate preferentially medial septal GABAergic cells, but also, to a lesser extent, medial septal cholinergic cells (Toth *et al.*, 1993). These backprojections may convey information about the activity of many pyramidal cells back to the medial septum (Takacs *et al.*, 2008). Hippocampal cells projecting to the medial septum receive extremely dense septal innervation, predominant inhibitory inputs probably arise from the GABAergic medial septum cell population (Takacs *et al.*, 2008). Based on parallel recordings a regulation of theta rhythmic activity of medial septum neurons by the hippocampus was suggested (Dragoi *et al.*, 1999; Manseau *et al.*, 2008). The hippocampal feedback to the medial septum is important for producing widespread synchrony (King *et al.*, 1998).

1.6. Hippocampal theta oscillations and locomotion

1.6.1. Behavioural correlates of the hippocampal theta rhythm

A variety of behavioural correlates of the hippocampal theta rhythm were reported after its discovery (reviewed in Buzsaki, 2005 and Korotkova *et al.*, 2017). During waking, theta occurs when an animal is moving, when it is exploring an environment or approaching an object (Grastyan *et al.*, 1959). Theta occurs when an animal is sensing, i.e. when it is attentive towards the environment and upon sensory stimulation (Buzsaki, 2005). Sensory stimulation can elicit theta in an anaesthetized animal (Green and Arduini, 1954) and *in vivo* (Whishaw and Dyck, 1984), and sensory inputs can reset the phase of the ongoing intrinsic theta oscillation (Buzsaki *et al.*, 1979). Theta further occurs in the presence of a predator (Green and Arduini, 1954) and upon presentation of a fear-conditioned stimulus (Seidenbecher *et al.*, 2003). Theta was hypothesized to be involved in the organization of voluntary motor behaviours, but not automatic motor behaviours, such as chewing, licking, shivering, grooming (Vanderwolf, 1969; Sainsbury, 1970). Locomotor behaviour is very strongly correlated with hippocampal theta (Vanderwolf, 1969).

Theta features, such as frequency and amplitude, correlate with aspects of locomotor behaviour, such as instantaneous running speed.

1.6.2. The relationship between theta frequency and running speed

A positive correlation between the instantaneous frequency of hippocampal theta and running speed was first presented by Vanderwolf (1969) in rats which freely explored an environment, and was confirmed in many following studies (e.g. Ahmed and Mehta, 2012; Rivas *et al.*, 1996; Hinman *et al.*, 2011; Maurer *et al.*, 2005; Jeewajee *et al.*, 2008). The correlation between speed and theta frequency exists for all hippocampal layers (Montgomery *et al.*, 2009). Along the hippocampal axis it is most pronounced at septal sites (Hinman *et al.*, 2011). Running speed also correlates with the frequency of hippocampal gamma oscillations (30-120 Hz), while frequencies between the theta and gamma range (18-30 Hz) are negatively correlated with speed (Ahmed and Mehta, 2012). Running speed does not correlate with gamma power (Montgomery and Buzsaki, 2007) or theta-gamma coupling (Tort *et al.*, 2009).

Under certain experimental conditions the correlation of theta frequency and running speed was absent. Vanderwolf himself reported an absence of a speed-theta frequency correlation in rats running on a treadmill. Here, the treadmill was set to a constant speed during one trial and speed was changed between trials. Theta frequency was the same across trials, although the instantaneous speed was different (Whishaw and Vanderwolf, 1973). Instead, a linear correlation between running speed and theta frequency was found in guinea pigs moving on a conveyor belt (Rivas *et al.*, 1996). The difference was that here the speed of the conveyor belt was increased gradually while the animal was running.

1.6.3. The relationship between theta amplitude and running speed

Theta amplitude increases with the magnitude of the movement (Whishaw and Vanderwolf, 1973; Hinman *et al.*, 2011; Oddie and Bland, 1998; Bland *et al.*, 2006b; Jeewajee *et al.*, 2008) and with running speed at the septal hippocampal pole (Rivas *et al.*, 1996; Bouwman *et al.*, 2005) for CA1 layers of the dorsal and intermediate hippocampus (Montgomery *et al.*, 2009). The correlation between running speed and theta amplitude also exists during forced running on a wheel or treadmill (McFarland *et al.*, 1975; Li *et al.*, 2012). Theta amplitude has been related to different aspects of locomotor behaviour and can vary considerably from one behavioural episode to the next (Whishaw and Vanderwolf, 1973). Theta amplitude is sensitive to movement changes. Rapid acceleration and deceleration is accompanied by a sharp reduction in theta amplitude, which is more prominent during decelerations which precede movement termination (Long *et al.*, 2014). Accelerations significantly contribute to momentary theta amplitude. Upon movement termination theta amplitude declines more rapidly than frequency (Sinnamon, 2006). Theta amplitude increases during the sensory processing period which precedes movement onset but then declines rapidly together with frequency during the movement preparation period before movement initiation (Bland *et al.*, 2006b). Movement onset is associated with an increase in theta power in humans and the degree of power increase is associated with the length of the path of the following movement epoch (Bush *et al.*, 2017).

Along the septo-temporal axis of the hippocampus the running speed-theta power correlation diminishes gradually (Montgomery *et al.*, 2009). Theta amplitude and neuronal firing rates are much more velocity-modulated in the dorsal than in the ventral hippocampus (Maurer *et al.*, 2005). The power of ventral hippocampal theta correlates weakly with locomotion velocity and varies largely independently from theta power in the dorsal hippocampus (Patel *et al.*, 2012; Hinman *et al.*, 2011). The finding is not surprising, as the ventral hippocampus carries largely non-spatial information (Royer *et al.*, 2010). Also the relation of theta amplitude and accelerations decreases along the septotemporal axis of the hippocampal CA1 area (Long *et al.*, 2014). The relationship between theta power and speed is also stronger in the dorsal hippocampus when compared to the entorhinal cortex (Hinman *et al.*, 2011) or to the neocortex (McFarland *et al.*, 1975).

The sensorimotor integration model suggests that the increase in theta amplitude allows for the integration of sensory inputs with motor outputs during navigation (Bland and Oddie, 2001; Bland and Bland, 1986; Caplan *et al.*, 2003).

In humans, hippocampal theta power increases during goal-directed virtual movement (Cornwell *et al.*, 2008). Human theta power increases proportionally with running speed during virtual navigation (Watrous *et al.*, 2011), which derives from increased optic flow. Movement-related theta was the highest in amplitude during movement initiation and a power reduction in novel environments was observed in humans (Kaplan *et al.*, 2012).

The shape of the theta wave also changes with running speed, with faster runs being accompanied by a more asymmetric sawtooth shape and slower runs by a sinusoid shape (Buzsaki *et al.*, 1983; Terrazas

et al., 2005). Sheremet *et al.* (2016) reported a correlation between the nonlinearity in theta shape and movement speed.

1.6.4. Hippocampal neuronal activity and running speed

Almost all hippocampal neurons, except for approximately 1 %, increase their firing rate during the theta state (Buzsaki *et al.*, 1983; Mizumori *et al.*, 1990). Hippocampal pyramidal cells typically fire below 3 Hz and interneurons above 7 Hz (Buzsaki *et al.*, 2003). Hippocampal CA1 and CA3 cells, pyramidal cells as well as interneurons, increase their firing rate with running speed (Czurko *et al.*, 1999; McNaughton *et al.*, 1983). That happens even when the frequency of theta remains constant when rats are running on a treadmill (Czurko *et al.*, 1999). Entorhinal cortex cells can also increase firing rate with speed (Kropff *et al.*, 2015). Increase in cell firing probability and firing rate are reflected in increases of theta amplitude and frequency during faster runs.

1.6.5. Guidance of spatial navigation by the dorsal hippocampus

The spatial map is encoded by the dorsal hippocampus (O'Keefe, 1978). During locomotion hippocampal "place cells" encode specific positions in the environment called "place fields". Hippocampal theta activity increases with the complexity of the environment and increased theta activity in the dorsal hippocampus correlates with better performance, e.g. a shorter length of the path taken to a platform, during virtual navigation in humans (Cornwell *et al.*, 2008).

Allocentric, or "map-based", navigation is based on environmental landmarks. Hippocampal place cells are sensitive to external cues, such as landmarks, contextual cues, geometric boundaries or reward associations. Hippocampal theta activity is correlated with whisking activity (Macrides *et al.*, 1982) and head movement oscillations (Ledberg and Robbe, 2011) which are believed to support optimal processing of sensory information. Visual, auditory, somatosensory and olfactory information is forwarded from the respective association cortices via the lateral entorhinal cortex (Knierim *et al.*, 2014). Associations are formed in the hippocampal area CA3 and forwarded to area CA1. The primate hippocampus comprises spatial view cells in CA1 and CA3 (Rolls *et al.*, 1997). Activity at electrodes in the human hippocampus greatly increases during vision of landmarks while subjects are navigating a virtual reality (Watrous *et al.*, 2011). Interestingly, hippocampal theta power increases much more when subjects are viewing a landmark that guides towards the goal than when they are viewing the goal itself, providing evidence for the primary role of hippocampal theta in guidance of locomotion in comparison to goal directed behaviour.

Egocentric navigation, or "path integration", requires active movement to integrate locomotor speed and elapsed time (Buzsaki and Moser, 2013). Spatial information is relayed via the medial entorhinal cortex (Knierim *et al.*, 2014). Movement-related information about momentary speed derives from the visual, the vestibular system and reafferent signals from muscles and muscle tendons. This information is relayed via ascending pathways to estimate the current position in relation to a starting

point via summation of locomotion vectors (length and direction) for optimal path integration (Bures *et al.*, 1997). The "oscillatory interference" theory suggests that theta oscillations are generated by velocity-controlled oscillators (VCOs) which encode distance travelled along a specific direction (Burgess *et al.*, 2007). In VCO neurons the firing frequency varies as a cosinus function of running direction and as a linear function of running speed. Dorsal hippocampal place cells are VCOs (Geisler *et al.*, 2007). Afferent speed-related signaling is relayed via the medial septum (Bland and Oddie, 2001). It has been suggested that VCOs are located in the medial septum (Hasselmo, 2014; Welday *et al.*, 2011). When the fornix, the major medial septum to hippocampus projection pathway, is lesioned, the selectivity and reliability of hippocampal place cells is reduced (Shapiro *et al.*, 1989), and also spatial periodicity of entorhinal cortex grid cells is lost upon medial septum inactivation (Brandon *et al.*, 2011; Koenig *et al.*, 2011).

Different functions were attributed to the ventral and dorsal hippocampus. While the dorsal hippocampus performs primarily cognitive functions, the ventral hippocampus relates to stress, emotion, and affect (Fanselow and Dong, 2010). Locomotor guidance during navigation is assigned to the dorsal, but not the ventral hippocampus. Evidence is summarized briefly in the following. First, place field density and selectivity is much higher in the dorsal than in the ventral hippocampus (Jung *et al.*, 1994). Neurons in the ventral hippocampus fire at multiple locations in the environment and thus carry less precise spatial information (Royer *et al.*, 2010). Second, spatial learning relies much more on the dorsal than on the ventral hippocampus: dorsal hippocampal lesions, but not ventral hippocampal lesions of the same size, strongly impair performance during a spatial learning task (Moser *et al.*, 1993). Third, the dorsal hippocampus is much stronger connected with sensory cortices and the entorhinal cortex, where grid cells are located (Strange *et al.*, 2014), than the ventral hippocampus. Fourth, theta rhythmic firing of cells is much more pronounced in the dorsal than in the ventral hippocampus (85 % vs 25 % of cells, Royer *et al.*, 2010).

1.6.6. The hippocampus and the concept of behavioural inhibition

The important role of the dorsal hippocampus during explorative behaviours is well established. Counterintuitively, lesions of the hippocampus induce impulsivity (Cheung and Cardinal, 2005) and increase overall activity (Coutureau *et al.*, 2000), locomotor behaviour (Gray and McNaughton, 1983; Jarrard and Bunnell, 1968) and running speed (Kim and Frank, 2009).

According to Gray (1978) the septohippocampal system is the central structure of the behavioural inhibition system which expresses theta during arousal. In favour of this theory, hippocampal function is impaired by anxiolytics which reduce theta frequency (McNaughton *et al.*, 2007; Seidenbecher *et al.*, 2003; McNaughton and Gray, 2000). One has to keep in mind, however, that anxiolytics also reduce the frequency of gamma and ripple oscillations (Ponomarenko *et al.*, 2004; Scheffzuk *et al.*, 2013). GABA antagonists, in general, can reduce the drive to networks and thereby affect frequency of oscillations and probably firing rate of many cells. Moreover, Gray (1978) suggested that the septohippocampal

system functions as a comparator, considering outcomes before happening. Indeed, the hippocampus plays a role in response inhibition when a conflicting stimulus is encountered or during interval operant tasks (Chan *et al.*, 2001; Ponomarenko *et al.*, 2008).

In summary, the hippocampus plays an essential role during explorative locomotion and may integrate conflicting stimuli, including anxiolytic stimuli, during behavioural guidance.

1.6.7. Hippocampal slow rhythmic oscillations during immobility

In the immobile, awake state the hippocampus expresses slow "sensory-related" oscillations when the animal is highly attentive and aroused (Sainsbury *et al.*, 1987). This slow sensory-related oscillation occurs upon visual, auditory or tactile stimulation and when the vestibular system is stimulated upon a passive rotation of the animal, and its frequency correlates with rotation speed (Tai *et al.*, 2012). Under anaesthesia it can be elicited by sensory stimulation. The sensory-related slow oscillation has been detected during movement preparation, for a time frame of several seconds before a movement was initiated (Bland *et al.*, 2007). Its frequency and amplitude could be related to subsequently executed behaviour (Whishaw and Vanderwolf, 1973).

This rhythm has been referred to as "sensory-related theta" as the frequency band overlaps with that of classical "movement-related" theta (Kramis *et al.*, 1975). However, while the sensory-related slow oscillation is abolished by the acetylcholine receptor antagonist atropine, locomotor theta is not (Kramis *et al.*, 1975). There is recent evidence that not only atropine sensitivity, but also current-source density and voltage-depth profiles differ. The slow rhythmic oscillation occurring during alert immobility was thereafter referred to as a respiration-entrained rhythm (Yanovsky *et al.*, 2014; Zhong *et al.*, 2017).

1.7. Relation of hippocampal theta to sleep, memory and time coding

1.7.1. Hippocampal theta during sleep

During sleep, when the brain does not integrate signals from the environment, the hippocampus displays theta oscillations during REM sleep when the EEG appears similar to that during the wake state (Jouvet, 1969). Rapid eye movements can then be detected by the EOG (Aserinsky and Kleitman, 1953). During REM sleep the cholinergic system becomes activated, as during the wake state (Vazquez and Baghdoyan, 2001). Due to muscle atonia the execution of movements is precluded, only heart beats and occasional twitches are detected by the electromyogram (EMG, Chase and Morales, 1990). Motoneurons receive barrages of IPSPs and show decreased input resistance during REM sleep, which makes them relatively insensitive towards excitatory inputs (Chase and Morales, 1990). In human adults REM sleep composes 20-25 % of total sleep. The duration of a REM epoch correlates with the duration of the following NREM epoch of the next NREM/REM cycle (Barbato and Wehr, 1998). REM epochs typically terminate by transitions to wakefulness (Jego *et al.*, 2013). Wake transitions are regulated by the ARAS, the ascending reticular activating system, which connects the brainstem to the

cortex (Moruzzi and Magoun, 1949). The fimbria-fornix carries ascending fibres from ARAS systems including the medial septum (Lewis and Shute, 1967).

REM sleep plays an important role in memory. According to the sequential hypothesis, during REM sleep processed memories are stored and integrated with pre-existing memories (Giuditta *et al.*, 1995). Temporal sequences of firing patterns of tens of seconds to minutes during wakefulness are reproduced during REM sleep at equivalent time scales (Poe *et al.*, 2000; Louie and Wilson, 2001). Hence, the behaviour-dependent modulation of subcortically driven theta is reproduced during REM sleep. Information is transferred from the hippocampus to the neocortex during REM sleep (Buzsaki, 1996; Diekelmann and Born, 2010). According to the ontogenetic hypothesis, REM sleep protects the brain from excessive experience-dependent plasticity (Roffwarg *et al.*, 1966). REM sleep plays an important role in regulating discharge rates and synchrony in the hippocampus during overall sleep (Grosmark *et al.*, 2012).

Differences in movement- and REM sleep theta have been reported. Theta synchrony across hippocampal regions and theta coherence across hippocampal layers is higher during REM sleep theta compared to movement theta (Montgomery *et al.*, 2008). Theta amplitude is higher during REM sleep- than during movement theta (Montgomery *et al.*, 2008). Theta frequency is lower during REM sleep compared to movement theta (Patel *et al.*, 2012; Montgomery *et al.*, 2008). Many CA1 pyramidal cells shift their preferred firing phase from the through to the peak during REM sleep (Mizuseki *et al.*, 2011; Poe *et al.*, 2000). Neuronal firing rate during REM sleep increases in CA1 but decreases in CA3 (Montgomery *et al.*, 2008; Mizuseki *et al.*, 2009). Reversed theta signaling flow, namely from subiculum to CA3, can be observed more frequently during REM sleep (Jackson *et al.*, 2014). Across the hippocampus theta frequency is consistent and amplitude decreases along the septohippocampal axis during REM sleep as during movement (Patel *et al.*, 2012).

Mechanisms which underlie movement and REM sleep theta rhythm generation may be different. The medial septum to hippocampus pathway is essential for hippocampal theta generation during locomotion and REM sleep (Brown *et al.*, 2012). However, posterior septal lesions suppress theta during waking, anterior septal lesions suppress theta during REM sleep (Monmaur *et al.*, 1979). During REM sleep atropine sulfate abolishes the lower frequency component of theta, while the intermittent and higher frequency components still occur during short intermitted periods with muscle twitches. REM sleep theta might consist of atropine-sensitive and atropine-resistant theta (Robinson *et al.*, 1977).

1.7.2. Hippocampal theta and memory

Episodic memory, hence storage of the temporal sequence of events of an experience, is attributed to the hippocampus in animals (Fortin *et al.*, 2004; Buzsaki and Moser, 2013) as well as in humans (Vargha-Khadem *et al.*, 1997). In humans, hippocampal electrical stimulation reactivates visual, and sometimes auditory or olfactory memories. Hippocampal theta power and memory performance are

correlated (Kaplan *et al.*, 2012; Cornwell *et al.*, 2008). Retrieval and encoding of events takes place online during explorative locomotion when the hippocampus exhibits theta rhythm. The theta state facilitates long-term potentiation (LTP) and enables learning (Otto *et al.*, 1991). Theta phase might temporally separate the retrieval and encoding phase (Hasselmo and Stern, 2014). Memory retrieval is suggested to take place during the theta through, when pyramidal cells are most active and the network is driven by previously potentiated synapses (reviewed in Hasselmo and Stern, 2014). Indeed, LTP is maximal at Schaffer collaterals when they were stimulated during the theta peak, while stimulation during the theta trough can induce long-term depression. Encoding is suggested to take place during the theta peak, when inputs are maximal and LTP occurs in CA3-CA3 recurrent connections and CA3-CA1 Schaffer collateral connections. The right hippocampal hemisphere in humans appears to have a greater role in spatial processing, while the left hippocampal hemisphere represents sequential aspects of episodic experiences (Burgess *et al.*, 2002; Maguire *et al.*, 1997). A similar lateralization may also exist in rodents (Shipton *et al.*, 2014; Klur *et al.*, 2009). The functional role of hippocampal theta may change depending on the momentary requirements of the task that the animal performs.

1.7.3. The hippocampus and time coding

The hippocampus guides behaviour across space and time. It has been implicated that the hippocampus codes time (Pastalkova *et al.*, 2008; Ponomarenko *et al.*, 2008). In rodents, multiple studies have reported that neurons in the hippocampal CA1 region sequentially fire as if they maintain memories or represent elapsed time (Kraus *et al.*, 2013; Pastalkova *et al.*, 2008; MacDonald *et al.*, 2011; MacDonald *et al.*, 2013). The peak firing rates of these "time cells" are typically above 10 Hz (MacDonald *et al.*, 2013). During wheel running, neuronal CA1 pyramidal cell assemblies can predict elapsed time (15-20 seconds) with a precision of 0.5 s (Itskov *et al.*, 2011). Timing could be either generated by a central mechanism (Church, 1984) or different subsystems could produce their own timing (Mauk and Buonomano, 2004). Different time scales include the supra-second (gamma frequency) range for cognitive processes, such as integration of auditory and visual stimuli, and the subsecond (theta frequency) range for motor coordination and integration of proprioceptive signals (Mauk and Buonomano, 2004).

1.8. The medial prefrontal cortex

Cytoarchitectonically the medial prefrontal cortex (mPFC) is organized into the medial, precentral cortex, the anterior cingulate cortex, as well as the prelimbic and infralimbic prefrontal cortex (Heidbreder and Groenewegen, 2003). The rodent mPFC comprises layers I,II/III, V/VI, but lacks the classical input layer IV (Uylings *et al.*, 2003). Pyramidal cells comprise 80-90 % of mPFC cells and show a wide range of firing properties (Wang *et al.*, 2006; Riga *et al.*, 2014). The mPFC contains a variety of interneurons (Riga *et al.*, 2014).

Incoming projections arrive from the cortex and hippocampus, as well as from the medial basal forebrain, amygdala, thalamus, brainstem, and few projections from the hypothalamus (Hoover and Vertes, 2007; Conde *et al.*, 1995). The mPFC has an important function in predicting the outcome, such as reward, of a situation on the bases of memories (Alexander and Brown, 2011; Schoenbaum *et al.*, 2009). It integrates the common aspects of a certain situation, which is repeated over time, and generates predictions, such as sensory and contextual features and value, of eventual outcomes based on the reward history (Rushworth and Behrens, 2008).

It is suggested that the PFC functions as a control board and guides goal-directed behaviour according to the momentary context (Miller and Cohen, 2001). Heavy projections to other cortical and subcortical regions, which are directly involved in sensory perception, memory processes, motivation and motor control, enable the mPFC to exert control over visceral, autonomic, limbic and cognitive functions (Hoover and Vertes, 2007; Miller and Cohen, 2001; Heidbreder and Groenewegen, 2003; Fuster, 2004). The time frames during which representations are maintained in the active state are guided by the mPFC, which simultaneously modulates the computation in the respective brain areas. The mPFC is suggested to mediate top-down executive control through the dense interconnectivity with subcortical regions (Miller and Cohen, 2001). Top-down processing is theory-driven: the brain is regarded an active, adaptive system which takes into account background information and experience to select meaningful inputs which agree with intrinsic goals and motivations (Fries, 2015). Attention can be focused volitionally by "top-down" signals (Fries, 2015).

Functionally, the mPFC is different from and complementary to the hippocampus. The brain has to maintain the structure of experience over time. While the hippocampus is believed to encode sequences that unfold over time, thereby linking time and space, the mPFC is suggested to represent events that extend in time (Eichenbaum, 2017b). It has been suggested, that the hippocampus leads the mPFC during context exploration, while the mPFC leads the hippocampus during object discrimination (Eichenbaum, 2017b). Both structures are bidirectionally connected through cortico-cortico and subcortical routes (Eichenbaum, 2017b).

1.9. Gamma oscillations

Gamma oscillations (30-100 Hz) are ubiquitous throughout the brain (Gray and Singer, 1989; Bragin *et al.*, 1995; Csicsvari *et al.*, 2003; Kay, 2003), and have been recorded previously in the mPFC (Sirota *et al.*, 2008; O'Neill *et al.*, 2013; Colgin, 2011). Gamma is the most characteristic pattern of the neocortex during the awake and active state. Time windows of gamma oscillations, 15-30 ms, are suggested optimal for efficient integration of excitatory inputs by pyramidal cells and neuronal coordination into assemblies (Csicsvari *et al.*, 2003; Harris *et al.*, 2003; Harris, 2005). Slow gamma (~25–60 Hz) and fast gamma (~60–100 Hz) oscillations can be distinguished (Colgin *et al.*, 2009; Colgin, 2016). How gamma oscillations are generated is still incompletely understood but likely involves gap junctions. The

periodicity of gamma oscillations reflects local competition of inhibitory and excitatory signals. Gamma can be synchronous across distant brain regions (Engel *et al.*, 1991).

Cognitive processes are linked to gamma oscillations (Canolty *et al.*, 2006, Kim *et al.*, 2016, Cardin *et al.*, 2009). Gamma oscillations play a major role during attention (Fries *et al.*, 2001; Kim *et al.*, 2016), stimulus selection (Fries *et al.*, 1997), sensorimotor integration (Womelsdorf and Fries, 2006), movement preparation (Sanes and Donoghue, 1993) and for memory (Yamamoto *et al.*, 2014; Igarashi *et al.*, 2014). Gamma oscillations allow binding of sensory inputs - visual, sensory, olfactory etc. - into a complex representation (Singer and Gray, 1995). Gamma band synchrony across brain regions increases with attention (Fries *et al.*, 2001; Lakatos *et al.*, 2008). Increase in gamma band synchronisation across brain areas was detected during performance in cognitive tasks (Engel *et al.*, 2001; Varela *et al.*, 2001)

1.10. The lateral septum

1.10.1. Anatomy of the lateral septum

The lateral septum (LS) is located in the middle of the brain, adjacent to the lateral ventricles. It is divided into a caudal, rostral and ventral part, based on chemoarchitecture (Risold and Swanson, 1996).

The LS is the main subcortical output region of the hippocampus (Risold and Swanson, 1997b; Swanson and Cowan, 1979). These projections arrive via the fimbria-fornix (Swanson and Cowan, 1977). They are routed ipsilaterally along a dorsoventral and rostrocaudal gradient and mainly project to the dorsolateral nucleus and only sparsely to the mediolateral nucleus of the septum (Swanson and Cowan, 1979; Staiger and Nurnberger, 1989; Risold and Swanson, 1996; Swanson, 1977). Hippocampal area CA1 projects mainly to the rostral lateral septum and area CA3 to the caudal lateral septum. The ventral hippocampus projects to the ventral lateral septum (Risold and Swanson, 1996). The lateral septum receives further strong projections from the prefrontal cortex (Carus-Cadavieco *et al.*, 2017; Sheehan *et al.*, 2004). Besides, the entorhinal cortex, amygdala, hypothalamus, brain stem areas, SUM, ventral tegmental area, raphe nuclei, locus ceruleus, laterodorsal tegmentum and bed nucleus project to the lateral septum (Sheehan *et al.*, 2004; Risold and Swanson, 1997b). The hypothalamus contacts those lateral septum cells which send projections back to the hypothalamus and receive innervation from the hippocampus (Jakab and Leranth, 1993; Varoqueaux and Leranth, 1997; Holderith *et al.*, 1998).

Main outgoing projections from the lateral septum are subcortical (Risold and Swanson, 1997b). The lateral septum projects to diencephalic and mesencephalic regions. Major descending projections from the lateral septum are to the hypothalamus and motor systems in the upper brain stem (Risold and Swanson, 1997b; Swanson and Cowan, 1975; Swanson and Cowan, 1977). The caudal lateral septum projects to the lateral hypothalamus (Risold and Swanson, 1996). Virtually all LS efferents are GABAergic (Risold and Swanson, 1997a). These cells provide recurrent axon collaterals to

neighbouring projection neurons (McLennan and Miller, 1974a). Intraseptal GABA release might have an autoinhibitory function, by coordinating firing within the lateral septum itself (McLennan and Miller, 1974b). The lateral septum also projects to the SUM and raphe nuclei (Staiger and Nurnberger, 1991a; Risold and Swanson, 1997b). Re-examination of lateral septum outputs revealed that it does not, as previously believed, project to the medial septum (Leranth *et al.*, 1992).

1.10.2. The lateral septum and behaviour

The lateral septum is referred to as an interface between cortical and subcortical structures. Its basic function is that of an integrator: cognitive and sensory information, relevant for guidance of motivationally aspects of behaviours, is gaged according to affective relevance and valence and is then relayed to subcortical regions, which control appropriate behaviours according to environmental context and current demands.

Locomotor behaviour is modulated by the lateral septum. It forms part of the behavioural control columns (Swanson, 2000), mediating controlled and conscious behaviours by regulating motor systems. A portion of lateral septum cells displays speed-related activity (Zhou *et al.*, 1999). The lateral septum further plays a major role in the expression of affective behaviours. In the absence of dangers, in environments which are considered safe as contextual cues indicate the absence of danger, lateral septum cells increase firing rate (Wong *et al.*, 2016). An increase in lateral septum cell firing inhibits abrupt fear-related behaviours. Increased firing of lateral septum cells suppresses the exploration of threatening environments and defence responses via direct LH projections (Schwerdtfeger and Menard, 2008). In accordance, benzodiazepines increase firing of lateral septum neurons. When the lateral septum is electrically stimulated, in contrast, neuronal activity diminishes fear responses, such as abrupt freezing or flight, reduces anxiety and is positively rewarding. Instead, a decrease in lateral septum cell firing is related to enhanced defensive readiness (Sheehan *et al.*, 2004; Behrendt, 2010) and inhibition of lateral septum neuronal activity enables fear responses, maybe established via hypothalamic projections (Sheehan *et al.*, 2004). Animals with lateral septum lesions are highly attentive, receptive and alert and show enhanced readiness to defence reactions or approach. The lateral septum further plays a role in mood and motivation, social functions, such as aggression, dominance behaviours and subordination, bonding, mating, parental attachment, as well as drug craving, psychosis and depression (Sheehan *et al.*, 2004). It contains various hormone receptors and maintains the balance between endocrine and emotional information flows (Numan, 2000; Sheehan *et al.*, 2004).

1.10.3. The septal nuclei in humans

In humans the medial and lateral septum are not, as originally believed, atrophic. On the contrary, septal nuclei are larger, relative to the overall body size, in humans than in lower primates (Andy and Stephan, 1968). Their dysfunction is suggested to underlie abnormal brain rhythms in the distinctly

human disorder schizophrenia (Butler *et al.*, 2014). Schizophrenia is associated with GABAergic dysfunction in the hippocampus that affects predominantly the fast-spiking PV+ cells, which play an important role in theta generation (Gisabella *et al.*, 2005). PV+ immunoreactivity is reduced in hippocampi of schizophrenic patients (Zhang and Reynolds, 2002) and the hippocampal hyperactivity probably underlies the hallucinations and abnormal thought processes (Heckers, 2001). Besides, enlargement of septal nuclei was reported in human patients with temporal lobe epilepsy (Butler *et al.*, 2014). Septal function is further implicated to play a role in amnesia (Voncramon and Schuri, 1992), anxiety (Gray, 1978), addiction (Sheehan *et al.*, 2004) and movement disorder (Levy *et al.*, 1997).

1.11. The locomotor regions of the brain

The behavioural control columns according to Swanson (2000) comprise the spinal cord motoneurons communicating with local central pattern generators (CPGs), which control timing of muscle contractions and communicate with locomotor regions of the central nervous system, including the mesencephalic locomotor region and the hypothalamic locomotor region. CPGs have a core of excitatory neurons which generate recurring bursts of activity (Yuste *et al.*, 2005; Sillar, 1991).

The lateral hypothalamus comprises the diencephalic locomotor region (Sinnamon, 1993; Grillner *et al.*, 2008). The posterior hypothalamus exhibits theta rhythmicity during locomotion (Slawinska and Kasicki, 1995; Slawinska and Kasicki, 1998). Hypothalamic locomotor regions control CPGs via the reticular formation, the somatomotor system, the autonomic visceromotor system and the neuroendocrine secretomotor system (Kiehn and Dougherty, 2013). Locomotor stepping can be induced via electrical stimulation of the lateral hypothalamus (Levy and Sinnamon, 1990). The hypothalamus projects to locomotor initiation regions in the brainstem reticular formation (Kiehn and Dougherty, 2013). Information from the hippocampus to the hypothalamus is mainly relayed via the lateral septum (see above). The subiculum projects directly to the hypothalamus. The hypothalamus projects back to the hippocampus (Leranth and Nitsch, 1994) and to the medial septum (Cullinan and Zaborszky, 1991).

Several other brain regions play an important role in locomotor control. The basal ganglia control the selection of locomotor behaviours (Graybiel *et al.*, 1994), which is further enabled by circuits in the lateral and medial hypothalamus (Levy and Sinnamon, 1990; Sinnamon, 1993; Schwerdtfeger and Menard, 2008). The basal ganglia project to the motor cortex via the thalamus, as well as to the mesencephalic locomotor region. Basal ganglia were suggested to encode familiar repeated sequences, while the hippocampus encodes new episodic sequences (White and McDonald, 2002). Cortical activity serves to correct motor activity in response to visual information via the posterior parietal cortex. Initiation of locomotion is mediated by the mesencephalic locomotor region which projects to the medial reticular formation in the lower brainstem. The medial reticular formation projects to the locomotor CPG in the spinal cord. The vestibular and rubrospinal pathways modulate ongoing movement. The cerebellum coordinates movement. It generates movement-related as well as internal

feedback and modulates the activity of descending reticulospinal, rubrospinal and vestibularspinal pathways. The cerebellum receives proprioceptive sensory feedback relayed via the CPGs and is updated about the CPG activity (Kiehn and Dougherty, 2013).

If the hippocampus guides locomotor speed it may be enabled via prominent projections to the lateral septum which targets locomotor regions in the brain, such as the hypothalamus, which communicate with the CPGs.

1.12. Optogenetic and pharmacogenetic methods

1.12.1. Optogenetic stimulation and inhibition

Optogenetics refers to the use of microbial opsins which can be activated by light of a certain wavelength to activate or inhibit synaptic transmission with a high temporal and spatial resolution (reviewed in Tye and Deisseroth, 2012). It can be applied in the behaving animal (Carter and de Lecea, 2011).

Channelrhodopsin 2 (ChR2), a cation channel, enables time-locking of action potentials to the applied light pulses (Nagel *et al.*, 2002; Nagel *et al.*, 2003). ChR2 passes preferentially protons and sodium, but also calcium and potassium (Nagel *et al.*, 2003). It is extracted from the green algae *Chlamydomonas reinhardtii* (Nagel et al., 2002). In hChR2 the algal codon has been replaced by a mammalian codon. Here, the algal codon was substituted by the mammalian codon to achieve higher expression levels. Peak activation of ChR2 (H134R) is at 470 nm (Nagel *et al.*, 2003; Yizhar *et al.*, 2011a; Yizhar *et al.*, 2011b) and off kinetics (tau) are 18 ms (at room temperature (RT), Yizhar *et al.*, 2011b). ChR2 does not enable reliable firing above 40 Hz (Lin *et al.*, 2009; Boyden *et al.*, 2005). For ultrafast, up to 200 Hz, optogenetic control the opsin ChETA was designed (Gunaydin *et al.*, 2010). ChETA allows for the entrainment of fast oscillations, such as gamma and ripple oscillations (Carus-Cadavieco *et al.*, 2017).

Halorhodopsin (NpHR) enables hyperpolarisation and thereby the inhibition of neuronal signaling (Zhang *et al.*, 2007). It is a fast light-activated electrogenic chloride pump, which pumps chloride into the cells and protons out of the cells (Han and Boyden, 2007). Peak activation is at 590 nm wavelength of light and off kinetics are 4.2 ms (at RT, Yizhar *et al.*, 2011b). ChR2, in contrast, does not respond to light of a wavelength of 590 nm (Yizhar *et al.*, 2011b). Halorhodopsin is extracted from *Natronomonas pharaonis*. It requires constant light, as it is a pump. Surface membrane localization was enhanced in eNpHR, "e" stands for "enhanced" (Gradinaru *et al.*, 2008), as NpHR-YFP expressing cells showed accumulation of fluorescence colocalized with the endoplasmatic reticulum (Gradinaru *et al.*, 2008).

Adeno-associated viral vectors (AAV, Monahan and Samulski, 2000) are widely used to introduce opsins and other genes into mouse cells and enable high expression levels over long time periods and little or no adverse effects have been observed. AAV-based vectors display low immunogenicity. Different serotype packaging systems can be used. For instance, AAV5 will lead to more diffuse expression in the entire target structure, while AAV2 can result in a restricted, more local, expression pattern.

Different promoters enable specific expression of opsins in the desired target neuronal population. For a broad targeting of excitatory neurons, the CamKIIα (calcium calmodulin-dependent kinase II α) promoter can be used (Zhang *et al.*, 2007; Dittgen *et al.*, 2004). Cre recombinase driver lines are used in conjunction with Cre-dependent opsin-expressing viral vectors to allow for specific expression of the opsin in the target neuronal population. Parvalbumin (PV)::Cre mice express Cre-recombinase in PV+ cells (Hippenmeyer *et al.*, 2005). Parvalbumin is a calcium-binding albumin protein, which acts as a slow calcium buffer.

Regarding the light source, lasers offer a very narrow spectral linewidth (typically below 1 nm) which can be matched to the peak activation wavelength and can be modulated at high frequencies. Diode-pumped solid-state (DPSS), lasers are considered most appropriate (Adamantidis *et al.*, 2007; Aravanis *et al.*, 2007). Light power densities of 1-5 mW/mm^2 were sufficient to elicit action potenials in ChR expressing neurons in wildtype (WT) mice (Boyden *et al.*, 2005).

1.12.2. Pharmacogenetic inhibition using DREADDs

DREADDs, designer receptors exclusively activated by designer drugs, were developed by directed evolution of muscarinic acetylcholine (ACh) receptors in yeast. In neurons these GPCR, G protein-coupled receptors, are trafficked to dendrites and axon terminals (Alexander *et al.*, 2009). They can be activated by nanomolar concentrations of the metabolically inert CNO, clozapine-N-oxide, while at the same time being insensitive to the native ligand acetylcholine (Zhu and Roth, 2014).

The human M4 muscarinic (hM4) modified DREADD (hM4Di) is coupled to Gi signaling and mediates neuronal and synaptic silencing (Stachniak *et al.*, 2014; Armbruster *et al.*, 2007). The hM4Di-DREADD upon CNO administration activates the G protein inwardly rectifying potassium (Girk) channel which leads to the hyperpolarization of the neurons and decreases neuronal activity *in vitro* and *in vivo* (Armbruster *et al.,* 2007*)*.

Combination of DREADDs and optogenetics has been successfully implemented in previous studies (Stachniak *et al.*, 2014).

1.13. Aims of the study

The aims of this study were the following.

1. To develop a preparation for optogenetic entrainment of hippocampal theta oscillations in behaving mice.

2. To determine the causal relationship between hippocampal theta oscillations and locomotor speed.

3. To verify the relevance of the lateral septum for locomotor regulation by the hippocampus.

4. To test whether mPFC to LS entrainment at gamma frequencies affects performance in a food rewarded spatial learning task.

2. Methods

2.1. Experimental subjects

In this study PV-Cre knock-in mice (The Jackson Laboratory, Hippenmeyer *et al.*, 2005) and WT (C57BL/6) male mice, 10-25 week old, were used. They were housed under standard conditions in the animal facility according to the institutional guidelines and kept on a 12 h light/dark cycle. All procedures were performed in accordance with national and international guidelines and were approved by the local health authority (Landesamt für Gesundheit und Soziales (LaGeSo), Berlin).

2.2. Virus injections

For virus injections mice were deeply anesthetized with isoflurane and placed in a stereotactic head frame (David Kopf instruments). After a midline incision craniotomy (coordinates according to Franklin and Paxinos, 2008) was performed using a dental drill the virus was infused via a metal needle (34 gauge) connected via a tube with a microsyringe pump (PHD Ultra, Harvard Apparatus) at a rate between 100 and 250 nl/min, depending on virus viscosity. To prevent excessive spread of the virus, the needle was kept for 10 minutes at the injection site before it was carefully withdrawn. The incision was sutured. Different preparations used in different sets of experiment are described in the following.

In the medial septum (AP (anterior-posterior) 0.98; L (lateral) 0.0; V (ventral) -5.0 and -4.5 mm) of PV-Cre mice a total of 1 μl of Cre-dependent ChR2 (*AAV2/1.CAGGS.flex.ChR2.tdTomato*, Penn Vector Core, titer: 1.42×10^{13} vg/ml) was injected. ChR2 was expressed under the Cre promoter in PV-Cre mice, which expressed Cre recombinase exclusively in PV^+ neurons, to ensure selective expression in GABAergic medial septal cells which co-express PV, but not in cholinergic or glutamatergic medial septal cells.

In the hippocampus (AP -1.7; L ±1.05; V -2.05 and -1.4 mm; AP -1.7; L ±1.7; V -2.05 and -1.55 mm; AP -2.3; L ±1.5; V -2.2 and -1.3 mm; AP -2.3; L ±2.2; V -1.65 and -2.45 mm) of PV-Cre mice expressing ChR2 in PV^+ medial septal cells a total of 2.4 μl of CaM kinase II dependent halorhodopsin (eNpHR3.0, *AAV2/1.CamKIIa.eNpHR3.0-EYFP.WPRE.hGH*, Penn Vector Core, titer: 2.08×10^{12} vg/ml) was injected bilaterally. For control experiments 2.4 μl of CaM kinase dependent YFP (*AAV2-CaMKIIa-eYFP*, UNC vector core, titer: 5×10^{12} vg/ml) was injected bilaterally in the hippocampus (same coordinates). The promoter CaM kinase II α restricted expression to hippocampal pyramidal cells, which project to the lateral but not to the medial septum.

In the hippocampus (same coordinates as described above) of PV-Cre mice expressing ChR2 in PV^+ medial septal cells a total of 2 μl of CaM kinase dependent inhibitory DREADD (*AAV8.CaMKIIa.hM4D(Gi).mCherry*, construct from Dr. Bryan Roth, UNC Gene Therapy Center Vector Core, titer: 2×10^{12} vg/ml) was injected bilaterally. The promoter CaM kinase II α restricted expression to hippocampal pyramidal cells, which project to the lateral but not to the medial septum.

In the mPFC (AP 1.7, L ±0.35, V -2.85 mm) of WT mice a total of 0.5-1 µl of CaM kinase dependent ChR2 (*AAV2-CaMKIIa-hChR2(H134R)-eYFP*, Penn Vector Core, titer: 2.55×10^{12} vg/ml) or ChETA (*AAV5-CaMKIIa-ChETA(E123T/H134R)-eYFP-WPRE-hGH*, Penn Vector Core, titer: 1.26×10^{13} vg/ml) was bilaterally injected. For control experiments a total of 1 µl of CaM kinase dependent YFP (AAV2-CaMKIIa-eYFP, UNC vector core, titer: 5×10^{12} vg/ml) was bilaterally injected in the mPFC (same coordinates) of WT mice.

2.3. Implantation of optic fibers and guide cannulas

For optogenetic as well as for pharmacogenetic experiments, animals were implanted after allowing at least 6 weeks of expression time of the virus to ensure that opsin expression reached the distal axons of the infected cells, as has been reported in previous studies (e.g. Ciocchi *et al.*, 2010).

Optic fibers were fabricated from fiber (100 µm, 0.22 NA, Thorlabs) and zirconia ferrules (Precision Fiber Products) and implanted chronically for optogenetic stimulation experiments. For hippocampal theta entrainment experiments one optic fiber was implanted in the right hippocampus above the CA1 pyramidal layer or bilaterally for contralateral stimulation experiments (AP -1.94, L ±1.4, V -1.4 mm). For optogenetic inhibition of the hippocampus to lateral septum projections or stimulation of mPFC to lateral septum projections experiments optic fibers were implanted bilaterally in the lateral septum (AP 0.1, L 0.25, V -2.25 mm and AP 0.5, L -0.3, V -2.7 mm).

Guide cannulas (22 gauge, PlasticsOne) for pharmacogenetic inhibition experiments were implanted bilaterally (4 mice) or unilaterally (2 mice) above the lateral septum (right: AP 0.5, L 0.3, V -1.7, left: AP 0.38 mm, L -1.5 mm, V -0.66 mm, 20° L).

Electrodes were implanted chronically to record LFP and/or unitary discharge. Reference and ground electrodes were miniature stainless-steel screws in the skull above the cerebellum. EEG electrodes were screws above the frontal lobe. The implants were secured on the skull with dental acrylic. Wire arrays were fabricated using 40 µm tungsten wires (angular cut, California Fine Wire Company), microdrives (Minidrive-8, BioSignal Group) were loaded with 8 independently movable tetrodes (12 µm tungsten wire, California Fine Wire Company). Linear probes (CM32, NeuroNexus Technologies) were mounted on a custom-made microdrive and implanted as described in Fidzinski *et al.*, 2015 and Bender *et al.*, 2015.

Implantation sites were the following: In the dorsal hippocampus (AP -1.94, L 1.4, V -1.4 mm, tungsten wire arrays), or above the dorsal hippocampus (AP -1.94, L 1.4, V -1 mm,) with subsequent lowering to the CA1 pyramidal cell layer with LFP and unitary activity as reference (tetrodes, CM32), in the ventral hippocampus (AP -3.16, L 2.5-3.5, V -4 mm, tungsten wire arrays), in the lateral septum (AP 0-0.5, L 0.2-0.45, V -2.3 to -3.4 mm, tungsten wire arrays) and in the mPFC (AP 1.4–1.9, L 0.3, V -3.0 mm, tungsten wire arrays).

2.4. Data acquisition

Chronically implanted electrodes were connected to headstages (HS-8, Neuralynx, or Noted B.T.) before the start of the experiments. Operational amplifiers eliminated cable movement artefacts. Electrophysiological signals were differentially amplified, band-pass filtered (1 Hz –10 kHz, Digital Lynx, Neuralynx) and acquired continuously at 32 kHz. A light-emitting diode was attached to the headset to track the animal's position (at 25 Hz) for hippocampal theta entrainment experiments. Timestamps of laser pulses were recorded together with electrophysiological signals.

2.5. Behavioural setups

After surgery animals were allowed several days of recovery before first experiments were conducted. Subjects were randomly assigned to the experimental conditions. Patch cord and headstage cables were light and flexible and did not restrict the behaviour of the mice. When connected mice were able to run, rear, climb, jump, groom, eat and sleep normally.

For locomotor behaviour experiments PV-Cre mice were recorded in a familiar, rectangular chamber (48 x 30 cm^2) constructed with pieces of wood which were painted dark grey. Mice could explore the enclosure freely. Mice were not food or water restricted.

For sleep experiments PV-Cre mice were recorded in a sleep-promoting clay flower pot with soft paper bedding for 2 hours after a 30 minute exploration session in an enriched environment (48 x 30 cm^2). Experiments were conducted during the dark cycle (between 11 am and 4 am).

For learning and decision making experiments mice were recorded in a T-maze constructed with pieces of wood which were painted dark grey. The dimensions of the start arm were 46 x 11 x 10 cm^3 and of the choice arm 80 x 11 x 10 cm^3. Mice were habituated to the set-up before first experiments were conducted. The spatial non-matching to place task was performed as described in Korotkova *et al.* (2010). During the sampling run the left or right arm, according to a pseudo-random sequence with equal numbers, of the T-maze was blocked. The food (20 mg pellet) or water reward was located at the end of the arm which could be explored. Between the sampling run and the test run the mouse rested for 10-15 seconds in a familiar chamber. For the test run both arms were accessible, however the reward was placed at the end of the previously not visited arm. Thus, if the arm was alternated between the sample and the test run the mouse was rewarded. If the mice entered the previously visited arm with all four paws the test run was terminated and the mouse did not receive the reward. Between trials the mouse rested in the familiar chamber for 3-5 minutes. One mouse conducted 10 trials per day and 20-40 trials in total.

2.6. Optogenetic protocols

For optogenetic experiments chronically implanted optic fibers were connected to fiberoptic patch cords with protective tubing (Thorlabs) via a zirconia sleeve (Precision Fiber products). Patch cords were connected with a FC/PC adapter (Thorlabs) to a 473-nm (R471005FX) or with a multimode fiber

optic coupler (FCMM50-50A-FC, Thorlabs) to a 593-nm (R591005FX) diode-pumped solid-state laser (Laserglow technologies). Time stamps of laser pulses were controlled using a stimulus generator and MC_Stimulus software (Multichannel Systems) and customized protocols. Light power output at the tip of the optic fiber was estimated using a power meter (PM100D, Thorlabs). Blue light with a wavelength of 473 nm is optimal for activation of channelrhodopsin or ChETA (Nagel *et al.*, 2005; Gunaydin *et al.*, 2010). Yellow light with a wavelength of 593 nm is optimal for activation of halorhodopsin (Gradinaru *et al.*, 2010).

Rhythmic optogenetic stimulation of hippocampal theta pacemaker cells consisted of 30 ms blue light pulses (10-15 mW). Baseline recording preceding optogenetic stimulation was at least 2 minutes in each experiment. Three different stimulation protocols were designed as follows (see Fig. 2.1). Stimulation was applied at 7-10-7-10 Hz with 2 minutes duration per stimulation epoch when the mouse was spontaneously active for at least 20 seconds (protocol 1). Stimulation was applied at 2-4-6-8-10-12 Hz with 45 seconds duration per stimulation epoch when the mouse was not moving for at least 20 seconds (protocol 2). Stimulation was applied at 2, 9 or 20 Hz for 1 minute, respectively, and each stimulation epoch was preceded by a 20 second baseline epoch during which the mouse was not moving (protocol 3). By using different protocols described here the effects of theta entrainment during ongoing movement or upon immobility could be tested and whether different stimulation frequencies have different behavioural effects. To control for visual effects by light itself on behaviour light with the same wavelength, frequency and power was delivered to a dummy ferrule connected to the headstage in control light experiments (see Fig. 3.1d). For behavioural analysis speed data for control light application and optogenetic stimulation were compared for the same protocol, stimulation frequency and epoch.

The jittered theta frequency protocol consisted of 30 ms blue light pulses delivered with varying inter-pulse-intervals. The distribution of the stimulation frequency followed a gaussian distribution with a mean frequency of 7.8 Hz. Eleven different stimulation protocols were applied with increasing standard deviation (σ=1.56 to σ=15.07) of interpulse intervals distributions for one minute each, respectively.

One second pulses of blue light were applied with 2.7 seconds inter-pulse-intervals for 2 minutes to apply the same amount of light over time as during theta entrainment but using a non-theta rhythmic stimulation protocol.

Varying light output intensity. During hippocampal theta entrainment light output was varied between 2-20 mW manually.

For optogenetic inhibition of the hippocampus to lateral septum pathway continuous yellow light (~20 mW) was delivered bilaterally to the lateral septum. Inhibition was initiated 15 seconds before blue light was delivered at 7 or 9 Hz to the hippocampus (as in protocols described above) and lasted for one minute in total. Control experiments with the same light conditions but no optogenetic stimulation were performed as described above and for additional control experiments yellow light

was delivered to the lateral septum of mice expressing only the fuorophore mCherry but not the opsin eNpHR3.0.

For hippocampal theta entrainment experiments during sleep 30 ms blue light pulses were delivered at 6, 7, 8, 9, or 10 Hz to the hippocampal CA1 region. In one set of experiments optogenetic stimulation was applied during random vigilance states over the course of a 90 minute sleep session. Stimulation epochs lasted 2 minutes and inter-stimulation-intervals were 5 minutes. In a second set of experiments optogenetic stimulation was applied during REM sleep epochs. Vigilance state was detected online according to EEG, EMG and CA1 LFP signals. Upon onset of a REM sleep epoch optogenetic stimulation was initiated manually and terminated when a transition from REM sleep was detected. Control light experiments were performed as described above. Per mouse 1-2 baseline recordings with no stimulation were conducted.

Optogenetic gamma rhythmic stimulation during the T-maze task was applied throughout the time the mouse was in the T-maze. Blue light (10-25 mW) was delivered at 66.7 Hz with pulse durations of 5 ms bilaterally to the lateral septum. For non-gamma rhythmic stimulation experiments 167 Hz bursts of 4 ms pulses were repeated at 9 Hz, hence theta frequency. For control experiments blue light was delivered to the lateral septum of mice expressing only the fuorophore YFP but no opsin.

Figure 2.1
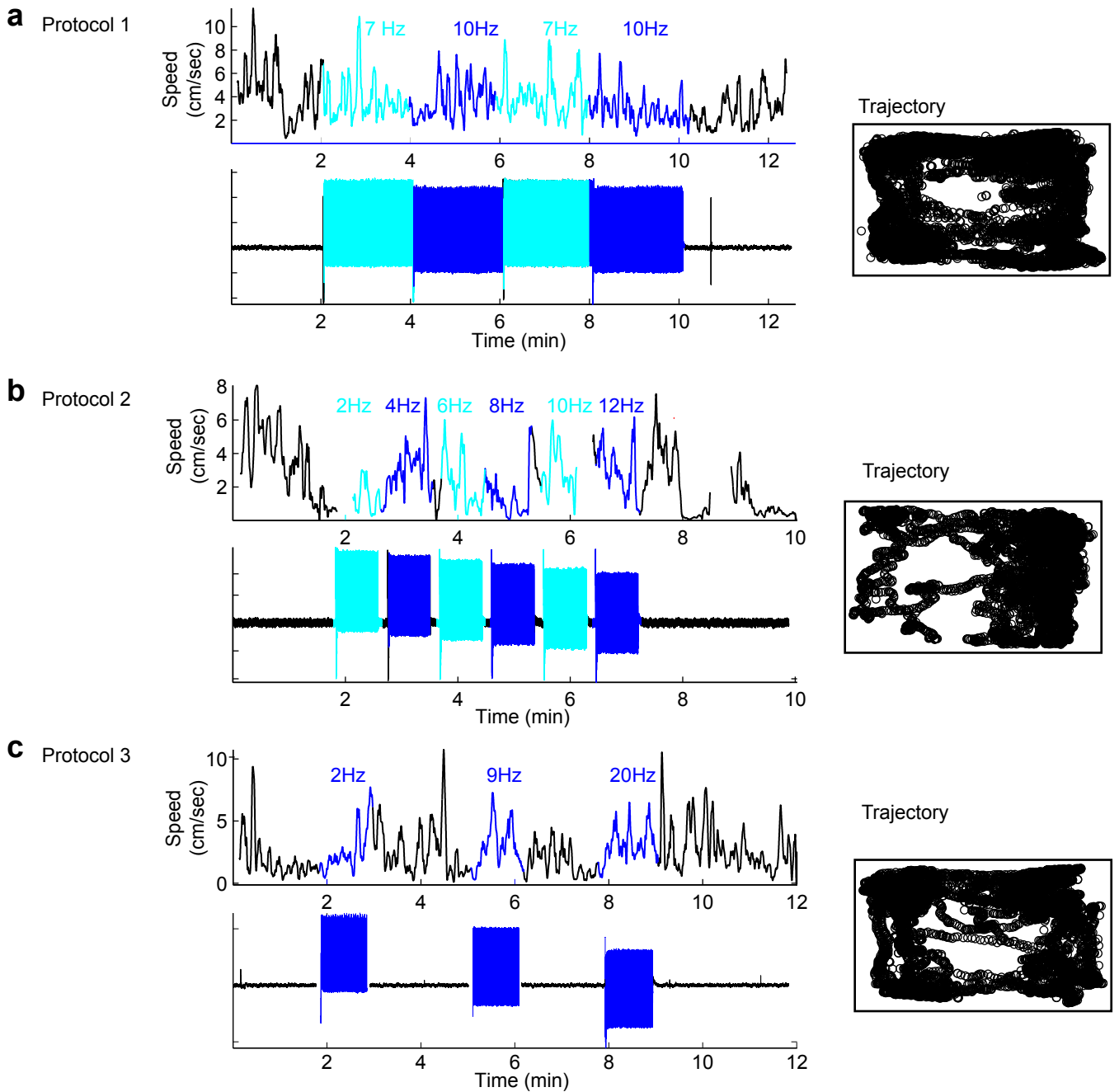
Fig. 2.1. Hippocampal theta entrainment protocols during behaviour.
Mice were recorded in a chamber which they could explore freely. Speed was tracked via a LED connected to the headset. Illustrated here are protocols applied for theta rhythmic entrainment of hippocampal LFP for one example experiment, respectively. Speed traces are depicted above, stimulation epochs depicted below. Different shades of blue serve to distinguish stimulation epochs. On the right the trajectory of the mouse is depicted which was recorded during the experiment shown on the left. In protocol 1 (**a**) stimulation was set to 7 Hz, followed by 10 Hz, another 7 Hz epoch and another 10Hz epoch. Each epoch was 2 minutes long. Stimulation was initiated manually when the mouse was constantly active for at least 20 seconds. In protocol 2 (**b**) frequency was increased from 2 to 4 to 6 to 8 to 10 to 12 Hz. Each epoch was 45 seconds long. Stimulation was initiated when the mouse was not moving for at least 20 seconds. In protocol 3 (**c**), during the course of the experiment stimulation was set to 2 Hz upon an epoch of quiet wakefulness of at least 20 seconds. The epoch was 1 minute long. When the mouse was again not moving for a minimum duration of 20 seconds, 9 Hz stimulation was initiated, which also lasted for one minute. After another epoch of quiet wakefulness for a minimum duration of 20 seconds, 20 Hz stimulation was initiated, which also lasted 1 minute.

2.7. Pharmacogenetic protocol

For pharmacogenetic inhibition of the hippocampus to lateral septum pathway, CNO (Sigma-Aldrich, 100 μM in artificial cerebrospinal fluid (aCSF) + 5 % DMSO) or vehicle (aCSF + 5 % DMSO) was infused (300 nl per injection side for bilateral injections or 600 nl per injection side for unilateral injections) using a microsyringe pump (PHD Ultra, Harvard Apparatus) at a rate of 100 nl/min via an internal cannula (28 gauge), which was temporally inserted into the guide cannula and protruded it by 1 mm from the tip of the guide cannula. Two minutes after termination of infusion the injector was slowly withdrawn. During infusion the mouse rested in the home cage and experiments began after 10 minutes.

2.8. Histology and microscopy

Successful and localized virus expression and electrode positions were confirmed by histology post-mortem after completion of experiments. Mice were deeply anaesthetized, electrolytic lesions were performed and mice were perfused intracardially with 4 % PFA in PBS (ChemCruz). Brains were fixed over night in PFA and equilibrated in 1 % PBS (Bio-Rad) the following night. Brain slices were cut using an oscillating tissue slicer (EMS 4500, Electron Microscopy Science) to 40 mm slices and mounted (Flouromount Aqueous Mounting Medium, Sigma-Aldrich). Images were taken with an Olympus BX 61 microscope (2/0.06 numerical aperture (NA), 10/0.3 NA and 20/0.5 NA, dry) or a Leica DM 2500 microscope (20/0.7 NA, oil-immersion objective).

2.9. Data analysis

Electrophysiological signals and position tracking data were processed with Neurophysiological Data Manager (NDManager67, http://neurosuite.sourceforge.net/). The LFP was obtained by low-pass filtering and down-sampling of the wide-band signal to 1250 Hz. Data were further processed by custom-written MATLAB (Mathworks, Natick) algorithms (Wulff *et al.*, 2009; Korotkova *et al.*, 2010). Data were stored in a database (MySQL) and automatically selected from the database for analysis. Power spectral density (PSD) was computed using multitaper method (the time-halfbandwidth product 3, window size 8,192 or 1,024) for 10 second sequences. Timestamps of laser pulses and on- and offset of stimulation epochs were detected.

For hippocampal theta entrainment experiments one channel with maximal theta amplitude was selected from each recording. The efficacy of entrainment was quantified as the ratio of the cumulative PSD around the stimulation frequency (±0.5 Hz) to the cumulative PSD in the theta frequency band (5–12 Hz). If dominant power peak for a recording epoch was below 5 Hz this recording epoch was excluded from analysis. Stimulation epochs with entrainment fidelity below 0.3 (approximately 20 % of recordings) were excluded from analysis, due to insufficient theta control.

To determine phase-amplitude coupling of theta and gamma oscillations epochs of theta oscillations with the theta/delta power ratio of at least 6 were selected (see Korotkova *et al.*, 2010). Theta phase was determined using Hilbert transformation of the theta-frequency band (5-10 Hz) filtered signal. Peak of gamma oscillations were detected in the signal that was band-pass filtered at 35-85 Hz. Gamma amplitude and theta phase were computed. The modulation coefficient was computed for each bin of normalized theta amplitude (Wulff *et al.*, 2009).

The statistical significance of comparisons was determined by ANOVA (repeated measures analysis of variance), by F-test for comparisons of model fits or by the x^2-test for comparisons of proportions. Two-group comparisons were performed using Mann–Whitney t-test. P-values below 0.05 were considered to indicate significance. The instantaneous running speed was computed from the position of the mouse and low-pass filtered in order to eliminate speed swings due movements of the head of the mouse (Geisler *et al.*, 2007).

Average running speed, CV of the running speed, optogenetic entrainment fidelity, duration of continuous running, fraction of time running, length of the path, average amplitude of theta peaks and the CV of the theta amplitude were computed for running (>2 cm/s) within 10 second epochs.

3. Results

3.1. Entrainment of hippocampal theta oscillations using optogenetics

3.1.1. Channelrhodopsin-2 expression in the medial septum of PV-Cre mice

I entrained hippocampal theta via optogenetic theta-rhythmic stimulation of GABAergic medial septal axons and terminals in the hippocampus. In comparison to electrical stimulation, optogenetic stimulation allows for cell type and brain pathway specificity of stimulation (Tye and Deisseroth, 2012). Channelrhodopsin-2 (ChR2, *AAV2/1.CAGGS.flex.ChR2.tdTomato.WPRESV40*) was selectively targeted to the medial septum (Fig. 3.1a). ChR2 was expressed under the control of the Cre promoter in PV-Cre mice, which expressed Cre recombinase exclusively in PV$^+$ neurons. That way we ensured selective expression in GABAergic, but not cholinergic or glutamatergic medial septal cells. Bright fluorescence visualized in the medial septal cell bodies and axons projecting to the hippocampus confirmed successful expression of the opsin. The opsin was transported along the axons, which run within the fimbria-fornix bundle and to cell terminals in the hippocampus (Fig. 3.1b,c). An optic fibre was implanted above the CA1 pyramidal layer of the right hippocampal hemisphere (Fig. 3.1b), which allowed for illumination with blue light (473 nm) and thus stimulation of GABAergic projections from medial septum to hippocampus. Time windows of stimulation were set using the stimulator software (MC_stimulus) and applied automatically during the course of the experiments. For control experiments light of the same wavelength was not delivered directly to the brain tissue but outside the brain to a dummy ferrule attached to the headset of the mouse, so that opsin- expressing cells were not optogenetically stimulated (Fig. 3.1d). These control experiments accounted for possible effects on behaviour by the light captured via the visual tract. LFP was recorded during the course of the experiments using tungsten electrodes chronically implanted in the hippocampal CA1 area (Fig. 3.1e). After completion of experiments successful expression of opsins was confirmed post-mortem (Fig. 3.1f).

Figure 3.1.

a *Pv-Cre   AAV flex::ChR2.tdTomato*

b 473-nm light / Optogenetic stim.

c

d Control light

e 500µm

f F35 F42 F43 F44 F48 F51 F52 F53 F59 F61 F62 F63 F64

500 µm
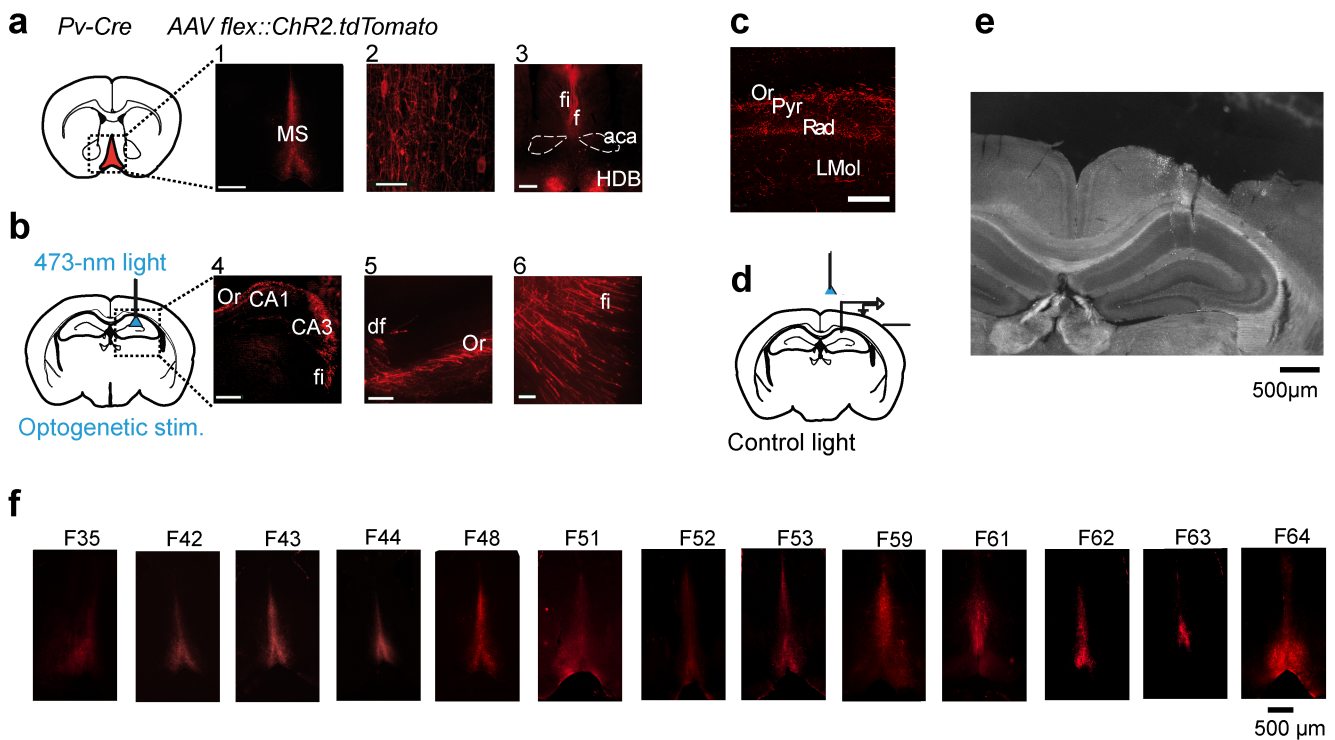
Fig. 3.1. An experimental preparation for optogenetic entrainment of hippocampal theta oscillations.
a, ChR2 expression was targeted to PV+ (GABAergic) MS cells of PV-Cre mice (scheme on the left and image 1). The viral construct *AAV2/1.CAGGS.flex.ChR2.tdTomato.WPRESV40* was delivered to the MS. Bright fluorescence confirms expression of somata in the MS (2). MS axons project via the fimbria (fi) and fornix (f) to the hippocampus (3). aca: anterior commissure, anterior part. HDB: nucleus of the horizontal limb of the diagonal band. **b**, MS ChR2 expressing axons projectiong to the hippocampus (4). Or: str. oriens. Fibres arriving to the hippocampal area CA1 via the dorsal fornix (df, 5). Fibres entering CA3 via the fimbria (6). An optic fibre was placed above the str. oriens layer of the hippocampal area CA1 for optogenetic excitation via illumination with blue light (wavelength: 473 nm) of MS PV+ cells projecting to the hippocampus (scheme on the left). **c**, GABAergic fibres from the MS enter the hippocampus primarily at the level of str. oriens (Or), str. radiatum (Rad) and str. lacunosum-moleculare (LMol), but also at the pyramidal layer (Pyr). **d**, For control experiments the patch cord of the laser was attached to a dummy fibre connected to the headset, which served to control for effects by the light itself, without optogenetic excitation of opsin-expressing cells. **e**, Trace of a recording electrode in the hippocampus. The hippocampal LFP was recorded during optogenetic entrainment to determine efficacy of entrainment for each recording. **f,** Opsin expression was confirmed after completion of experiments in all mice. Modified from Bender et al., *Nature Commun.* 2015 (panels a-c,f).

3.1.2. Hippocampal theta was entrained optogenetically at theta frequencies

We computed power spectral densities (PSDs) for 10 second epochs for spontaneous theta and opto-genetically entrained theta at 6, 8, 9, 10 or 12 Hz, respectively (Fig. 3.2a,b). Spontaneous theta occurred at 5-10 Hz (Fig. 3.2a, left image). Optogenetic stimulation at theta frequencies enabled entrainment of the hippocampal theta rhythm to the respective frequency: the dominant spectral frequency recorded in the hippocampus matched the respective stimulation frequency (Fig. 3.2a, images on the right). The entrainment fidelity is an estimate of how well stimulation frequency matched theta frequency within a 10 second epoch. A fidelity value of 0.3 indicates that the leading theta frequency matched the stimulation frequency, which was the case in ~80 % of recording epochs (Fig. 3.2c,d). Entrainment fidelity could vary within a stimulation epoch. The cumulative probabilities for theta entrainment fidelity of the 10 s epochs for stimulation frequencies within the theta band (6, 7, 8, 9 or 10 Hz) are depicted on Fig. 3.2d. For selected entrained epochs with a fidelity index of >0.3, stimulation frequency within the theta range matched leading theta frequency reliably (Fig. 3.2.e). If epochs were not selected according to entrainment fidelity (>0.3), leading theta frequency did not reliably match stimulation frequency (Fig. 3.2.e). Cumulative probability for different entrainment fidelity bins was similar across the differ-ent stimulation frequencies within the theta band. Theta was not elicited by continuous (e.g. 500 ms) light application (Fig. 3.2f).

Figure 3.2.
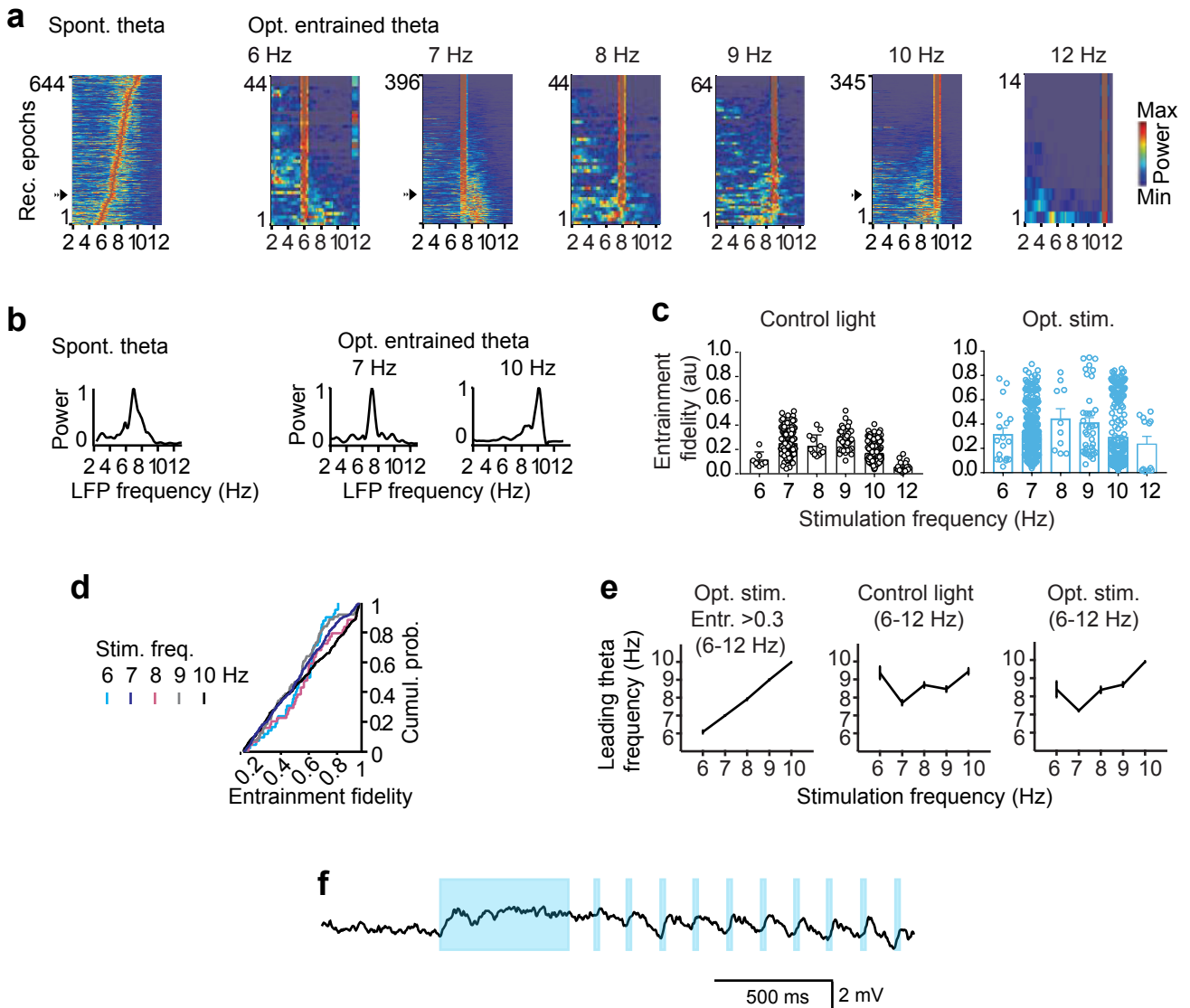
Fig. 3.2. Optogenetic entrainment of hippocampal theta oscillations.
a, Spontaneous theta occurs at frequencies of 5-10 Hz in the mouse. Plotted PSDs are generated for 10 second epochs. Rows include all recording epochs and are staged according to dominant frequency. Warmer colours indicate higher power. Optogenetic excitation of PV+ MS to hippocampus projections at 6, 7, 8, 9, 10 or 12 Hz, respectively, entrained the theta rhythm. Rows include all recording epochs and are staged according to entrainment fidelity. Power spectra of example epochs marked by an arrow are shown in **b** for spontaneously occurring theta and entrained theta at 7 Hz or 10 Hz, respectively. **c**, Entrainment fidelity values for epochs with 6-12 Hz control light delivery (black) or 6-12 Hz optogenetic stimulation (blue). **d**, Cumulative distribution of entrainment fidelity for entrained theta (6, 7, 8, 9 or 10 Hz, respectively). **e,** Stimulation frequency matched leading theta frequency, calculated for 10 second epochs, when only epochs with entrainment fidelity of above 0.3 were considered (left), but not during control light stimulation at same frequencies (middle). When all epochs with optogenetic stimulation, including epochs with entrainment fidelity below 0.3 were considered, leading theta frequency did not match reliably stimulation frequency (right). **f**, Theta entrainment required theta-rhythmic inputs, a continuous pulse would not elicit theta. Modified from Bender et al., *Nature Commun*. 2015 (panels a,b,d).

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3.1.3. The entrained hippocampal theta oscillation showed physiological features

First, we compared the hippocampal laminar phase profile during spontaneous and entrained theta oscillations. The laminar phase profiles showed characteristic features, such as a phase reversal between pyramidal layer and str. radiatum (Fig 3.3a). Further, the form of theta waves above the pyramidal layer, in str. oriens, had a sawtooth fast upstroke of positive polarity and a slower descending part with gamma waves, while in str. radiatum and str. L-M the fast component was negative and gamma waves were most clearly visible during the slow ascending phase, as described by (Buzsaki *et al.*, 2003). With faster frequencies (e.g. 10 Hz) the asymmetry of the theta waves increased and the positive component in the pyramidal layer consisted of only a sharp peak, followed by a so-called gamma envelope - several gamma cycles on the descending theta phase (Buzsaki *et al.*, 2003). The pulse fell to the ascending theta phase in the str. oriens and to the descending phase in layers below the pyramidal layer, such as str. radiatum. An example of a phase reset of the hippocampal network in response to the stimulation pulse is illustrated in Fig. 3.3a (arrow).

Next, we analysed whether theta-gamma coupling was preserved during theta entrainment (Fig. 3.3b-d). The amplitude of gamma oscillations was coupled to the theta phase during spontaneous and entrained theta. Amplitude-adjusted average coefficients of gamma amplitude modulation did not differ between spontaneous (*n*=8) and optogenetically entrained (*n*=19) theta (coefficient of modulation, F-test: $F_{4,8}=1.04$, *p*=0.42, *N*=3 mice, Fig. 3.3b). Gamma amplitude coupling did not differ between spontaneous theta and optogenetically entrained theta (*p*=0.42, *N*=3 mice, 8 and 19 recordings, respectively, *F*-test).

Next, we investigated whether theta frequency coherence of hippocampal hemispheres was preserved during theta entrainment. Not only the ipsilateral, but also the contralateral hippocampal hemisphere was entrained to the stimulation frequency. Stimulation of GABAergic projections from medial septum to hippocampus at theta frequencies (6, 8, 10 Hz) in the CA1 area of the right hippocampal hemisphere led to entrainment of theta also in the contralateral dorsal hippocampal hemisphere. Leading LFP theta frequency and stimulation frequency were correlated (Pearson's r=0.99±0.0004, *p*<0.05 in each of five out of six mice; Fig. 3.3e,f). The bilateral coordination of theta oscillations was also preserved (*p*<0.05, Pearson's correlation, *N*=5 mice). Fig. 3.3e shows example LFP traces recorded from the right and left hippocampus during stimulation at 10 Hz, which illustrates the effective entrainment of hippocampal theta oscillations in both hemispheres. Power spectra for epochs with stimulation frequencies within the theta band (6, 8, 10, 12 Hz) as well as frequencies outside the theta band (2 and 4 Hz) are shown for ipsilateral and contralateral hippocampal LFP. Fig 3.3f shows entrainment of contralateral str. oriens and str. radiatum hippocampal theta in another mouse. Note consistent theta phase reversal between hippocampal layers str. oriens and str. radiatum also during contralateral entrainment (Fig. 3.3f).

Figure 3.3.
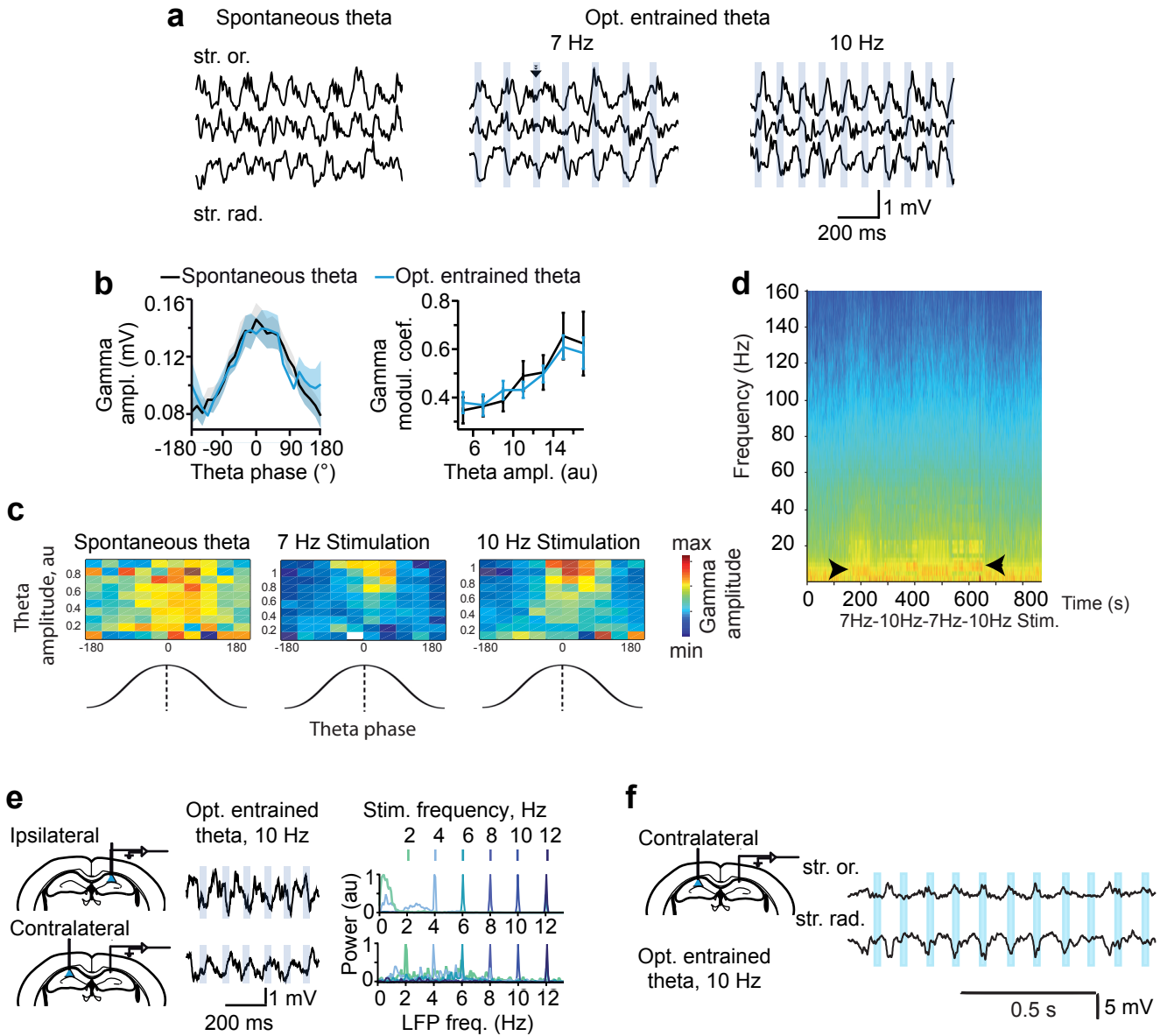
Fig. 3.3. Entrained hippocampal theta has physiological features.
a, Hippocampal LFP traces of str. oriens (str. or.) and str. radiatum (str. rad.) layers during spontaneous theta and theta entrained at 7 Hz or 10 Hz, respectively. A phase reset is indicated by the arrow. Note gamma envelopes during spontaneous as well as entrained theta and phase reversal of LFP recorded at str. oriens and str. radiatum. **b**, Gamma amplitude modulation according to theta phase (left), depending on theta amplitude (right), is unchanged during optogenetic entrainment. Data are presented as mean±s.e.m. **c**, Theta phase/amplitude-gamma amplitude coupling during an example experiment for spontaneous and 7 Hz as well as 10 Hz entrainment epochs. Gamma frequency is highest (indicated by warmer colour) during the theta peak and high amplitude theta. **d**, Hippocampal spectrogram (0-160 Hz) during one experiment with theta entrainment (7-10-7-10 Hz, arrowheads mark beginning and end of stimulation). **e**, Left: rhythmic excitation of PV⁺ MS fibres projecting to the right hippocampal hemisphere entrains LFP at the right (ipsilateral) as well as at the left (contralateral) hippocampal hemisphere. Middle: blue stripes mark time windows of blue light application to opsin expressing fibres projecting to the right hippocampal hemisphere. Right: power spectra show successful entrainment. **f**, Contralateral hippocampal theta entrainment at 10 Hz in another mouse. Note consistent phase reversal between hippocampal layers above (str. oriens) and below (str. radiatum) the pyramidal layer. Modified from Bender et al., *Nature Commun*. 2015 (panels a,b,e).

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Figure 3.4.
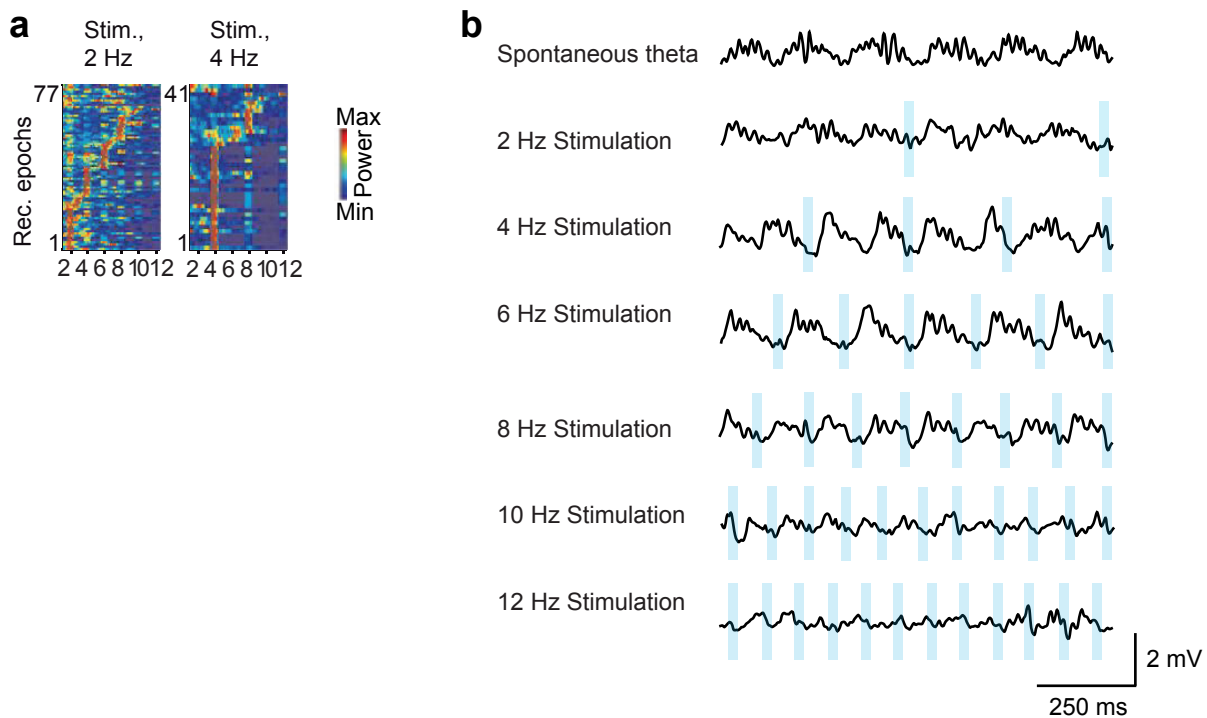
Fig. 3.4. Optogenetic stimulation at frequencies below the theta range.
a, Optogenetic stimulation of PV⁺ MS to hippocampus fibres at 2 Hz resulted in hippocampal LFP oscillations of either 2 Hz, or 4 Hz, if every second cycle would be phase-locked, or 6 Hz, if every 3rd cycle would be phase-locked, or 8 Hz, if every 4th cycle would be phase-locked, or did not affect frequency. Optogenetic stimulation at 4 Hz resulted in about 2/3 of recording epochs in hippocampal LFP oscillation of 4Hz, and otherwise of 8 Hz. **b**, Example traces of hippocampal LFP during baseline followed by consecutive 2, 4, 6, 8, 10 and 12 Hz optogenetic stimulation. Blue stripes mark time windows of blue light application to opsin expressing fibres. Modified from Bender et al., *Nature Commun*. 2015 (panel a).

3.1.4. Non-theta frequency stimulation failed to effectively entrain hippocampal theta oscillations

Stimulation frequencies outside the theta frequency band (2, 4, 20 Hz) were less effective in entraining hippocampal activity (Figures 3.4. and 3.5.). We computed PSDs for 10 second epochs during optogenetic stimulation at 2, 4 or 20 Hz. About 63 % of recording epochs with 4 Hz stimulation resulted in a maximal power at 4 Hz and only about 29 % of recording epochs with 2 Hz stimulation resulted in a maximal power of 2 Hz (N=9 mice). Setting the stimulation frequency to 2 or 4 Hz often resulted in entrainment at harmonic frequencies within the theta band, such as at 6 or 8 Hz (Fig. 3.4a,b). Stimulation at 20 Hz entrained hippocampal activity only in a subset of stimulation epochs (Fig. 3.5a-c).

Figure 3.5.

a

Stim., 20 Hz

Rec. epochs

26

1

2 5 10 15 20

Max

Power

Min

b Str. pyramidale

2 mV

250 ms

c Str. pyramidale

1 mV

250 ms
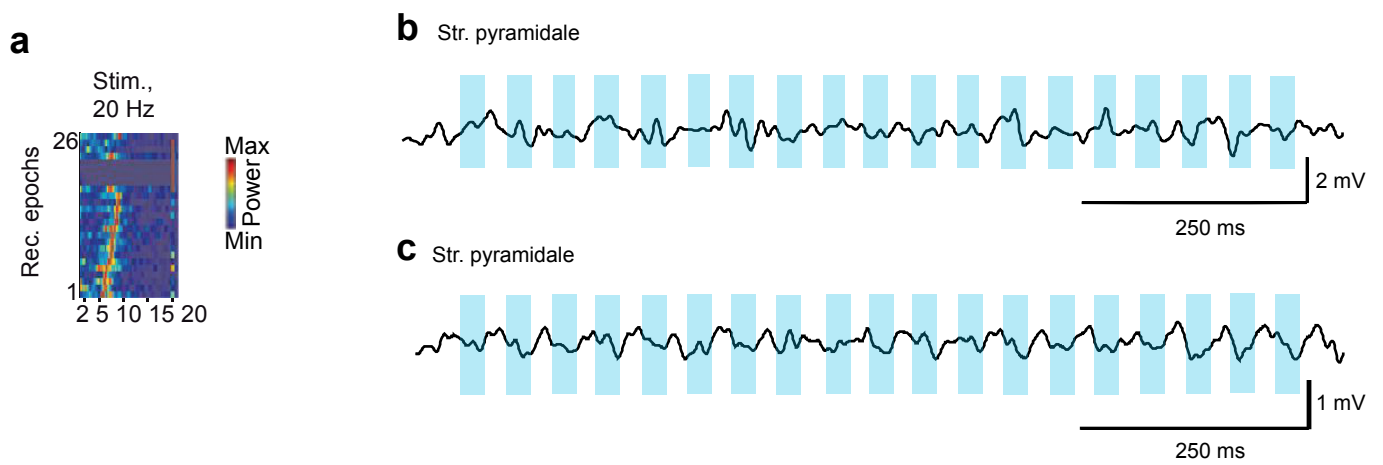
Fig. 3.5. Optogenetic stimulation at frequencies above the theta range.
a, Optogenetic stimulation of PV⁺ MS to hippocampus fibres at frequencies of 20 Hz resulted in hippo-campal LFP oscillation of 5 Hz, if every 4th cycle would be phase-locked, 6.7 Hz, if every 3rd cycle would be phase-locked, or, in some epochs, 20 Hz. Surprisingly, 20 Hz stimulation did not induce a theta rhythm of 10 Hz. Within one recording epoch with 20 Hz stimulation theta frequency could vary between 5-6.7 Hz. An example trace is shown in **b**. An example trace of entrainment at 20 Hz is shown in **c**. Blue stripes mark time windows of blue light application to opsin expressing axon terminals. Modified from Bender et al., *Nature Commun*. 2015 (panel a).

Figure 3.6.

a 6 Hz Control light stimulation

Str. oriens

Str. pyramidale

Str. radiatum
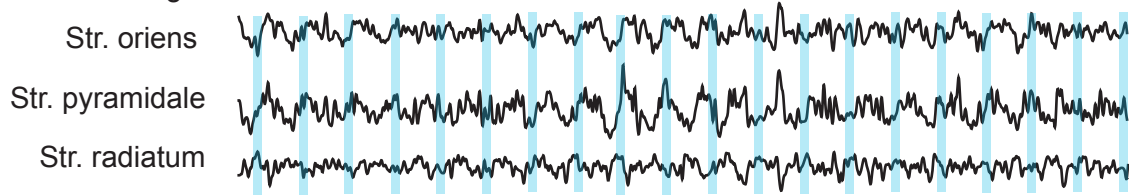
b 8 Hz Control light stimulation
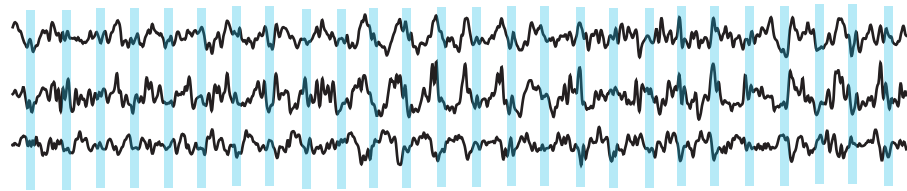
c 10 Hz Control light stimulation

0.5 mV

0.5 sec
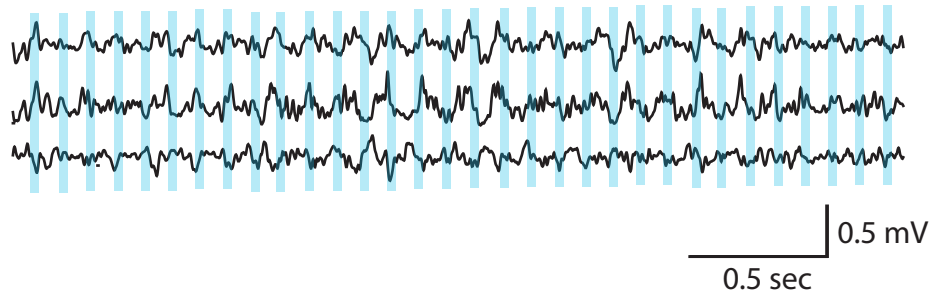
Fig. 3.6. Control light delivery did not entrain hippocampal theta oscillations.
Representative examples for hippocampal LFP traces during spontaneous theta in presence of control light delivery at 6 Hz (**a**), 8 Hz (**b**) or 10 Hz (**c**). Blue stripes mark time windows of blue light delivery via a dummy fibre outside the brain, attached to the head-set. Note that pulses match random phases of theta.

3.1.5. Control light delivery did not entrain hippocampal theta

Control light application (see scheme in Fig. 3.1d) at theta frequencies did not entrain hippocampal theta (Fig. 3.6). Mean entrainment fidelity of all stimulation epochs with control light stimulation in the theta frequency range (6-12 Hz) was below 0.3 (0.2±0.005). Mean entrainment fidelity of all stimulation epochs with optogenetic stimulation in the theta frequency range (6-12 Hz) was above 0.3 (0.422±0.007).

3.1.6. Hippocampal theta could be entrained during sleep

The hippocampus expresses theta oscillations during explorative behaviour and REM sleep. However, it is not known whether underlying mechanisms are the same. We recorded during 2-hour sleep sessions EEG, EMG and hippocampal LFP in the absence of light (Fig. 3.7a), or during optogenetic optogenetic hippocampal theta entrainment (Fig. 3.7b), or during control light delivery at the same frequency. We applied stimulation either automatically during the sleep session (automatic protocol), and hence during different vigilance states (Fig. 3.7c), or specifically during the course of the REM sleep epochs (Fig. 3.7d, manual protocol). We could successfully entrain hippocampal theta to the stimulation frequency during all vigilance states (Fig. 3.8a). Entrainment efficacy was on average 0.63±0.003 during active wakefulness, 0.62±0.002 during quiet wakefulness, 0.61±0.002 during NREM sleep and 0.59±0.003 during REM sleep (N=3 mice, n=8 experiments, all entrainment values were included here). Note that in these experiments mice were not exploring or running during active wakefulness. Cortical theta power increased upon hippocampal theta entrainment (automatic or manual protocol, stimulation frequencies: 6, 7, 8, 9, or 10 Hz) in comparison to control light application or baseline recordings during REM sleep, but not during quiet or active wakefulness or NREM sleep (Fig. 3.8b, control light: N=7 mice, n=18 experiments, optogenetic stimulation: N=5 mice, n=24 experiments with multiple stimulation epochs, preliminary results).

Figure 3.7.

a Baseline

CA1

EEG

EMG

1 s   1 mV

b *Pv-Cre*
AAV flex::ChR2.tdTomato

473-nm light

High entrainment

CA1

EEG

EMG

1 s   1 mV

c

Frequency (Hz)

Time (sec)

d

Frequency (Hz)

Time (sec)

Max
Power
Min
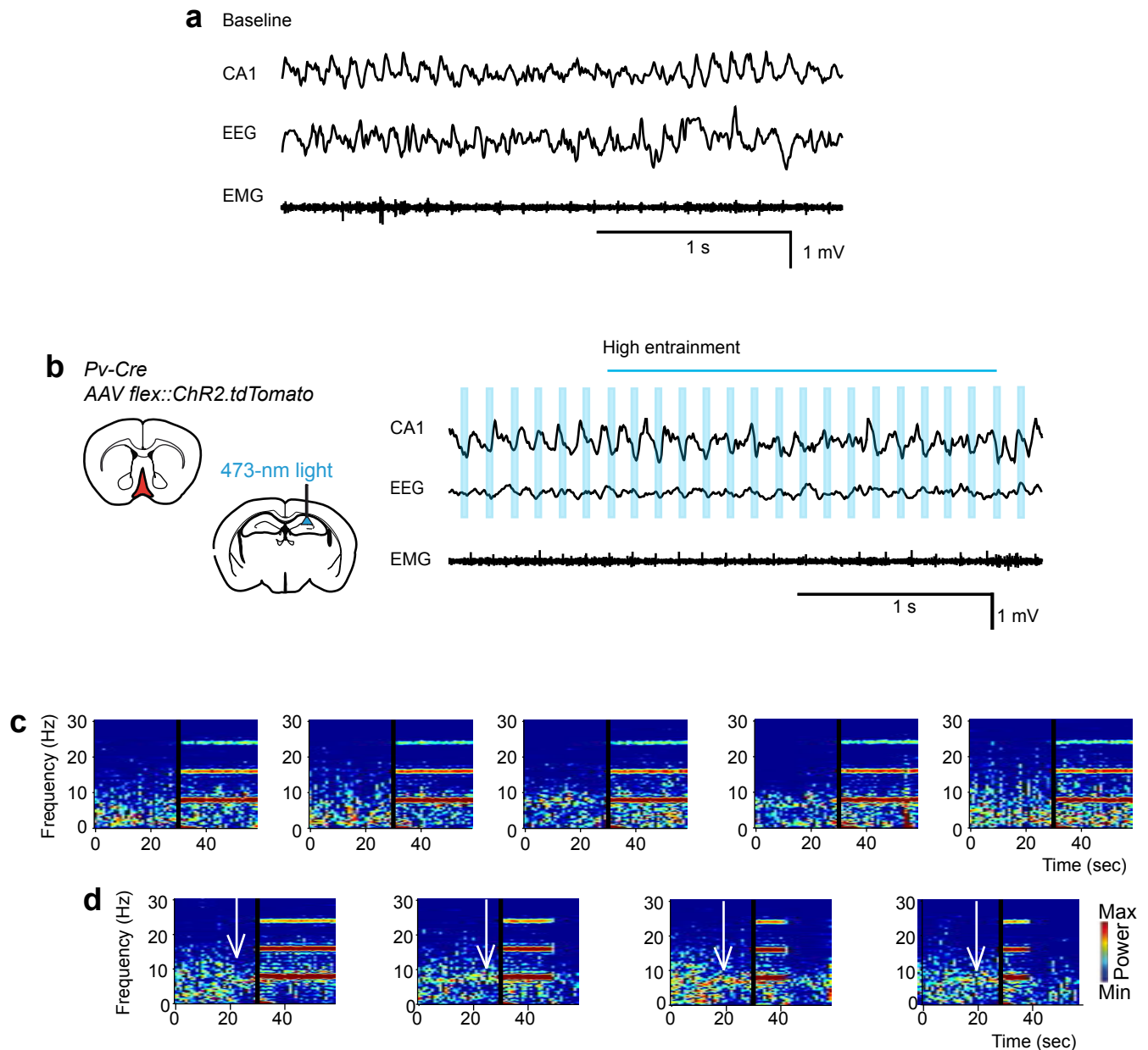
Fig. 3.7. A preparation for hippocampal theta entrainment during sleep.
a, Baseline hippocampal LFP, cortical EEG and EMG during REM sleep. **b**, Hippocampal theta entrainment during REM sleep. The preparation was the same as for entrainment during explorative behaviour: ChR2 was expressed in GABAergic medial septal cells and blue light pulses were applied at theta frequency to the hippocampal CA1 area via an optic fibre (scheme). Mice were recorded in a sleep-promoting environment. On the right: effective entrainment of hippocampal theta during REM sleep. Note the low muscle tone, a clear marker to identify the REM sleep state. Stimulation was applied either during random vigilance states (**c**) or initiated upon REM sleep detection (**d**). Plotted are spectrograms of hippocampal LFP. Warmer colours indicate higher power. White arrows in (**d**) indicate beginning of REM sleep. Note power concentration in the theta band (5-10Hz). Black lines mark time points of stimulation onset.

Figure 3.8.

a

Hippocampal LFP, spontaneous theta oscillation

Hippocampal LFP, 8 Hz optogenetic stimulation

Cortical PSD, spontaneous theta oscillation

Cortical PSD, 8 Hz stimulation in the hippocampus

b Quiet wakefulness   NREM   REM   Active wakefulness
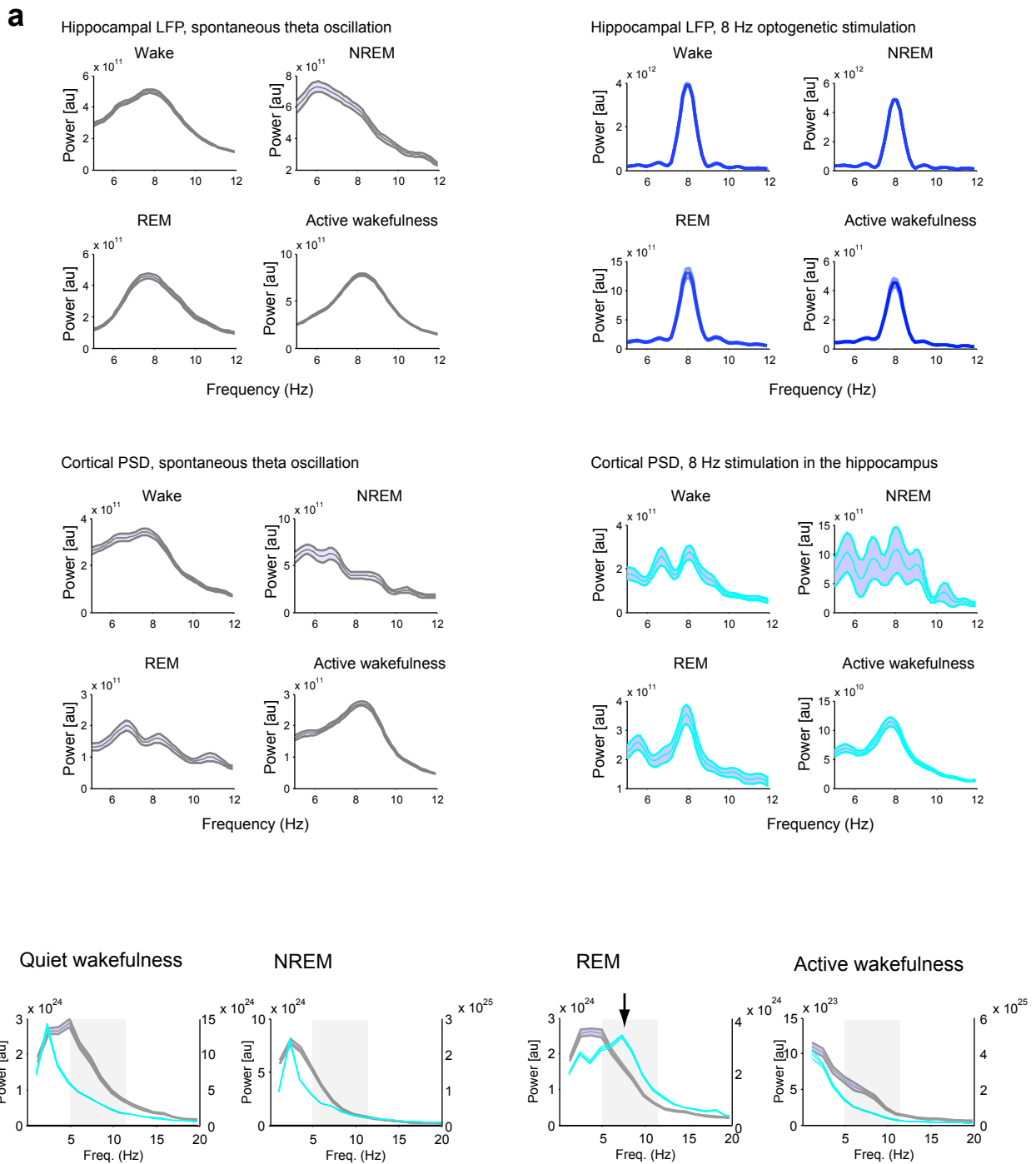
Fig. 3.8. State-dependent impact of hippocampal theta entrainment on cortical EEG.
a, Hippocampal and cortical LFP power spectral density in the theta frequency band during spontaneous theta oscillation (in grey) and optogenetic hippocampal theta entrainment at 8 Hz (hippocampal LFP: blue, cortical EEG: cyan) during different vigilance states. **b**, Cortical theta (5-12 Hz, grey shadow) relative power increased during epochs with optogenetic entrainment during REM sleep (indicated by arrow) but not during quiet or active wakefulness or NREM sleep (power (au) during control light epochs (grey) and during optogenetic entrainment (cyan)).

3.2. Behavioural effects of hippocampal theta entrainment

3.2.1. Causality behind speed and theta frequency correlation

A correlation of hippocampal theta frequency and running speed has been reported in many studies (e.g. Vanderwolf and Heron, 1964; Geisler *et al.*, 2007). However, the question about the causality of the relationship could not be addressed using electrical stimulation protocols, as electrical stimulation of the medial septum or hippocampus inevitably involves direct activation of many output pathways, among those subcortical pathways which exert direct control of locomotor behaviour. Thereby, behavioural motor responses, which may be conducted via computations in upstream cortical regions would be impossible to identify. Here, we used a preparation which allowed to excite solely projections of GABAergic medial septal neurons to the hippocampus, but not to other brain regions. This preparation enabled us to identify how hippocampal computations affect locomotion.

We first addressed the question whether hippocampal theta frequency causally adjusts locomotor speed. When hippocampal theta occurred spontaneously in the presence of control light, theta frequency correlated with running speed, as reported in previous studies (Fig. 3.9a, Pearson's r=0.82, *p*=0.013, *N*=6 mice, *n*=42 recording sessions). When hippocampal theta oscillations were optogenetically entrained, theta frequency was determined by the stimulation frequency and did not correlate with running speed (Fig. 3.9a, Pearson's r=0.04, *p*=0.7, *N*=8 mice, *n*=72 recording sessions). During theta entrainment, the impact of ascending inputs on theta frequency was minimized (scheme in Fig. 3.9b). The average running speed did not differ between 7 Hz or 9 Hz optogenetic stimulation (see Fig. 11a, $F_{1,248}$=0.82, *p*=0.37). Consequently, we suggest that the correlation between theta frequency and running speed derives from ascending speed-controlled afferents which modulate theta frequency and that hippocampal theta frequency by itself does not regulate running speed. Entrainment also mediated a dissociation of theta amplitude and running speed. Theta amplitude and running speed were correlated during baseline recordings (Fig. 3.10a, Pearson's r=0.23, *p*=0.0005). Theta amplitude and running speed were not correlated during optogenetic hippocampal theta entrainment (Fig. 3.10b, Pearson's r=0.058, *p*=0.21).

Figure 3.9.
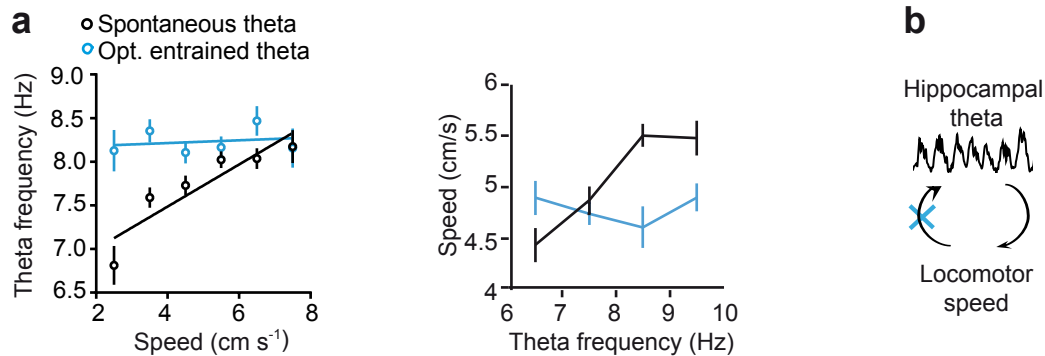
Fig. 3.9. Theta frequency and running speed relation during optogenetic theta entrainment.
a) A positive correlation between theta frequency and running speed was detected for spontaneous theta in absence of optogenetic entrainment in line with previous studies (black). During optogenetic entrainment of hippocampal theta speed did not correlate with theta frequency (blue).
b) Scheme illustrates that optogenetic stimulation determines theta frequency and hence speed-related afferents do not modulate theta (blue cross), which allows to study the reverse influence - the impact of hippocampal theta on running speed. Modified from Bender et al., *Nature Commun.* 2015 (left panel in a, panel b).

Figure 3.10.
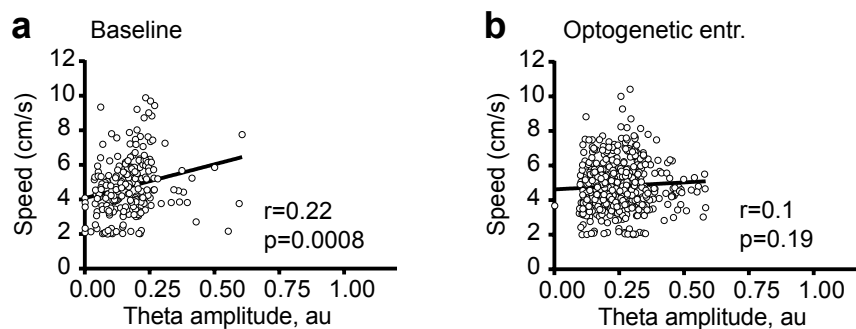
Fig. 3.10.: Theta amplitude and running speed relation during optogenetic theta entrainment.
a) Theta amplitude correlated with running speed during baseline epochs before the start of optogenetic hippocampal theta entrainment. **b)** During optogenetic entrainment at theta frequencies (6-12 Hz), theta amplitude did not correlate with running speed

3.2.2. Entrainment of hippocampal theta oscillations led to a reduction in average running speed

Next, we analysed the influence of hippocampal theta entrainment on locomotor speed. We found that the entrainment of hippocampal theta led to a reduction in average running speed (Fig. 3.11a-e). The effect was independent of the stimulation frequency within the theta range: both 7 Hz or 9 Hz stimulation led to a speed reduction in comparison to spontaneous theta epochs in the presence of frequency-matched control light (Fig. 3.11a, $F_{1,248}$=17.85, p=0.00003; 7 Hz: p=0.0002, N=5 mice; 9 Hz: p=0.0002; N=3 mice, Repeated measures ANOVA, with factors "experimental subject", "type of optical stimulation (optogenetic theta entrainment vs. control blue light stimulation" and "stimulation frequency", Bonferroni tests). Average running speed was lower upon entrainment at 7 Hz compared to spontaneous theta of 5-10 Hz in the presence of control light ($F_{1,323}$=18.88, p=0.00002; p=0.000019, N=5 mice, Fig. 3.11b). Average running speed was lower during optogenetic entrainment at 6-12 Hz compared to control light application of 6-12 Hz (Fig. 3.11c, p=0.0005, Mann Whitney t-test). Fig. 3.11.d illustrates the reduction in running speed during 10 second epochs of optogenetic theta entrainment (N=9 mice, n=41 experiments, n=250 epochs) compared to 10 second epochs of spontaneously occurring theta in presence of control light (N=6 mice, n=20 experiments, n=118 epochs). Fig. 3.11e shows five respective example speed traces at the beginning of either 7 Hz control light application (left) or 7 Hz optogenetic entrainment (right). Furthermore, higher entrainment fidelity was associated with a lower variability (CV) of theta amplitude across theta cycles (Fig. 3.11.f, Spearman rank correlation, r=-0.953, p=0.01, N=9 mice, n=62 experiments, n=731 epochs).

Figure 3.11.
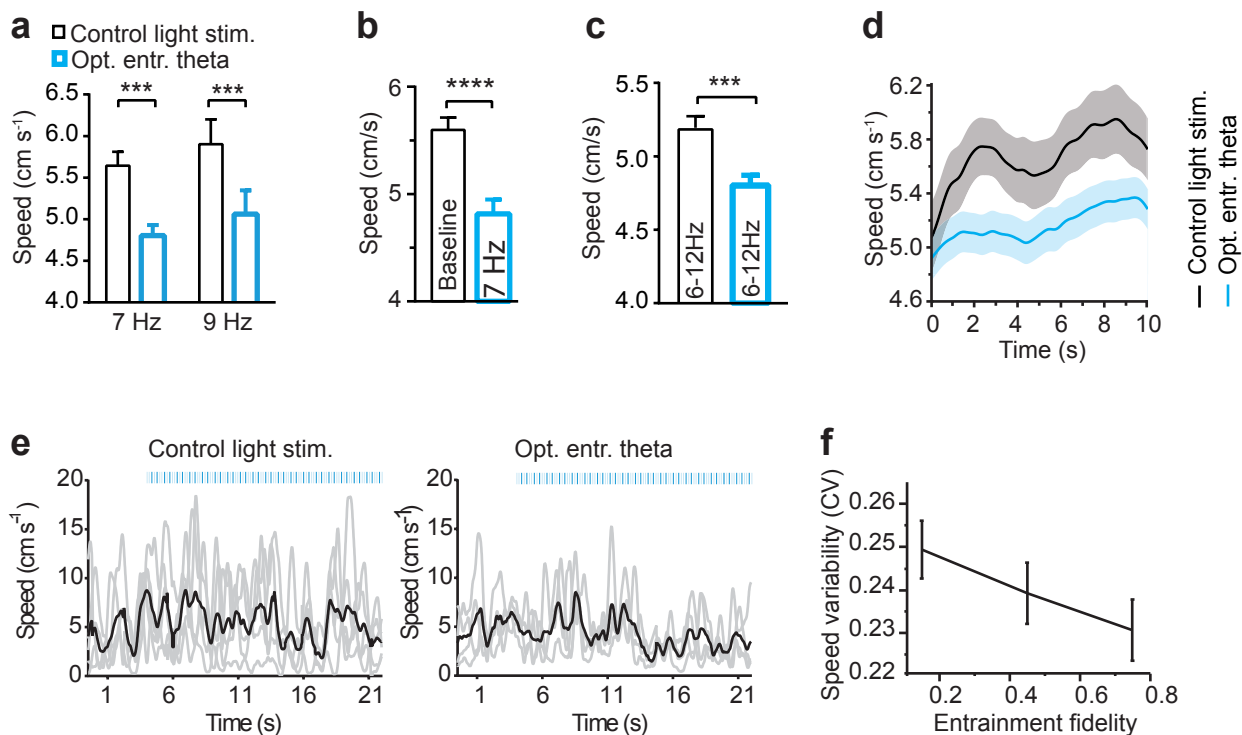
Fig. 3.11. Optogenetic hippocampal theta entrainment at constant frequency slowed down the animals speed.
a, During optogenetic entrainment at 7 Hz as well as at 9 Hz average running speed was reduced in comparison to epochs with control light stimulation of the same respective frequency. **b**, Average running speed was lower when theta was entrained (at 7 Hz) compared to spontaneous running epochs with theta frequency between 5-10 Hz. **c**, Average running speed was lower when theta was entrained (6-12 Hz) compared to epochs with control light stimulation (6-12 Hz). **d**, Average speed for 10-second epochs during control light stimulation (black) or optogenetic theta entrainment (blue). **e**, Five speed traces upon control light application (left) or optogenetic theta entrainment (right), illustrating lower speed during entrainment. Blue stripes mark time points of blue laser pulses. **f**, Speed variability for epochs of theta entrainment (6-12 Hz stimlation frequency) decreased significantly with entrainment fidelity (bin size: 0.3). ***p<0.001, ****p<0.0001. Modified from Bender et al., *Nature Commun*. 2015 (panels a,b,d,e).

3.2.3. Optogenetic stimulation at non-theta frequencies did not influence running speed

Importantly, the effect on speed was dependent on theta frequency optogenetic stimulation, hence effective entrainment of the hippocampal theta rhythm. Upon optogenetic stimulation with the same duration of the light pulses but at frequencies outside the theta band (2, 4 or 20 Hz) running speed during the baseline was not different from running speed during optogenetic stimulation (Fig. 3.12a, $F_{2,84}$=0.07, p=0.8; 2 Hz: N=7 mice, n=47 recording epochs, 4 Hz: N=5 mice, n=19 recording epochs, 20 Hz: N=5 mice, n=29 recording epochs, repeated measures ANOVA, with factors "experimental subject", "type of optical stimulation (optogenetic stimulation vs. control blue light delivery)" and "stimulation frequency"). The stimulation frequency (2, 4, or 20 Hz) did not affect running speed ($F_{2,84}$=2.8, p=0.06) and no interaction between factors was detected ($F_{2,84}$=1.5, p=0.23). For this comparison experiments with an average baseline speed of 5 cm/s, similar as in experiments with optogenetic stimulation, were selected. Also for the overall running speed (>2 cm/s), with all experiments included, no difference in running speed was detected between type of stimulation ($F_{1,131}$=0.31, p=0.58), or stimulation frequency ($F_{2,131}$=0.08, p=0.93) and no interaction between the factors was detected ($F_{2,131}$=1.17, p=0.32; 2 Hz: n=73 recording epochs, N=8 mice; 4 Hz: n=28 recording epochs, N=6 mice; 20 Hz: n=43 recording epochs, N=6 mice). When the light pulse duration was set to 1 second, repeated every 3.7 seconds for 2 minutes, running speed was also not reduced during optogenetic stimulation epochs (N=10 mice, n=20 recording sessions) in comparison to spontaneous running epochs in presence of control light (Fig. 3.12b, N=9 mice, n=10 recording sessions, $F_{1,15}$=1.22, p=0.29; Two-way repeated measures ANOVA, with factors "experimental subject" and "type of optical stimulation" (optogenetic stimulation vs. control blue light delivery)).

Figure 3.12.
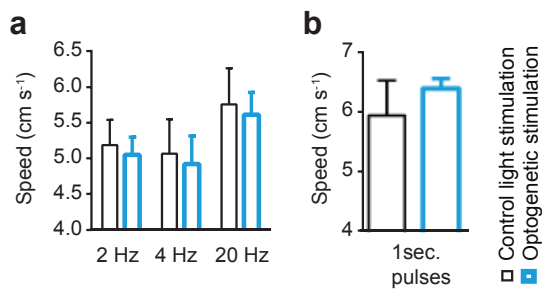
Fig. 3.12. Running speed was not affected by non-theta rhythmic optogenetic stimulation.
a, Average running speed during epochs of optogenetic stimulation of PV$^+$ MS to hippocampus projections at 2 Hz, 4 Hz or 20 Hz was not lower compared to running epochs accompanied by spontaneous theta epochs in presence of control light of the same respective frequency. **b,** Average running speed during optogenetic stimulation with 1 second pulses did not differ from average running speed in presence of control light 1 second pulses. Modified from Bender et al., *Nature Commun.* 2015.

3.2.4. Induction of hippocampal theta did not evoke exploratory behaviour

We next tested whether hippocampal theta entrainment affected explorative behaviour per se. To do so we compared the time the mice spend exploring the chamber and the likelihood of movement onset in response to light onset between optogenetic stimulation and control light experiments. Our analysis revealed that optogenetic theta entrainment did not affect the average duration of a continuous run (Fig. 3.13a, $F_{1,244}$=0.002, p=0.97) or the total time a mouse was exploring the environment (Fig. 3.13b,c, $F_{1,244}$=0.37, p=0.54, Repeated measures ANOVA, with factors "experimental subject", "type of optical stimulation (optogenetic theta-entraining stimulation vs. control blue light delivery)" and "stimulation frequency", with stimulation frequency of 7 Hz or 9 Hz). Values were compared with spontaneous theta epochs at 5-10 Hz during application of control light. Hippocampal theta could be induced in the absence of movements (Fig. 3.14a). The presence of control light did also not evoke locomotor behaviour in the quietly awake mouse. Presence of control light in the absence of movement did not evoke hippocampal theta oscillations. Fig. 3.14b illustrates the absence of locomotion and absence of optogenetic theta entrainment (hippocampal LFP recording shows large irregular activity) in the presence of control light. No difference in the likelihood of movement onset was found upon onset of optogenetic stimulation at theta frequencies or presence of control light in the quietly awake mouse. The number of trials during which the mouse remained immobile or presented locomotor behaviour within 15 seconds upon light onset did not differ between experiments with optogenetic entrainment or onset of control light (Fig. 3.14c, X^2-test, $X^2(1)$=2.33, p=0.13; N=8 mice, optogenetic entrainment; N=5 mice, control). In these experiments the mouse stayed immobile at least for 20 seconds before control light delivery or optogenetic stimulation onset. In conclusion, we found that hippocampal theta entrainment did not affect exploratory behaviour per se, but rather regulated the speed of ongoing locomotor activity. Entrainment fidelity was higher when the mouse was actively running (speed >2 cm/s, n=751 epochs, N=9 mice) than when it was immobile (p<0.0001, n=550 epochs, N=9 mice, t-test).

Figure 3.13
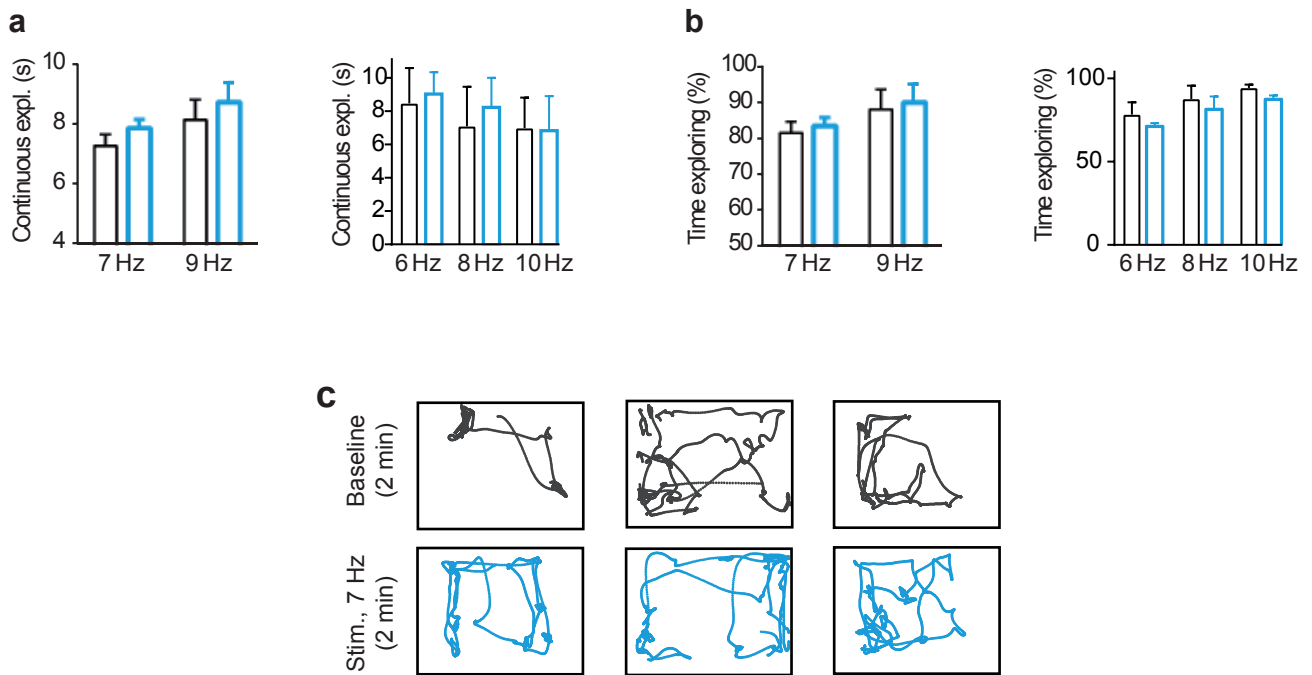
Fig. 3.13. Optogenetic hippocampal theta entrainment did not affect duration of explorative behaviour.
a, The average duration of continuous exploration was similar for running epochs accompanied by spontaneous 7 Hz hippocampal theta oscillation or entrained 7 Hz hippocampal theta oscillation. The same was true for 9 Hz, as well as 6, 8 and 10 Hz. **b**, The total time exploring was similar for running epochs accompanied by spontaneous 7 Hz hippocampal theta oscillation or entrained 7 Hz hippocampal theta oscillation. The same was true for 9 Hz, as well as 6, 8 and 10 Hz. **c**, Three example traces showing mouse trajectory during baseline with no stimulation (black) or optogenetic entrainment at 7 Hz (blue), respectively. Rectangles mark the borders of the recording chamber. Modified from Bender et al., *Nature Commun*. 2015 (left panels in a and b).

Figure 3.14.
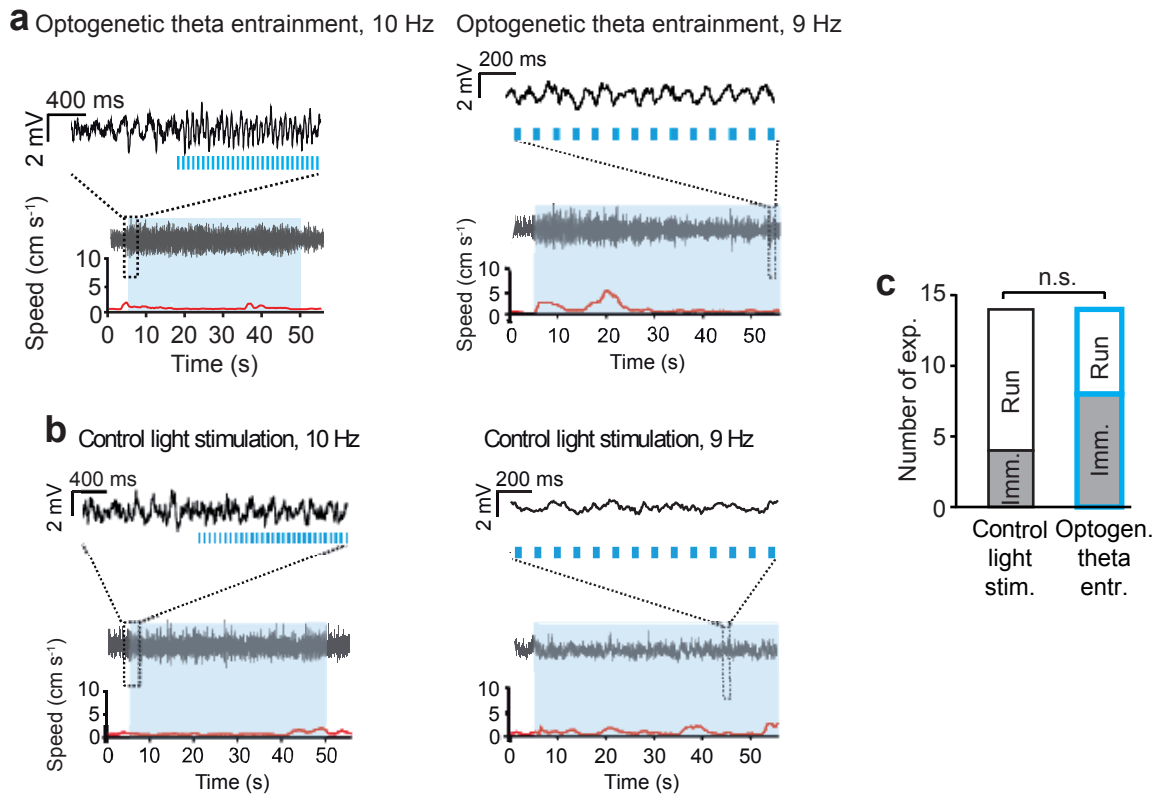
Fig. 3.14. Optogenetic inducation of hippocampal theta did not evoke movement.
a, Hippocampal theta could be successfully induced in the mouse in absence of movement via optogenetic entrainment, see excerpts of LFP traces above. Occurrence of hippocampal theta was not strictly linked to occurence of running epochs. Example traces showing no effect on running speed (red trace) of hippocampal theta entrainment at 10 Hz (left), or 9 Hz (right). **b**, Presence of control light at 10 Hz or 9 Hz did not evoke theta oscillations (upper traces) and was not linked to movement onset. **c**, The proportion of experiments in which a quietly awake mouse stayed immobile (Imm.) upon light onset or began to move (Run) was not different for control light onset or optogenetic entrainment onset. Modified from Bender et al., *Nature Commun.* 2015.

3.2.5. The ventral hippocampus did not show consistent locomotion-dependent theta rhythmic activity

In the preparation developed in this study optogenetic stimulation was targeted to the dorsal hippocampus. In several publications the dorsal hippocampus has been attributed a much greater role in spatial navigation than the ventral hippocampus (Royer *et al.*, 2010; Moser and Moser, 1998). We performed simultaneous dorsal and ventral hippocampal LFP recordings during baseline and optogenetic stimulation. In line with previous studies our results showed that ventral hippocampal theta oscillations were not consistently related to locomotor activity (Fig. 3.15). Theta oscillation amplitude (5-10 Hz bandpass filtered, amplitude derived from Hilbert transform, Fig. 3.15a) correlated with running speed in the dorsal hippocampus (r=0.59, p<0.0001, n=6 recording session), but not in the ventral hippocampus (r=-0.12, p=0.45, n=6 recording sessions, N=3 mice, Fig. 3.15b). Here, experiments with parallel recordings in dorsal and ventral hippocampus were analysed and epochs with a speed below 2 cm/s were included. Fig. 3.15c illustrates the absence of a clear correlation of theta amplitude and running speed in the ventral hippocampus. Cumulative theta power was generally higher in the dorsal hippocampus compared to the intermediate and ventral hippocampus during optogenetic entrainment of the hippocampal theta (p=0.0063) as well as during spontaneous theta oscillations (p=0.0063, Fig. 3.15d, Repeated measures ANOVA, with factors "experimental subject", "light delivery (optogenetic theta entrainment vs. light off)" and "part of hippocampus (dHip vs. vHip)", $F_{1,15}$=14.48, p=0.0017; Tukey-Kramer post-hoc test). The power relation between dorsal and ventral hippocampus was not affected by theta entrainment in the dorsal hippocampus ($F_{1,15}$=0.25, p=0.622). The coherence between the dorsal and ventral hippocampus was not affected by theta entrainment in the dorsal hippocampus, the cumulative theta power difference did not differ (p=0.31, signed rank test, N=3 mice, n=5 recordings). Fig. 3.15e shows recording tracks in the ventral hippocampus.

Figure 3.15.

a LFP (vHip)

b Theta amplitude (mV) vs Speed (cm s⁻¹)

— dHip spontaneous theta
— vHip spontaneous theta

c Theta amplitude (mV) — vHip

Speed (cm s⁻¹) vs Time (s)

d 473-nm light

Cumulative theta power (au)

** **

Optogenetic theta entrainment in dorsal Hip
— dHip
— vHip

Spontaneous theta
— dHip
— vHip

e F88   −1.94   −2.92

F76   −1.94   −3.40

F90   −2.06   −2.80

LV

500 µm
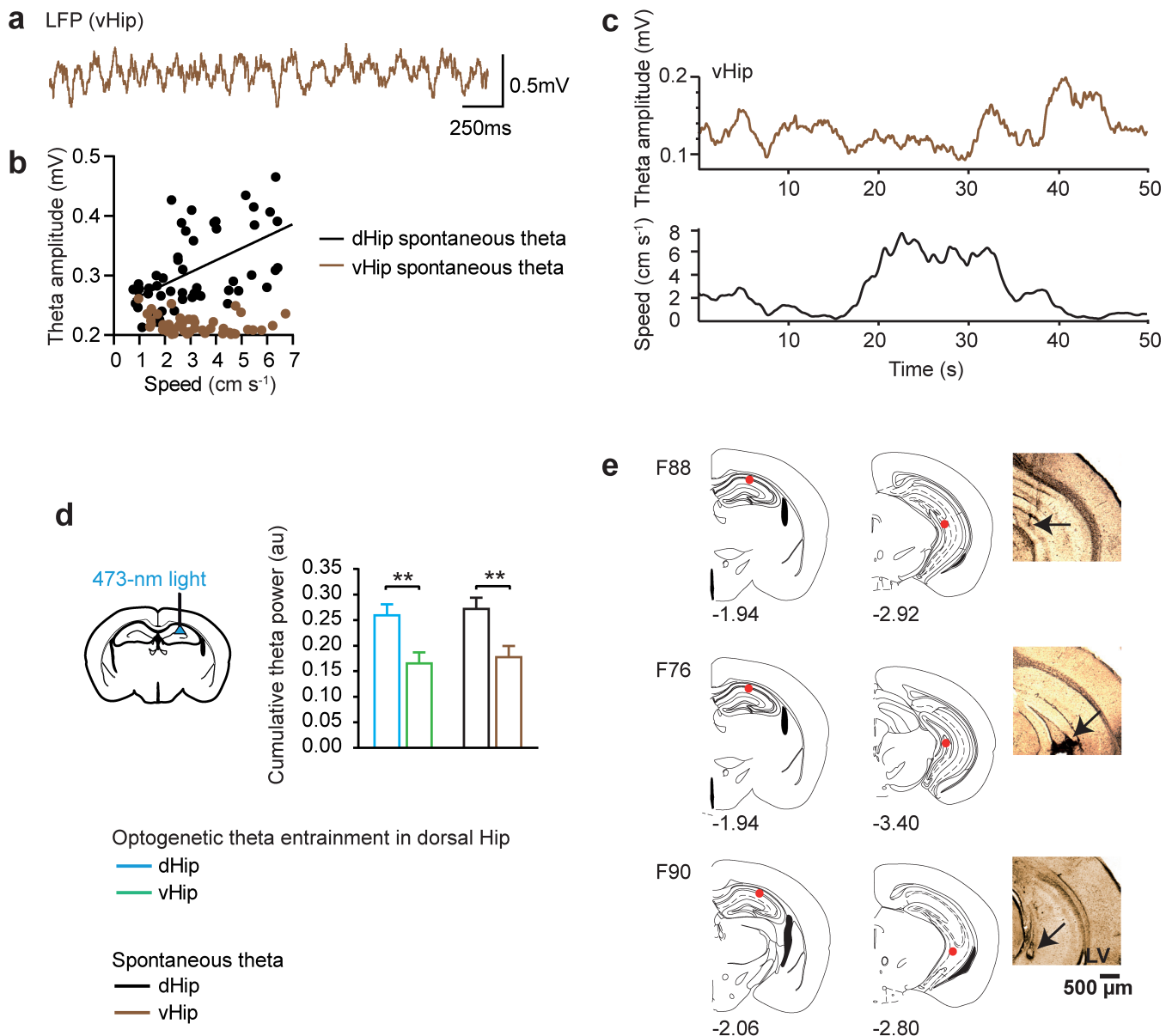
Fig. 3.15. Ventral hippocampal theta-band LFP power did not correlate with movement speed.
a, Example trace of ventral hippocampal theta oscillation. **b**, Theta amplitude of the dorsal (black), but not of the ventral (brown), hippocampal LFP during theta was correlated with movement speed. **c**, Amplitude of theta-band LFP recorded in the ventral hippocampus (upper trace) and simultaneously recorded movement speed (lower trace) showing no relation. **d**, Difference in cumulative theta power between dorsal and ventral aspects was similar during entrained theta (blue and green bar) and spontaneous theta (black and brown bar). **e**, Left, schemes: verified positions of electrodes in the dorsal (left) and ventral (right) aspects are marked by red dots in coronal schemes. Right, histology images: electrode tracks in the ventral hippocampus. **p<0.01. Modified from Bender et al., *Nature Commun*. 2015.

3.2.6. A lower theta amplitude variability was associated with a lower running speed

Next, we aimed at unraveling which aspect of hippocampal theta oscillations was modulated during entrainment and mediated the effect on behaviour. We discovered that higher entrainment fidelity correlated with lower variability (CV) of theta amplitude across theta cycles which predicted a slower and more steady running speed of the animal (see Bender *et al.*, 2015).

Theta amplitude variability was lower during optogenetic theta entrainment (at 6, 7, 8, 9, 10 or 12 Hz, n=797 recording epochs, n=70 experiments, N=9 mice) compared to spontaneous theta during control light delivery of the same respective frequencies (Fig. 3.16a, n=421 recording epochs, n=32 experiments, N=7 mice, p<0.0001, unpaired t-test). Theta amplitude variability was not different during optogenetic stimulation at non-theta frequencies (2, 4 or 20 Hz, n=318 recording epochs, n=42 experiments, N=9 mice) compared to spontaneous theta during control light delivery of the same respective frequencies (Fig. 3.16a, n=32 recording epochs, n=12 experiments, N=5 mice, p=0.88, unpaired t-test).

We found that running epochs with a lower amplitude variability of the hippocampal theta rhythm were correlated with a lower running speed (Fig. 3.16b). The correlation was detected not only for optogenetically entrained hippocampal theta oscillations during running (r=0.1, p=0.002, Spearman correlation), but also, importantly, the same correlation was found for spontaneously occurring hippocampal theta oscillations and running speed during control light delivery of the same respective frequencies (6, 7, 8, 9, 10 or 12 Hz). Also here, epochs with a lower variability of theta amplitude correlated with a lower running speed (r=0.17, p<0.0001, Spearman correlation). The relation of running speed and theta amplitude variability was not different between spontaneous (n=384 recording epochs, N=8 mice) and optogenetically entrained theta epochs (n=818 recording epochs, N=8 mice, $F_{4,10}$=2.14, p=0.14, F-test). As reported above, theta frequency was not correlated with running speed during entrainment. We concluded that theta amplitude variability, but not theta frequency, modulated running speed during entrainment. Illustrated in Fig. 3.16c is the association of lower theta amplitude variability with lower and more regular speed.

An increase in theta frequency variability could be experimentally achieved by introducing variability of inter-pulse interval durations during an optogenetic stimulation epoch (Fig. 3.17a-d, preliminary data). An increase in theta amplitude variability could be experimentally achieved by modifying light intensity output during the course of an optogenetic stimulation epoch (Fig. 3.18, preliminary data).

Figure 3.16.
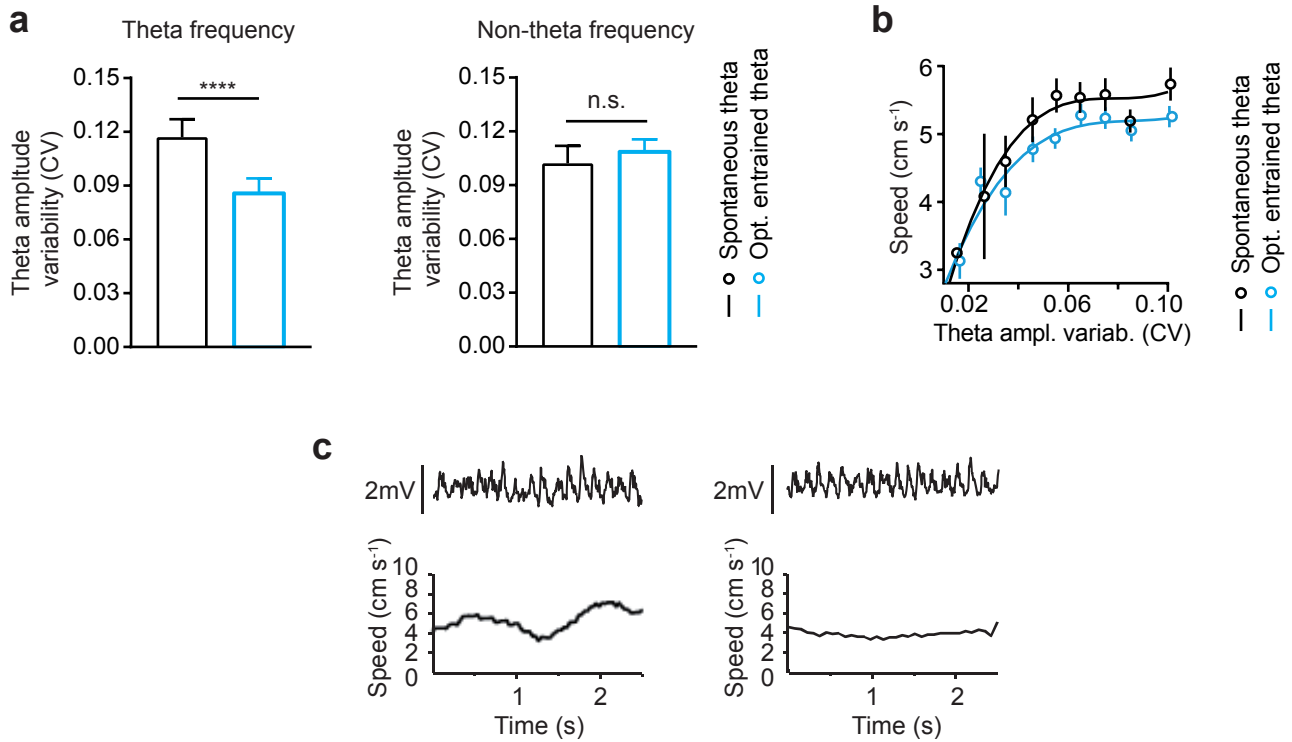
Fig. 3.16. Theta amplitude variability predicted running speed and running speed variability.
a, Left: theta amplitude variability (CV) was significantly lower during optogenetic stimulation at theta frequencies, compared to control light application of same respective frequencies. Right: theta amplitude variability (CV) was not different between optogenetic stimulation at non-theta (2, 4 or 20 Hz) or control light delivery of the same respective frequencies. **b**, Speed correlated with theta amplitude variability for entrained (blue), as well as spontaneous (black) theta epochs. **c**, Example epochs illustrating that higher speed is accompanied by higher theta amplitude variability and lower speed is accompanied by lower variability in theta amplitude. ****$p < 0.0001$. Modified from Bender et al., *Nature Commun.* 2015 (panels b,c).

Figure 3.17.
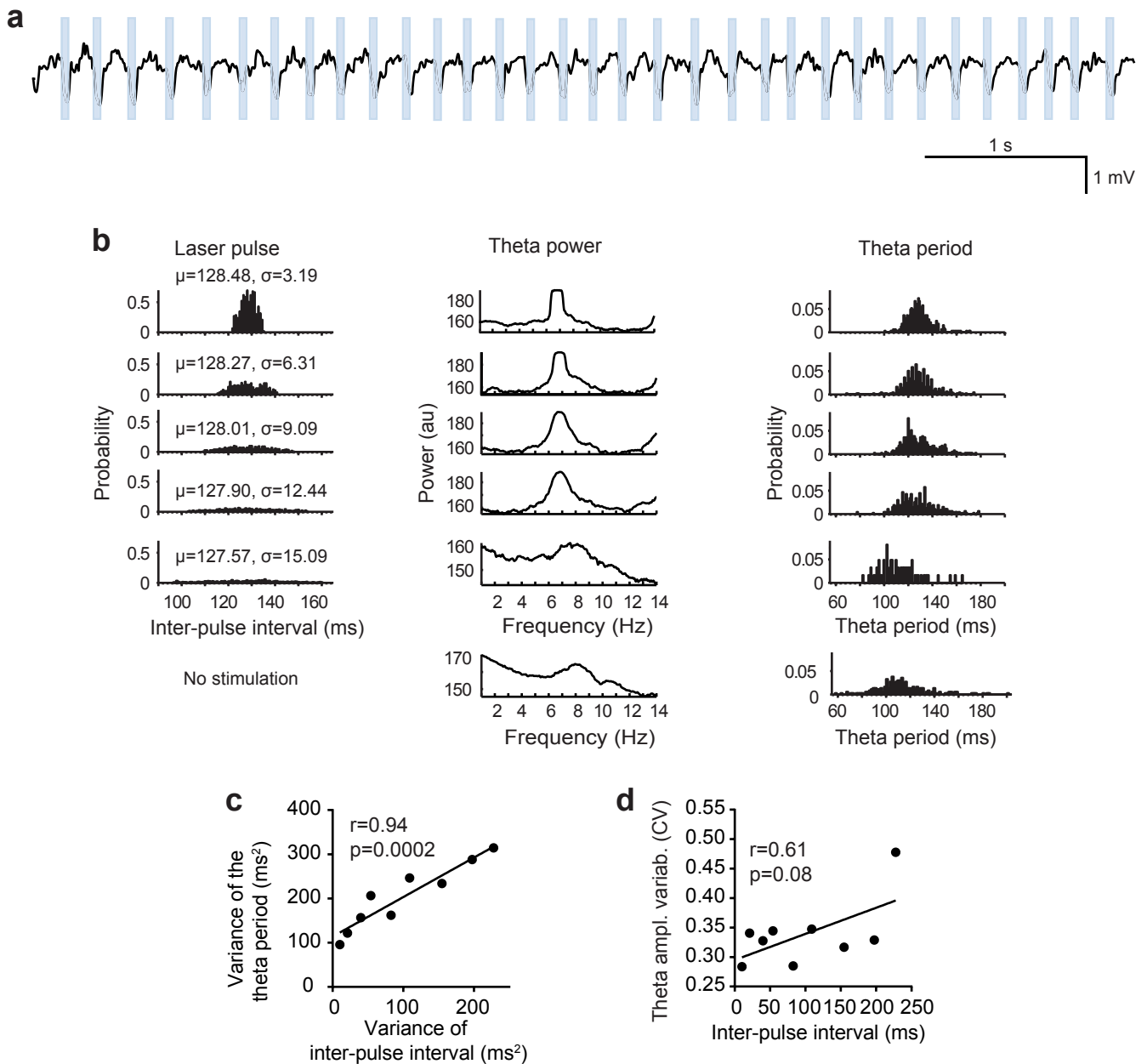
Fig. 3.17. Direct control of stimulation frequency variability.
a) Representative example trace of hippocampal theta LFP during entrainment with variable inter-pulse intervals. **b**) The probability distribution of the stimulation frequency followed a gaussian distribution with a mean frequency of $\mu=7.8$ Hz. Eleven different stimulation protocols with increasing standard deviation ($\sigma=1.56$ to $\sigma=15.07$) of the inter-pulse intervals were applied, each of 1 minute total duration. The first column shows the probability distribution of the inter-pulse interval of 5 protocols with different standard deviations. The second column shows the power spectral density (1-14 Hz) of the recorded hippocampal LFP during application of the respective protocol shown on the left. The third column shows the probability distribution of the theta period duration of the same hippocampal LFP recording shown on the left. **c**) Variance of the theta period adapted according to the variance of the inter-pulse interval of the respective stimulation protocol applied in one pilot experiment (Pearson's r=0.94, p=0.0002). **d**) Theta amplitude variability increased, but not significantly, with the probability distribution of the inter-pulse intervals in a pilot experiment (Pearson's r=0.61, p=0.08). Adapted from Korotkova and Ponomarenko, 2017.

Figure 3.18.

Laser light

Time stamps laser pulses

Hippocampal LFP

2 mV

10 s
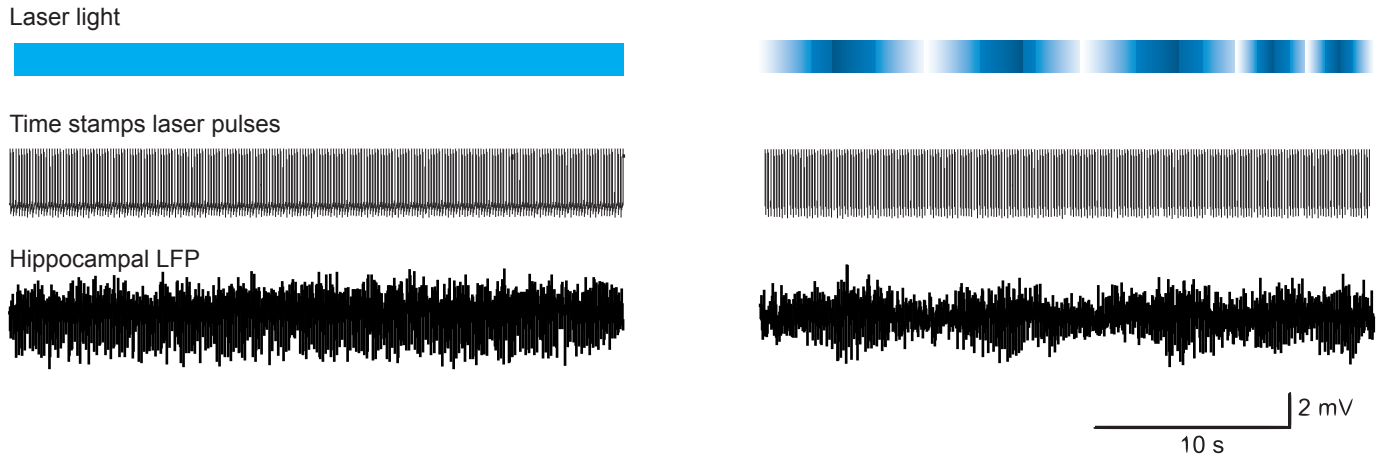
Fig. 3.18. Theta amplitude can be modulated during hippocampal theta entrainment by modulation of blue light output intensity.

Increase in amplitude variability during optogenetic hippocampal theta entrainment can be achieved by varying the light output intensity during the stimulation epoch. Constant light output mediates theta oscillations with constant amplitude across cycles (left). Varying the light intensity can modulate the amplitude of theta oscillations and hence control its variability (right). Colour shades of upper blue stripes (top) indicate light intensity output (colour code: blue-max, white-low). Trace with detected laser pulses is shown in the middle. Lower traces are hippocampal LFP traces during theta oscillations.

3.3. Relevance of the hippocampus to LS pathway for locomotor speed control

We next addressed the question of how hippocampal output could be relayed to downstream locomotor regions. The lateral septum is the major subcortical output region of the hippocampus and lateral septum lesions affect locomotor behaviour (Risold and Swanson, 1997b; Sheehan *et al.*, 2004). To study the role of the hippocampal subcortical output to the lateral septum for the modulation of locomotor speed, we tested whether hippocampal theta entrainment would modulate running speed also while signal transmission from the hippocampus to the lateral septum was inhibited. We applied two experimental approaches. We inhibited the hippocampus to lateral septum pathway pharmacologically by using the DREADD system. The effects of inhibition upon CNO application to hM4Di-expressing cells last for several hours (Alexander *et al.*, 2009). Therefore, we found the DREADD system suitable to study long-lasting effects on the average running speed of the animals. A change in speed variability upon theta entrainment was most evident within the first tens of seconds upon stimulation onset. To be able to initiate inhibition with high temporal precision during the course of the experiment and trigger inhibition onset according to the onset of stimulation of medial septum to hippocampus projections for theta entrainment, we choose an optogenetic approach in a second set of experiments. Halorhodopsin (eNpHR3.0) was expressed in hippocampus, and opsin-expressing projections to lateral septum were activated via yellow light application 15 seconds before the start of optogenetic hippocampal theta entrainment. As the yellow laser necessitates approximately 10-15 seconds to reach full light power output required for efficient pathway inhibition, we thereby ensured that fibres were inhibited when theta entrainment was initiated. Yellow light was switched off automatically after 1 minute.

3.3.1. Pharmacogenetic inhibition of the hippocampus-LS pathway

Pharmacologically inhibition was achieved by expressing the inhibitory DREADD hM4Di (*AAV-CaMKIIa-hM4D(Gi)-mCherry*) bilaterally in pyramidal cells of all subregions of the dorsal hippocampus (Fig. 3.19a). Pyramidal cells expressed hM4Di in somata in the hippocampus, axons projecting to the lateral septum and axon terminals in the lateral septum (Fig. 3.19a-c). ChR2 was expressed in PV$^+$ medial septum cells as in previous experiments. Guide cannulas were chronically implanted above the lateral septum (Fig. 3.19d). We combined bilateral pharmacogenetic inhibition of the lateral septum to hippocampus pathway with optogenetic hippocampal theta entrainment. I administered CNO or vehicle via an injection cannula fitting the guide cannula 5-10 minutes before the start of the experiment.

The average running speed during experiments with optogenetic entrainment of hippocampal theta upon lateral septum vehicle injections, hence where signals could be transmitted from the hippocampus to the lateral septum, was significantly lower than during experiments with optogenetic entrainment of hippocampal theta upon CNO administration with inhibited signal transmission from the hippocampus to the lateral septum (Fig. 3.19e, $F_{1,18211}$=514.94, p<0.00001, N=5 mice, Vehicle: n=17, CNO: n=30, Two-way repeated measures ANOVA, with factors "experimental subject" and "drug treatment (CNO or vehicle in LS)"). Running speed did not differ between the respective baseline recordings ($F_{1,39739}$=0.09, p=0.77). Spontaneous running speed even increased in response to lateral septum CNO administration in comparison to baseline recordings (Fig. 3.19f, $F_{1,10809}$=29.23, p<0.00001, N=6 mice, Two-way repeated measures ANOVA, with factors "experimental subject" and "drug treatment (CNO or vehicle in LS) "). Speed variability did not increase significantly upon lateral septum CNO administration during spontaneous running epochs (Fig. 3.19g, $F_{1,13}$=2.64, p=0.15; N=6 mice).

Figure 3.19.
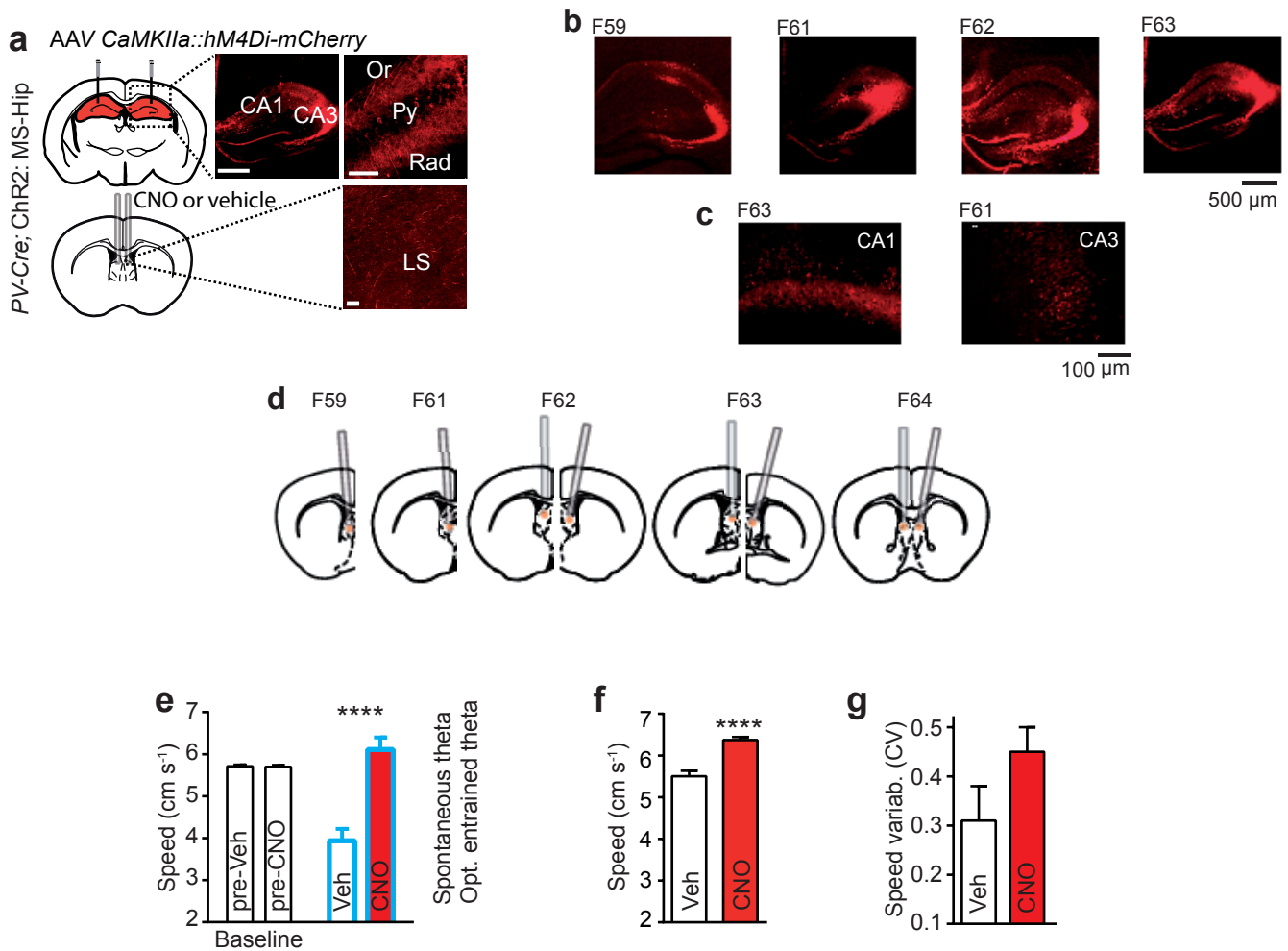
Fig. 3.19. Pharmacogenetic inhibition of the hippocampus to LS pathway.
a, DREADD viral construct hM4Di (*AAV-CaMKIIa-hM4D(Gi)-mCherry*) was delivered to pyramidal cells of the dorsal hippocampus of PV-Cre mice (upper scheme). The receptor was expressed under the CaM kinase II promoter, hence expression was confined to pyramidal cells (upper images). CNO or vehicle was delivered via internal cannulas inserted into permanently implanted guide cannulas (lower scheme). Axons in the LS expressed the receptor hM4Di as verified by bright fluorescence in the LS. **b,c**, mCherry fluorescence in the hippocampi of mice used for pharmacogenetic inhibition experiments. **d**, Positions of guide cannulas verified post-mortem and infusion sites in the LS are marked in orange. **e**, Running speed was significantly higher during optogenetic hippocampal theta entrainment in combination with pharmacogenetic inhibition of the hippocampus to LS pathway via CNO delivery to the LS (red bar with blue contours) than during optogenetic hippocampal theta entrainment and vehicle delivery to the LS (white bar with blue contours). **f**, Running speed was higher when the hippocampus to LS pathway was inhibited via CNO delivery to the LS (red) compared to vehicle delivery to the LS (white). **g**, Running speed variability did not significantly change when the hippocampus to LS pathway was inhibited via CNO delivery (red) to the LS compared to vehicle delivery to the LS (white, p=0.15). ****p<0.0001. Modified from Bender et al., *Nature Commun*. 2015.

3.3.2. Optogenetic inhibition of the hippocampus-LS pathway

For optogenetic inhibition, halorhodopsin (*AAV2/1.CamKIIa.eNpHR3.0-EYFP.WPRE.hGH*) was expressed bilaterally in pyramidal cells in all subregions of the dorsal hippocampus (Fig. 3.20a,b). eNpHR3.0-EYFP was detected at somata in the hippocampus (Fig. 3.20b), axons projecting to the lateral septum and at axon terminals in the lateral septum (Fig. 3.20c). ChR2 was expressed in PV$^+$ medial septal cells as in previous experiments. We combined optogenetic inhibition of the lateral septum to hippocampus pathway using yellow light (wavelength: 593 nm) applied bilaterally to the lateral septum via chronically implanted optic fibres above the lateral septum (Fig. 3.20a) with optogenetic hippocampal theta entrainment via unilateral delivery of blue light (wavelength: 473 nm) via a fibre chronically implanted above the hippocampus. During optogenetic inhibition of this major hippocampal output pathway hippocampal theta could still be entrained optogenetically. Fig. 3.21a shows all LFPs for recording epochs when 9 Hz stimulation theta entrainment was combined with optogenetic inhibition of the hippocampus to lateral septum pathway. The rows are sorted according to entrainment fidelity. When the hippocampus to lateral septum pathway was intact during optogenetic theta entrainment speed variability was reduced ($F_{1,94}$=14, *p*=0.0003). When the hippocampus to lateral septum pathway was inhibited optogenetically during theta entrainment speed variability was not reduced ($F_{1,66}$=0.48, *p*=0.49). Speed variability during experiments with optogenetic entrainment of hippocampal theta and no yellow light application to the lateral septum, hence with intact signal transmission from hippocampus to lateral septum, was significantly lower than during experiments with optogenetic entrainment of hippocampal theta and inhibitory yellow light stimulation above the lateral septum (Fig. 3.21b, $F_{1,36}$=8.38, *p*=0.0073, *N*=8 mice, theta entrainment: *n*=19, theta entrainment and hippocampus to LS inhibition: *n*=18, Two-way repeated measures ANOVA, with factors "experimental subject" and "type of optical stimulation (intra-LS yellow light or no yellow light stimulation)). During the respective baselines before optogenetic intervention, running speed variability was not different between experiments in both conditions ($F_{1,66}$=0.48, *p*=0.49, *N*=8 mice, baseline before theta entrainment: *n*=19, baseline before theta entrainment and hippocampus to LS inhibition: *n*=15). The average running speed variability was similar between experiments with optogenetic hippocampal theta entrainment and no additional yellow light application, or additional yellow light application in the lateral septum of mice which did not express eNpHR3.0 but only the fluorophore mCherry, or additional application of yellow control light but outside the brain (Fig. 3.21c,d, $F_{2,66}$=0.037, *p*=0.96, *N*=13 mice, Two-way ANOVA, with factors "experimental subject" (random factor, different mice in groups) and "yellow light (off vs. intra-LS in eNpHR3.0$^-$ mice vs. dummy patch cord in eNpHR3.0$^+$ mice)", fixed factor).

Altogether, these results suggest that an intact hippocampus to lateral septum pathway is required for the modulatory influence of hippocampal theta oscillations on ongoing running speed.

Figure 3.20.
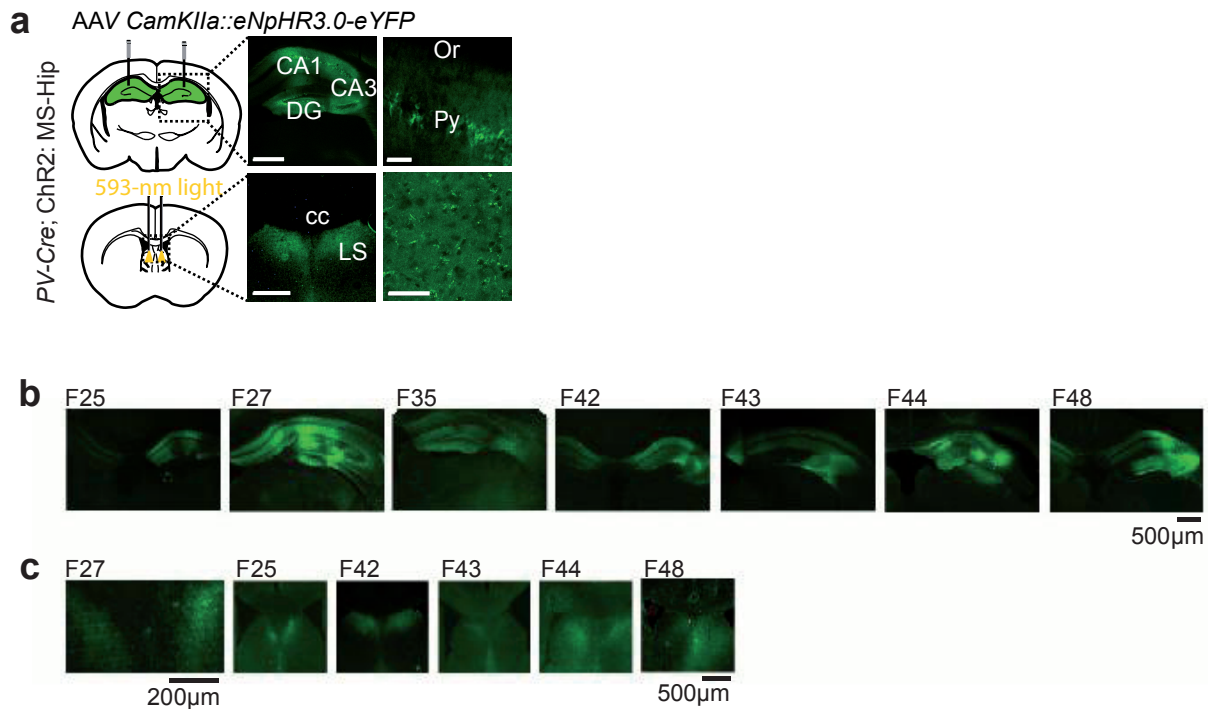
Fig. 3.20. Optogenetic preparation for inhibition of the hippocampus to LS pathway.
a, Halorhodopsin was expressed under the CaM kinase promoter in hippocampal pyramidal cells (upper scheme). Bright fluorescence confirmed successful expression of the opsin in the dorsal hippocampus (upper left image). Pyramidal cells expressed the opsin (upper right image). Cell bodies in the pyramidal layer (Py) but not in the str. oriens (Or) express opsin. Optic fibres were implanted bilaterally above the LS for delivery of yellow light to the axon terminals of hippocampal pyramidal cells in the LS (lower scheme). Hippocampus to LS fibres expressed the opsin (lower left image). The LS is located below the corpus callosum (cc). Axons expressing the opsin are visible in the LS (lower right image). **b,c**, successful expression of the opsins at somata in the hippocampus (**b**) and axon terminals in the LS (**c**) was confirmed post-mortem after completion of the experiments. Modified from Bender et al., *Nature Commun*. 2015.

Figure 3.21
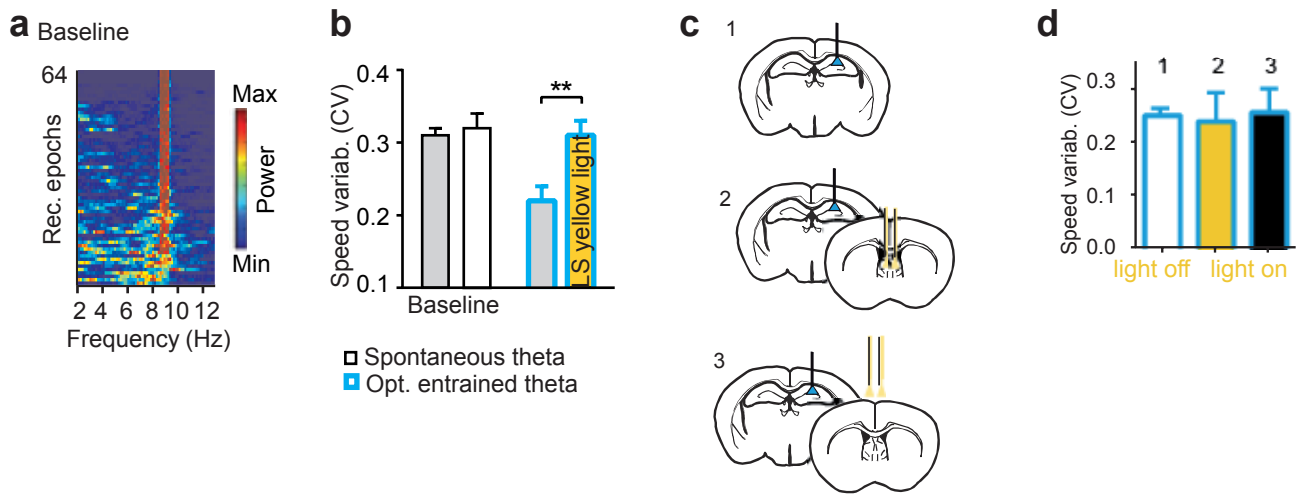
Fig. 3.21. Optogenetic inhibition of the hippocampus to LS pathway prevents the reduction in speed variability during optogenetic theta entrainment.
a, Hippocampal theta was successfully entrained in experiments where simultaneously optogenetic inhibition of the hippocampus to LS pathway was performed. PSDs are stagged according to entrainment fidelity. Warmer colours indicate higher power. Stimulation frequency was 9 Hz. **b**, Inhibition of the hippocampus to LS pathway prevented the reduction in running speed variability. In grey: running speed variability during the baseline before optogenetic entrainment and no yellow light application (grey bar with blue contour). In white: baseline before optohenetic theta entrainment in combination with yellow light application to the LS (yellow bar with blue contour). **c**, Optogenetic theta entrainment via blue light application to the hippocampus in the absence of yellow light (1). Optogenetic theta entrainment via blue light application to the hippocampus and yellow light application to the LS in mice which did not express halorhodopsin but only the fluorophore mCherry in the hippocampus to LS pathway (2). Optogenetic theta entrainment via blue light application to the hippocampus and yellow light application to a dummy fibre outside the brain attached to the headset so that activation of the inhibitory opsin was prevented (3). **d**, The speed variability was similar in all experimental conditions described in **c**. Blue contours indicate application of blue light to the hippocampus. White filling indicates absence of yellow light (1). Yellow filling indicates yellow light application to the LS in mice which did not express halorhodopsin (2). Black filling indicates application of yellow light ouside the brain (3). **p<0.01. Modified from Bender et al., *Nature Commun*. 2015.

3.4. Role of gamma signaling within the mPFC to LS pathway during a T-maze task

3.4.1. mPFC to LS gamma rhythmic optogenetic stimulation did not decrease running speed

While the hippocampus and LS are highly coherent at theta frequencies, the mPFC and LS are highly co-herent at gamma frequencies (Carus-Cadavieco *et al.*, 2017). ChETA (*AAV2-CaMKIIa-hChR2(H134R)-eYFP*) or ChR2 (*AAV2-CaMKIIa-hChR2(H134R)-eYFP*) was expressed in pyramidal cells of the mPFC (Fig. 3.22a). We stimulated axonal projections to the lateral septum by light delivery via optic fibres im-planted above the lateral septum (Fig. 3.22a,b). Optogenetic gamma rhythmic stimulation of mPFC to lateral septum projections did not affect running speed (Fig. 3.22c, YFP: *N*=8 mice, opsin: *N*=7 mice; *p*=0.7, t-test).

3.4.2. mPFC to LS gamma rhythmic optogenetic stimulation improved performance in the T-maze

Further, we studied the role of gamma oscillations in the mPFC and lateral septum for performance in a cognitive task. In a food rewarded spatial non-matching to place T-maze task gamma occurrence is in-creased in the LS and mPFC while the mouse is in the choice, but not in the start arm (Carus-Cadavieco *et al.*, 2017, a scheme of the T-maze is depicted in Fig. 3.23a). Optogenetic gamma frequency stimula-tion (66.7 Hz) of mPFC pyramidal cells projecting to the lateral septum (Fig. 3.23b) could improve the performance in the T-maze. Mice which expressed the opsin and were optogenetically stimulated dur-ing the task made more correct choices, than mice which expressed the fluorophore YFP but not the opsin (Fig. 3.23c, trials 1–20: YFP: *N*=7 mice, opsin: *N*=9 mice; *p*=0.0096, t-test; trials 21–40: YFP: *N*=6 mice, opsin: *N*=5 mice; *p*=0.074, t-test). The fraction of repeatedly correct trials increased (cc, YFP: *N*=7 mice, opsin: *N*=9 mice; *p*=0.026, t-test), while the fraction of repeatedly incorrect trials decreased (Fig. 3.23c, YFP: *N*=7 mice, opsin: *N*=9 mice; *p*=0.014, unpaired t-test) when the optogenetic stimulation was conducted during the task. Performance was also improved when water instead of food was used as a reward in water-restricted mice: the number of correct trials (YFP: *N*=6 mice, opsin: *N*=6 mice, trials 1–20: *p*=0.0097, t-test; trials 21–40: *p*=0.4) and fraction of repeatedly correct trials (*p*=0.02, t-test) was increased (Fig. 3.23d). The fraction of repeatedly incorrect trials was not significantly reduced (YFP: *N*=6 mice, opsin: *N*=6 mice, *p*=0.052, t-test). In contrast, when mPFC to lateral septum projections were stimulated at theta frequencies the performance in the T-maze task did not improve (Fig. 3.23e). Neither was the number of correct trials (trials 1–20, opsin: *N*=7 mice, YFP, intensity-matched stimula-tion: *N*=7 mice, *p*>0.99, t-test), nor the fraction of repeatedly correct trials (cc, *p*=0.74, t-test) increased or the fraction of repeatedly incorrect trials decreased (*p*=0.7, t-test).

Figure 3.22.
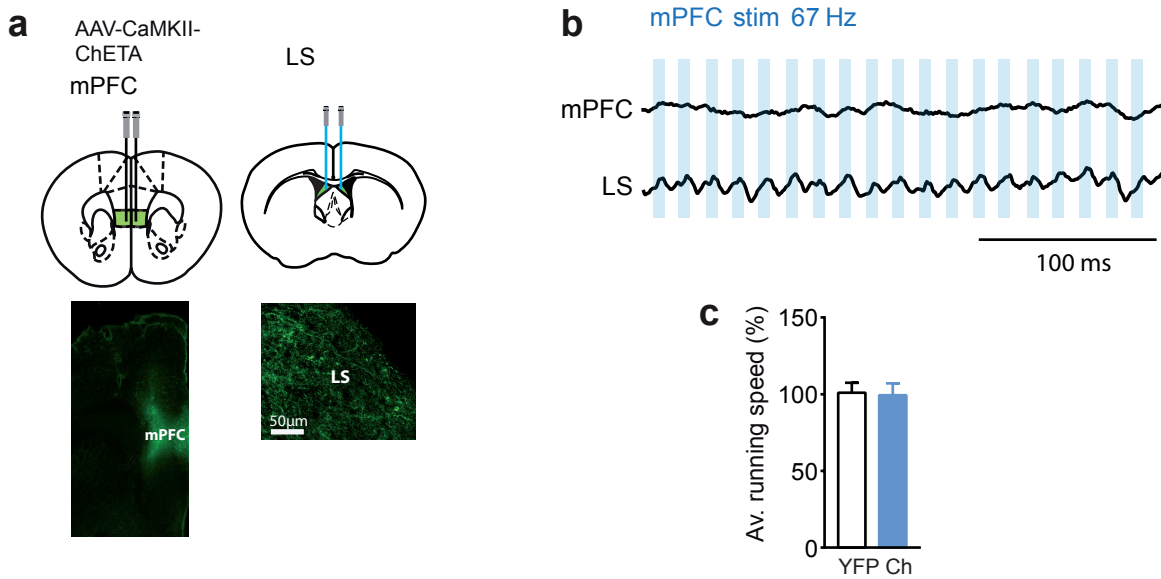
Fig. 3.22. Optogenetic stimulation of mPFC to LS fibres did not affect running speed.
a, ChETA or ChR2 was expressed under the CaM kinase II promoter in mPFC pyramidal cells for optogenetic stimulation experiments. For control experiments only the fluorophore YFP (AA-V2-CaMKIIa-eYFP), but not the opsin, was expressed. Optic fibres were implanted above the LS. Illustrated below is local expression of ChR2 in the mPFC (left) and at axon terminals in LS (right). **b,** mPFC and LS LFP during optogenetic gamma (66.7 Hz) stimulation of mPFC to LS projections with 5 ms blue light pulses. **c,** Average running speed was similar during optogenetic stimulation of mPFC to LS projections at gamma frequencies in mice expressing ChETA and sham stimulation in mice expressing only the fluorescent protein YFP in mPFC pyramidal cells. Modified from Carus-Cadavieco, Gorbati, Ye, Bender et al., *Nature* 2017 (schemes in panel a, panel c).

Figure 3.23
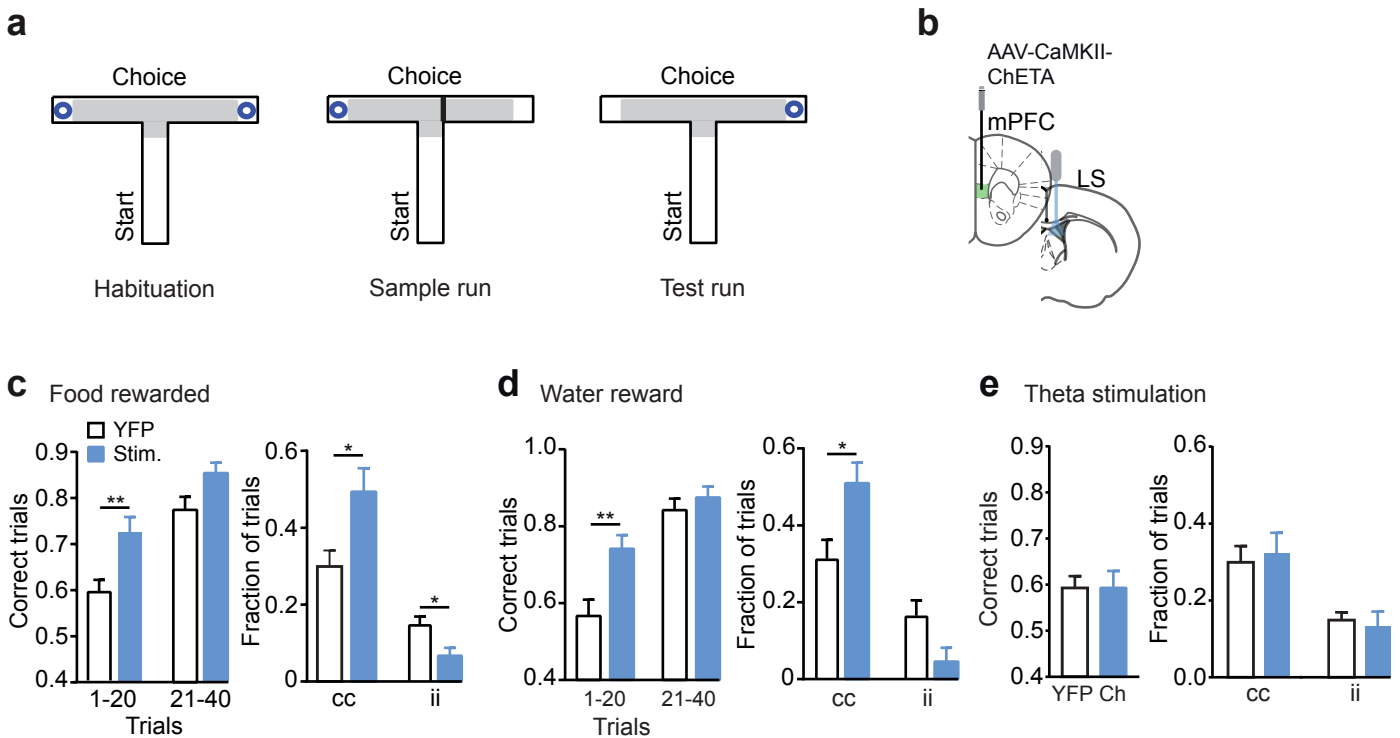
Fig. 3.23. In trials with optogenetic stimulation of the LS to mPFC pathway at gamma frequencies mice performed better in the spatial non-matching to place T-maze task.
a, Mice performed in a non-matching to place T-maze task. The choice segment is marked in grey. The food/water is indicated by a blue circle. **b**, ChETA or ChR2 was expressed under the CaM kinase promoter in mPFC pyramidal cells for optogenetic stimulation experiments. Only the fluorophore YFP was expressed for control experiments. Optic fibres were implanted above the LS. **c**, The fraction of correct decisions within the first 20 trials increased significantly when the mPFC to LS pathway was optogenetically stimulated at gamma frequencies in mice expressing ChETA or ChR2 compared to sham stimulation in mice expressing only YFP when correct decisions were rewarded with food (left). The fraction of repeated correct trials increased significantly and the fraction of repeated incorrect trials decreased significantly when the mPFC to LS pathway was stimulated at gamma frequencies during the experiment when correct choices were rewarded with food (right). **d**, The fraction of correct decisions within the first 20 trials increased significantly when the mPFC to LS pathway was optogenetically stimulated at gamma frequencies in mice expressing ChETA compared to sham stimulation in mice expressing only YFP when correct decisions were rewarded with water in water restricted mice (left). The fraction of repeated correct trials increased significantly and the fraction of repeated incorrect trials decreased when the mPFC to LS pathway was stimulated at gamma frequencies during the experiment when correct choices were rewarded with water in water restricted mice (right). **e**, Number of correct trials (left) and fraction of repeated correct and repeated incorrect trials was similar when the mPFC to LS pathway was stimulated at theta frequencies during the experiment when correct choices were rewarded with food. *p<0.05, **p<0.01. Modified from Carus-Cadavieco, Gorbati, Ye, Bender et al., *Nature* 2017 (panels b-e).

4. Discussion

4.1. Summary of results

The goal of this study was to develop a preparation which enables entrainment of hippocampal theta oscillations in behaving mice, to identify the causal relationship between theta frequency and running speed, to unravel whether hippocampal theta causally influences running speed and exploratory behaviour in general, and to clarify the role of the lateral septum in transmitting behaviour-relevant information from the hippocampus to subcortical regions which control locomotor output. My final aim was to examine the relevance of rhythmic activity of the mPFC to the lateral septum for spatial goal-directed behaviour. We achieved entrainment of physiological hippocampal theta oscillations during all vigilance states by optogenetic theta-rhythmic stimulation of GABAergic medial septum neurons projecting to the right dorsal hippocampus. Entrainment did not change the behavioural state. Instead, our data indicate that the hippocampus modulates ongoing movement. The frequency of hippocampal theta oscillations, when set by the stimulation frequency, was no longer correlated with running speed. We then found that hippocampal theta entrainment at constant theta frequency slowed down the speed of the mouse. Our data indicate that constant frequency is associated with a more stable theta amplitude across theta cycles. A correlation between theta amplitude variability and running speed was detected for entrained and spontaneous theta. We further provide evidence that the hippocampal output is transmitted downstream via the lateral septum, as inhibiting the hippocampus to lateral septum pathway abolished the effect of hippocampal theta entrainment on running speed. Optogenetic stimulation of mPFC to lateral septum fibres at gamma frequencies did not affect running speed but improved performance in a spatial working memory task. Overall, our data suggest that theta rhythmic signaling from the hippocampus to the lateral septum supports regulation of locomotor speed while gamma rhythmic signaling from the mPFC to the lateral septum supports goal directed behaviour during spatial learning.

4.2. Methodological considerations for hippocampal theta entrainment

4.2.1. Optogenetic stimulation parameters

Light intensity was set to 10-15 mW from the tip of the optic fiber. I used this light intensity in order to activate a sufficient number of axon terminals to achieve theta entrainment (Cardin *et al*, 2010, Yizhar *et al*, 2011b, Boyden *et al*, 2005). In contrast, weak focal photostimulation using a LED with peak power in the µW range is applied to activate selectively a limited number of hippocampal cells (2-20) while leaving network dynamics unaffected *in vivo* (Stark *et al*, 2012; Stark *et al*, 2013).

Light pulse duration was 30 ms. Depending on frequency, inter-pulse intervals, including all protocols, were between 20 ms and 470 ms, and between 80 ms and 120 ms for theta entrainment protocols. These time windows fitted temporal dynamics of endogenous theta as well as kinetics of ChR2 and GABA$_A$ receptor. The duration of the positive theta component in the pyramidal layer is approximately 40 ms in the mouse when theta frequency is high (Buzsaki *et al*, 2003). ChR2 is activated within less than 2 ms and inactivated within about 18 ms (Yizhar *et al*, 2011b; Boyden *et al*, 2005). ChR2 requires pulse durations of above 5 ms for efficient activation and a recovery period of at least 10 ms (Yizhar *et al*, 2011b; Boyden *et al*, 2005; Nagel *et al*, 2003). The decay time constant of currents mediated by the GABA$_A$ receptor is approximately 10 ms (Wulff *et al*, 2009).

An *in vitro* study reported reliable theta entrainment with 2-70 ms duration of blue light pulses send at 8 Hz to ChR2 expressing hippocampal PV^{+} cells and an increase in the number of elicited action potentials with increasing pulse duration (Amilhon *et al*, 2015). In contrast, light pulse duration of 1 ms elicited action potentials in only a portion of interneurons and drove ongoing oscillations partially. Maximal theta power increase was reached with pulse durations of 40 ms. In contrast, continuous light delivery for 10 seconds decreased theta power *in vitro* (Amilhon *et al*, 2015). *In vivo* in behaving mice ChR2 expressing hippocampal PV^{+} neurons emitted reliably up to 4 spikes per 10 ms pulse (Siegle and Wilson, 2014; Siegle *et al*, 2014).

Optogenetic stimulation can also induce burst firing (Madisen *et al*, 2012; Cardin *et al*, 2010; Cardin *et al*, 2009), which is a physiological activity mode of medial septum GABAergic cells (Hangya *et al*, 2009). Our protocol mimics high excitation, as occurs during burst firing. ChR2 expressing medial septum PV^{+} cells were demonstrated to spike reliably upon excitation of somata with 20 ms pulses at frequencies between 5-40 Hz *in vitro* using a light intensity of 2 mW (Dannenberg *et al*, 2015).

4.2.2. Targeting GABAergic medial septum cells

The medial septum is suggested to be the main hippocampal theta generator (Petsche *et al*, 1962). It integrates signals coming from various brain regions (Dutar *et al*, 1995), including the ascending brain stem synchronizing pathway (Bland and Oddie, 2001), as well as feedback signals from the hippocampus (Dragoi *et al*, 1999; Manseau *et al*, 2008). Different medial septum cell populations have different firing properties and were attributed different functions. The GABAergic medial septum

population has been suggested to provide the theta rhythmic input to the hippocampus (Freund and Antal, 1988; Dragoi *et al*, 1999, Hangya *et al.*, 2009). Optogenetics allows to selectively stimulate GABAergic medial septum projections to the hippocampus with high spatial and temporal precision.

We achieved hippocampal theta entrainment *in vivo* in behaving mice via optogenetic theta rhythmic excitation of GABAergic (PV+) medial septum to hippocampus projections (Bender *et al*, 2015). Medial septum GABA cells rhythmically discharge bursts at theta frequencies during the theta state (Hangya *et al*, 2009). They synapse exclusively onto hippocampal interneurons and have been suggested to disinhibit hippocampal pyramidal cells rhythmically from inhibition (Freund and Antal, 1988, Hangya *et al.*, 2009). Rhythmic inhibition of PV+ cells in the hippocampus by medial septum PV+ cells has been suggested pivotal for hippocampal theta generation (Wulff *et al*, 2009). Rhythmic optogenetic silencing or stimulation (4-10 Hz) of hippocampal PV+ cells entrains hippocampal theta *in vitro* while their continuous silencing disrupts theta in anesthetized and behaving mice (Amilhon *et al*, 2015; Gangadharan *et al*, 2016; Boyce *et al*, 2016). Other hippocampal interneuron types, such as O-LM interneurons, which also receive rhythmic input from medial septum GABA cells, probably contribute to theta generation or modulate network responses (Amilhon *et al*, 2015; Gloveli *et al*, 2005; Gillies *et al*, 2002). This work is the first report of the selective control of hippocampal theta.

Optogenetic theta rhythmic excitation of glutamatergic medial septum cell somata entrains hippocampal theta *in vitro* and *in vivo* in behaving mice (Fuhrmann *et al*, 2015; Robinson *et al*, 2016). Medial septum glutamatergic projections to the hippocampus, however, are sparse and selective activation of projections to the hippocampus does not affect hippocampal theta frequency *in vivo* in behaving mice (Robinson *et al*, 2016). Hence, glutamatergic medial septum cells modulate hippocampal theta indirectly, probably through modulation of other neuronal populations within the medial septum, and/or through activation of other subcortical regions which form part of the ascending hippocampal synchronizing pathway. Robinson *et al*, 2016, suggested that medial septum glutamatergic cells synchronize activity of GABAergic and, to a minor extent, cholinergic medial septum cells, as they project to the majority of local GABAergic and a minority of local cholinergic cells (Manseau *et al*, 2005; Leao *et al*, 2015; Robinson *et al*, 2016; Hajszan *et al*, 2004; Xu *et al*, 2015).

Optogenetic theta rhythmic excitation of cholinergic medial septum cell somata increases firing of hippocampal interneurons and mediates more precise coupling of pyramidal cell firing to theta phase in anesthetized animals (Vandecasteele *et al*, 2014; Dannenberg *et al*, 2015). However, during active behaviour the effect of stimulation on hippocampal network dynamics is much less pronounced than during quiet wakefulness (Mamad *et al*, 2015) or even absent (Vandecasteele *et al*, 2014). Thus, the effect of optogenetic excitation of cholinergic medial septum cells on hippocampal theta rhythm is behavioural state dependent. It was argued that during the active behavioural state acetylcholine levels are already increased and therefore additional acetylcholine release has less impact (Vandecasteele *et al*, 2014; Mamad *et al*, 2015). Within the medial septum cholinergic cells excite local GABAergic and

glutamatergic cells (Dannenberg *et al*, 2015; Manseau *et al*, 2005). Lesioning cholinergic medial septum cells leads to a reduction of the number of cells within the medial septum which fire rhythmic bursts (Apartis *et al*, 1998) and attenuates hippocampal theta (Yoder and Pang, 2005). Vanderwolf *et al*, 1978, initially suggested that the lower frequency component of hippocampal theta is controlled via an ascending -reticuloseptal- cholinergic pathway. Accordingly, average firing frequency of medial septum cholinergic cells is in the low theta frequency range (5 Hz, Griffith and Matthews, 1986; Markram and Segal, 1990b). Cholinergic activation suppresses oscillations with a frequency below the theta frequency range (Vandecasteele *et al*, 2014; Dannenberg *et al*, 2015). Overall, the role of cholinergic medial septum cells in modulating hippocampal theta oscillations appears complex and is still incompletely understood.

Non cell-type selective optogenetic rhythmic excitation of projections from medial septum to hippocampus affects hippocampal activity *in vivo* in behaving rats (Laxpati *et al*, 2014) and mice (Robinson *et al*, 2016). However, Blumberg *et al.* (2016) found that entrainment efficacy *in vivo* in behaving mice depended on the animals momentary mobility and speed. When stimulating at low theta frequency (6 Hz), hippocampal theta frequency was more likely to match stimulation frequency when the animals speed was below 2 cm/s.

4.2.3. Targeting dorsal hippocampus CA1 area

The optic fiber, which transmitted the laser light, was implanted above the dorsal right hippocampal CA1 area for stimulation of medial septum GABAergic axon terminals in all hippocampal layers.

Functionally, the dorsal, but not the ventral hippocampus, has been attributed an important role in guidance of locomotor behaviour (Patel *et al*, 2012; Montgomery *et al*, 2009). In line with the present literature (Royer *et al*, 2010) we found that dorsal hippocampal activity was much stronger related to locomotor behaviour than ventral hippocampal activity. Power and frequency of ventral hippocampal theta did not relate to momentary running speed. It is known that the ventral hippocampus rather relates to anxiety and stress than locomotor guidance (Fanselow and Dong, 2010). Mechanistically, hippocampal theta is suggested to arise at the septal (hence dorsal) pole of the hippocampus and to travel along the septo-temporal axis (Patel *et al*, 2012; Zhang and Jacobs, 2015; Lubenov and Siapas, 2009) and CA1 pyramidal cell commissural projections form primarily at the septal hippocampal pole (Van Groen and Wyss, 1988; van Groen and Wyss, 1990). Therefore, I assume that optogenetic stimulation at the septal compared to the temporal pole better facilitates propagation of locally modulated network dynamics.

The CA1 isolated preparation exhibits spontaneous theta rhythm in the rat (Goutagny *et al*, 2009) as well as in the mouse hippocampus (Amilhon *et al*, 2015). Thus, an isolated CA1 network can generate and maintain theta oscillations. A uniquely biological data-driven full-scale computer model of the isolated CA1 network supported theta generation by the CA1 network (Bezaire *et al*, 2016). The CA3

network has long been suggested to harbour an intrinsic theta generator (Buzsaki, 2002). Hippocampal areas CA1 and CA3 may harbour two series of separate intrinsic theta generators (Goutagny *et al*, 2009; Jackson *et al*, 2014). Distinct intrahippocampal theta generators probably synchronize *in vivo*, as theta frequency and phase offsets are consistent across the hippocampus (Bullock *et al*, 1990). In general, *in vitro* and *in vivo* studies suggest that theta rhythm in the hippocampus is generated by interaction of intrinsic and external oscillators (Colgin, 2013).

In this study we have demonstrated that axon terminal stimulation of medial septum PV+ cells projecting to the dorsal hippocampal CA1 area can entrain theta oscillations. We predict that light delivery to the dorsal hippocampal area CA3 using the same preparation would also enable hippocampal theta entrainment and may mediate similar behavioural results, while light delivery at the ventral hippocampus may be less effective and may result in different behavioural effects. In this preparation light may also have excited a fraction of medial septum projections to CA3 passing near the fibre.

4.2.4. Dependency of stimulation efficacy on stimulation frequency

Non-theta frequency stimulation resulted in low entrainment efficacy. In line with our results, optogenetic activation of hippocampal PV+ interneurons *in vitro* at 2 Hz does not slow down hippocampal theta rhythm to that frequency and within a frequency range of 2-20 Hz theta power is maximal at 8 Hz stimulation frequency (Amilhon *et al*, 2015). Moreover, spiking of pyramidal cells is most efficiently elicited when hippocampal PV+ cells are stimulated at theta frequencies (Stark *et al*, 2013). Pharmacological blockade of HCN channels abolishes the suprathreshold spiking resonance and random noise activation of hippocampal PV+ cells does not induce theta rhythmic firing (Stark *et al*, 2013). HCN channels, expressed in hippocampal pyramidal cells, facilitate preferential amplification of signals repeated at theta frequencies. Resonance has been suggested to serve timing of neuronal activity and communication across brain regions and generation of brain states (Buzsaki, 2002; Llinas, 1988; Alonso and Llinas, 1989; Hasselmo, 2014; Pike *et al*, 2000; Buzsaki and Draguhn, 2004). Moreover, HCN channels have been assigned an important function in adjusting the gain for integrating movement related information to spatial firing fields during self-motion guided navigation (Giocomo *et al*, 2011). Pyramidal cell spiking is accompanied by postinhibitory rebound spiking, mediated via hyperpolarization mediated current, which supports synchronized firing of pyramidal cells and maintenance of persistent oscillatory dynamics (Lien *et al*, 2002; Bottjer, 2005).

Electrical stimulation studies previously provided evidence for increased responsiveness of septohippocampal cells towards inputs arriving at theta frequency. Electrical stimulation of the perforant path at 1 Hz frequency inhibited hippocampal theta-on cell activity (Bland *et al*, 1980) and theta rhythmic stimulation increased drastically the number of recruited pyramidal cells in the hippocampus in comparison to single pulse stimulation (Andersen *et al*, 1966; Andersen and Lomo, 1967; Andersen and Lomo, 1970). In the medial septum, electrical theta rhythmic, but not higher

frequency stimulation of the fimbria elicited burst firing (McLennan and Miller, 1974b).

Spectral harmonics within the theta range often were the result of stimulation at non-theta frequencies in our experiments. Occurrence of theta harmonics is a natural phenomenon that has been described for spontaneous theta as well (Czurko *et al*, 1999; Terrazas *et al*, 2005; Sheremet *et al*, 2016; Buzsaki *et al*, 2003). Theta harmonics in the hippocampal LFP also occurred in other preparations which optogenetically controlled hippocampal theta dynamics (Fuhrmann *et al*, 2015; Dannenberg *et al*, 2015; Robinson *et al*, 2016).

In a pilot experiment we introduced variability of inter-pulse intervals during optogenetic stimulation. With increasing variance of inter-pulse interval durations the variance of theta periods increased. Network activity adaptation to optogenetic stimulation with introduced variability of inter-pulse intervals has previously been demonstrated (Tchumatchenko *et al*, 2013; Laxpati *et al*, 2014). It has been argued that the introduced variability better mimics spontaneous network activity (Tchumatchenko *et al*, 2013).

4.2.5. Competition of optogenetic stimulation with intrinsic dynamics

Hippocampal theta could be successfully entrained in the preparation used in this study. However, competing signals, which may be sensory driven or intrinsic, could interfere with the optogenetic control over the local network dynamics. Resetting of hippocampal theta rhythm in response to electrical stimulation of the fornix, the fibre bundle projecting from the medial septum to the hippocampus, has been previously reported (Williams and Givens, 2003; Scarlett *et al*, 2004; Bland *et al*, 2006a).

Sensory stimulus-locked reordering of phase of ongoing oscillations is a common (Canavier, 2015). Indeed, in some trial sessions, which were not included in analysis, presentation of sounds, for instance hand clapping, disrupted entrainment for several theta cycles. Phase reset is expected from intrinsic properties of oscillators and represents an efficient mechanism for sensory modulation of network dynamics. Theta phase reset in response to stimuli across sensory modalities has been described previously (Givens, 1996; Adey, 1967; Canavier, 2015).

The momentary intrinsic state of the brain may also affect efficacy of entrainment. Dependence of entrainment efficacy on the momentary state of the animal has been demonstrated in ChAT-Cre (Mamad *et al*, 2015) and in WT mice (Blumberg *et al*, 2016). We found that entrainment efficacy was generally higher during running compared to immobility. It is therefore unlikely that the correlation between lower speed and entrainment efficacy could be explained by behavioural state dependency of entrainment efficacy in our data.

For analysis of behavioural data entrainment efficacy was calculated for 10 second stimulation epochs. The impact of optogenetic stimulation on behaviour was related to momentary entrainment efficacy.

4.2.6. Physiological features of entrained hippocampal theta rhythm

Physiological features of optogenetically entrained hippocampal theta oscillations were confirmed before analysis of behavioural effects. Electrical stimulation of the medial septum has previously been shown to induce hippocampal theta (Ball and Gray, 1971). However, the electrically induced theta differs from spontaneous theta, in particular, theta-related cellular activity was changed (Scarlett *et al*, 2004). The optogenetic approach implemented in our study allowed for higher selectivity as well as spatial and temporal precision, in agreement with reliable and physiological cellular responses produced by axonal stimulation of ChR2 expressing cells in other brain regions (Jackman *et al*, 2014).

Cross-frequency coupling of theta and gamma oscillations is an important feature of spontaneous hippocampal theta (Wulff *et al*, 2009; Buzsaki *et al*, 2003; Bragin *et al*, 1995; Korotkova *et al*, 2010; Colgin *et al*, 2009; Buhl *et al*, 2003). It is, for instance, impaired when excitatory drive or inhibitory inputs onto PV+ interneurons are ablated (Wulff *et al*, 2009; Korotkova *et al*, 2010). Infusion of the $GABA_A$ receptor agonist muscimol into the medial septum reduced theta-gamma co-modulation (Shirvalkar *et al*, 2010). In our study, gamma amplitude was modulated by theta phase during spontaneous as well as optogenetically entrained theta oscillations in the hippocampus, i. e. cross-frequency coupling of theta and gamma oscillations was preserved. Intact cross-frequency coupling of theta and gamma oscillations has also been demonstrated *in vitro* upon theta rhythmic optogenetic stimulation of ChR2 expressing hippocampal pyramidal cells in CA1 slices in mice (Butler *et al*, 2016). Generally, theta and gamma oscillations and their mutual timing are signatures of normal activity dynamics in the hippocampus (Colgin *et al*, 2009; Bragin *et al*, 1995; Buzsaki, 2002; Hasselmo, 2005).

A characteristic LFP pattern across hippocampal laminae, which includes phase reversal below the pyramidal cell layer, has been described for spontaneous hippocampal theta rhythm (Buzsaki, 2002). Phase reversal is preserved in the isolated hippocampus (Goutagny *et al*, 2009). Buzsaki, 2002, suggested, that the gradual phase reversal derives from coordinated activity of multiple current generators. A computational model suggests that curved and layered structures like the hippocampus support functionally relevant clustering of transmembrane currents (Fernandez-Ruiz *et al*, 2013). During optogenetic entrainment of hippocampal theta gradual phase reversal across hippocampal layers was preserved in our experiments.

Coherence across hippocampal hemispheres is another landmark of the physiological theta rhythm (Buzsaki *et al*, 2003). In our study optogenetic stimulation at the ipsilateral hemisphere entrained ipsilateral as well as contralateral hippocampal theta. Previous studies have reported high coherence of theta across hippocampal hemispheres while coherence at frequencies within the gamma range could be low (Sabolek *et al*, 2009). Our data indicate that patterns evoked by optogenetic stimulation are rapidly transmitted within the hippocampus. In the hippocampus single interneurons innervate hundreds to thousands of pyramidal cells (Sik *et al*, 1995; Cobb *et al*, 1995). CA3 as well as CA1 hippocampal pyramidal cells have commissural projections. Transmission could be conducted via

commissural projections or relayed via a feedback circuit via connections with the medial septum or entorhinal cortex. Clear entrainment of the contralateral hemisphere provides further prove that optogenetic stimulation drives indeed network effects and that the observed effect is not a light artefact.

Additional data analysis not presented in this thesis revealed that firing rates and preferential firing phase of hippocampal pyramidal cells and interneurons was not changed during optogenetic entrainment (see Bender *et al.*, 2015). Moreover, positional firing properties of pyramidal cells were preserved, suggesting that spatial navigation was not impaired. Hence, while hippocampal theta frequency followed constant stimulation frequency in the theta band, spatial rate coding by the hippocampus was unaltered by optogenetic theta pacing. Proper spatial coding by individual neurons during constant frequency theta rhythm has been previously demonstrated in an analytical model (Geisler *et al.*, 2010). Phase precession is mediated by faster firing frequency of active individual neurons in comparison to the theta rhythm (O'Keefe and Recce, 1993; Skaggs *et al.*, 1996). We conclude that characteristic, physiological properties of theta are maintained during hippocampal theta entrainment via theta rhythmic optogenetic excitation of GABAergic medial septum to hippocampus fibres.

4.2.7. Hippocampal theta entrainment during sleep

Differences in oscillation pattern, neuronal activity and generation mechanisms between theta occurring during locomotion and REM sleep have been suggested (Patel *et al.*, 2012; Montgomery *et al.*, 2008; Jackson *et al.*, 2014; Mizuseki *et al.*, 2011). Here we have shown that theta during both states can be entrained via theta rhythmic optogenetic excitation of GABAergic medial septum to hippocampus projections. Stimulation frequency matched hippocampal theta frequency during all vigilance states for theta epochs. Complementary, a previous study demonstrated that silencing of PV+ medial septum cells strongly decreased hippocampal theta oscillations *in vivo* during sleep (Boyce *et al.*, 2016).

Our experiments showed that the cortical LFP was influenced by hippocampal theta entrainment during REM sleep. It has been previously shown that hippocampus and cortex present coherent theta oscillations and neocortical neurons can fire phase-locked to hippocampal theta (Kahana *et al.*, 2001; Siapas *et al.*, 2005; Sirota *et al.*, 2008). The direction of information flow and function of synchronization is still not fully understood. During REM sleep ensemble trajectories in the hippocampus may follow intrinsic dynamics according to unknown rules. The preparation can help understand generation and function of endogenous hippocampal theta oscillations. REM sleep theta has been attributed a central role in coordinating synchrony and firing rates of pyramidal cells and interneurons in the hippocampus (Grosmark *et al.*, 2012).

4.3. Hippocampal theta and locomotion

4.3.1. Causality of theta frequency-speed correlation

The correlation between theta frequency and running speed is well established (Vanderwolf, 1969; Bland and Vanderwolf, 1972). In accordance, we detected during baseline recordings and in presence of control light but no optogenetic stimulation, a positive correlation between theta frequency and running speed.

The causality of the correlation of theta frequency and running speed, however, has so far been difficult to address, as electrical stimulation of the medial septum or hippocampus inevitably involves direct activation of descending pathways. Precise timing of hippocampal pyramidal cell and interneuron activity underlies highly complex mechanisms which are still incompletely understood. Congruently, effects of direct manipulations of hippocampal cells is difficult to interpret. Therefore, in our experiments opsins were expressed in theta pacemaker neurons in the medial septum. The optogenetic approach allowed to activate exclusively the ascending pathway from the medial septum to the hippocampus without perturbing descending medial septum projections. We detected a dissociation of hippocampal theta frequency and running speed when theta frequency was determined by the stimulation frequency. Our finding suggests that ascending projections modulate theta frequency according to momentary running speed and not vice-versa, which is in agreement with the sensorimotor integration theory (Bland and Oddie, 2001). In accordance, correlation between speed and theta frequency diminishes when rats are locomoting in a self controlled vehicle in absence of self motion signals (Terrazas *et al.*, 2005), and also in presence of self motion signals but movement at a constant speed on a running wheel (Whishaw and Vanderwolf, 1973), indicating that moment to moment changes in speed are necessary to update the hippocampal theta frequency. One proposed function of brain oscillations is mediating optimal integration of sensorimotor signals. Oscillatory networks and synchronized bursting throughout the nervous system have been implicated in a variety of activity-dependent developmental processes (Ben-Ari, 2001; Shatz, 1990; Purves *et al.*, 1994). Moment-by-moment variation in hippocampal theta frequency has been linked to sensorimotor integration (Bland and Oddie, 2001; Sinnamon, 2006; Wyble *et al.*, 2004). The hippocampus dynamically integrates sensorimotor experience and cognitive processes at the subsecond scale (Montgomery *et al.*, 2009; Tort *et al.*, 2009; Hasselmo *et al.*, 2002) and relates them to ongoing brain activity to guide navigation. Our data is in accordance with studies that suggest necessity of sensory-, motor-, proprioceptive- and vestibular feedback for adjustment of theta frequency (Stackman and Taube, 1997; Bland and Oddie, 2001). Previous studies described higher theta frequency *in vivo* compared to *in vitro* (Buzsaki, 2002; Goutagny *et al.*, 2009), in awake compared to anesthetized animals (Perouansky *et al.*, 2010), during movement compared to REM sleep (e.g. Patel *et al.*, 2012) and in humans during actual navigation compared to virtual navigation (Bohbot *et al.*, 2017; Aghajan *et al.*, 2015). Theta frequency progressively increases during pre- and neonatal development upon onset of movements (Leblanc and

Bland, 1979; Mohns and Blumberg, 2008; Langston *et al*, 2010; Gramsbergen *et al*, 1970). Theta frequency is positively correlated with running speed from the earliest ages at which exploration occurs (Wills *et al*, 2010). Theta frequency *in vitro* and during first theta bouts during development is around 5 Hz, the lowest theta frequency detected during wakefulness, suggesting that ascending projections increase theta frequency upon higher behavioural activity levels, along with the overall increase in cell firing rates. Furthermore, intrinsic resonance of hippocampal pyramidal cells is at low theta frequency, below 7 Hz (Pike *et al*, 2000).

In contrast to our optogenetic stimulation of medial septum GABA cells, upon stimulation of cholinergic medial septum cells in behaving mice stimulation frequency does not determine hippocampal theta frequency. In that case the relation between locomotor speed and theta frequency or power is maintained (Vandecasteele *et al*, 2014).

To conclude, our data provide evidence that increases in theta frequency in the behaving animal are mediated through extrinsic signals, which are at least partially relayed to the hippocampus via the medial septum. We suggest that, in accordance with the sensorimotor integration theory, it provides a means by which the hippocampus is constantly updated about the speed of external signals and movement speed of the body.

4.3.2. Hippocampal theta entrainment during quiet wakefulness

Movement and hippocampal theta naturally co-occur. We next addressed the question whether hippocampal theta oscillations causally elicit movement. The preparation allowed for hippocampal theta entrainment during quiet wakefulness without direct stimulation of subcortical locomotor regions. Optogenetic theta entrainment did not elicit locomotion compared to control light stimulation. Neither did it affect the average durations of locomotor behaviour, hence maintenance of movement. Direct optogenetic stimulation of medial septum glutamatergic cell somata, in contrast to our preparation and electrical stimulation protocols (Bland *et al*, 2006a; James *et al*, 1977), elicited   locomotion onset and also hippocampal theta oscillations (Fuhrmann *et al*, 2015). The medial septum is bidirectionally connected with the brainstem, in particular with the posterior hypothalamus, the reticular formation and the supramammillary nucleus (SUM), and also innervates the lateral hypothalamus (Kalen and Wiklund, 1989). Stimulation of these areas is known to induce locomotor behaviour (Green and Arduini, 1954; Sinnamon *et al*, 1984; Grillner and Shik, 1973).

In conclusion, our result suggests that locomotion causes hippocampal theta and not vice versa. At the same time, it does not exclude a modulatory role of hippocampal theta during ongoing locomotion.

4.3.3. Hippocampal theta entrainment during running

Speed-related information did not determine theta frequency or amplitude when optogenetic control over hippocampal theta rhythm was high, which allowed us to study reverse effects, namely to unravel

how theta-related computations by the hippocampus modulate behaviour. We found that optogenetic entrainment of hippocampal theta oscillations via stimulation of medial septum GABAergic cells projecting to the hippocampus can indeed regulate movement speed, entrainment modified theta oscillations features -e.g. a more constant theta amplitude- which mediated slower and more regular locomotion (see Bender *et al*, 2015).

In contrast, cholinergic medial septum cell optogenetic stimulation has no consistent effect on locomotor behaviour in mice (Vandecasteele *et al*, 2014; Mamad *et al*, 2015). Medial septum cholinergic stimulation increases speed during the non-theta state, which may be mediated by direct activation of locomotor regions, but decreases speed during the theta state (Vandecasteele *et al*, 2014). Medial septum-mediated cholinergic release may have an important role in coordinating burst firing in the hippocampus. For instance, pyramidal cells discharge bursts synchronously upon pharmacological activation of acetylcholine receptors *in vitro* (Cobb and Davies, 2005; Roshan-Milani *et al*, 2003; Benardo and Prince, 1982; Kawasaki *et al*, 1999) and excessive activation of nicotinic acetylcholine elicits seizures *in vivo* (Damaj *et al*, 1999; Kriegstein *et al*, 1983). Furthermore, acetylcholine released in the hippocampus depolarizes neurons and blocks spike-frequency accommodation (Cole and Nicoll, 1983). The behavioural significance of these phenomena remains to be investigated.

Optogenetic stimulation of glutamatergic cells in the medial septum increased movement speed with increasing stimulation frequency (Fuhrmann *et al*, 2015), which may be mediated via descending projections to locomotor regions. Increases in running speed with higher stimulation frequencies of medial septum subcortical targets, such as the reticular formation (Grillner and Shik, 1973), the hypothalamus (Green and Arduini, 1954; Sinnamon *et al*, 1999) or the SUM (Sinnamon, 1984), using electrical stimulation have been previously reported. Glutamatergic medial septum cells coordinate pyramidal cells firing with running speed (Fuhrmann *et al*, 2015).

We suggest that the effects on ongoing running speed observed in our study are mediated by hippocampal network activity patterns introduced by the effective entrainment. While entrainment did not mediate a general decrease in theta amplitude, it did alter the unfolding of hippocampal computations over time –across and within theta cycles- reflected in the variability of theta amplitude (see Bender *et al*, 2015).

4.3.4. Theta amplitude variability and running speed relation

Amplitude of hippocampal theta is fluctuating during movement and also during REM sleep. The brains dynamics are inherently variable from moment to moment (Faisal *et al*, 2008; Stein *et al*, 2005). Theta amplitude can indicate the number of active neurons and their phase relationship in proximity to the recording electrode activated within the time window of a theta cycle. In our study, fluctuation of theta amplitude reflected fluctuations of pyramidal cell discharge probability across theta cycles (see Bender *et al*, 2015).

Higher consistency of theta frequency predicted lower amplitude variability which mediated lower speed and speed variability. Consistent theta amplitude reflects higher linearity in the temporal domain. Increase in nonlinearity of theta dynamics increases with higher running speeds has been reported (Sheremet *et al*, 2016). Steady frequency discharge of hippocampal pyramidal cells and interneurons was reported when rats were running at a steady speed on a running wheel (Czurko *et al*, 1999).

Neuronal spikes represent the basic module of brain operations. Elementary symbols of the neural code are firing rate or firing patterns of neurons. How single cell or population spike patterns develop over time can bear important information adding to the neural code (Riehle *et al*, 1997; O'Keefe and Recce, 1993; Brenner *et al*, 2000). Temporal variability of theta amplitude may be related to and indicate propagation of assembly trajectories. The sequential activation of cell assemblies, termed "phase sequences", via an internal mechanism has been suggested to provide the basis for the flow of cognitive processes, including memory recall planning and decision making, independently from internal or environmental cues (Buzsaki, 2010; Hebb, 1949). I hypothesize that windows of lower variability in amplitude across theta cycles may resemble consistency in assembly activity and a transient pause in the progress of the trajectory. In contrast, activation of runner-up-assemblies resonance (Stark *et al*, 2013) should be reflected in alteration of theta amplitude and neuronal firing probability at a certain electrode position. Hippocampal neurons generate evolving cell assemblies during spatial navigation, but also in the absence of environmental or bodily derived cues (Pastalkova *et al*, 2008; Itskov *et al*, 2011; MacDonald *et al*, 2011). Internal episodic trajectories parallel egocentric spatial trajectories through physical environments (Buzsaki and Moser, 2013). There is evidence that episodic cell assemblies depend on internally generated neural activity as firing patterns of place and episodic cells were similar on the running wheel or in the maze (Wang *et al*, 2015). It was suggested that hippocampal assembly trajectories resemble the principal mechanism of hippocampal function and provide the basis for space as well as time coding (Buzsaki, 2006).

Encoding and retrieving sequences of episodic experiences is an important hippocampal function (Hasselmo *et al*, 2002; Ergorul and Eichenbaum, 2004). During bottom-up control ever changing, partially unpredictable sensory stimuli impact on intrinsic hippocampal dynamics and increase entropy of neuronal activity patterns. These inputs are eliminated during entrainment and behaviour is modulated by top-down control of hippocampal theta rhythm. Hippocampal neuronal activity may then predict upcoming events and estimate the probabilistic structure of the environment according to stored previous experiences. Hippocampal entrainment may also mediate slower and more stereotyped, less variable, locomotion as the environment where experiments were conducted was safe and familiar and did not provoke fight or flight responses.

Described here are possible mechanisms of how hippocampal computations unfolding over time could correspond to and direct locomotor behaviour. The behavioural effect was specific for theta rhythmic stimulation which proves that the observed effect is not an artefact of the optogenetic interrogation.

Mimicking variability of theta amplitude and frequency experimentally during optogenetic stimulation may provide further insights into how hippocampal computations affect locomotor output.

4.3.5. The hippocampus and movement inhibition

Optogenetic hippocampal theta entrainment mediated slower speed. According to Gray (1978), the hippocampus functions as a behavioural inhibitor. Lesions of the hippocampus or septum increase locomotor activity (Jarrard and Bunnell, 1968), running speed (Kim and Frank, 2009) and orienting reactions (reviewed in Vinogradova, 1995). Electrical stimulation of the hippocampal formation inhibited movement in some studies (Bland and Vanderwolf, 1972; Kaada *et al*, 1953; Maclean, 1957), while other studies reported no effect on running speed and locomotor activity (La Corte et al., 2013). Differences in behavioural effects could result from different stimulation protocols, electrode positions and generally diverse effects on net excitation and feedback inhibition of pyramidal cells. Gray emphasized that all drugs which exhibited clinically effective anxiolytic properties impaired septo-hippocampal theta in rodents. One has to keep in mind, however, that anxiolytics not only impair theta but network dynamics in general, including ripple and gamma oscillations (Ponomarenko *et al*, 2004, Scheffzuk *et al*, 2013).

The medial septum translates increasing activity levels into faster theta rhythmicity which is converged to the hippocampus. The hippocampus sends inhibitory feedback to the medial septum, which may serve to synchronize theta rhythmicity across the septo-hippocampal formation. Hippocampal feedback via GABAergic projections to the medial septum may further prevent a build-up of overexcitation of the system mediated by continuous impinging of sensory inputs, to prevent seizures and hyperactivity and to maintain balanced activity levels in the limbic system. Selective optogenetic stimulation of hippocampus to medial septum backprojections and behavioural readout could help to clarify the role of this feedback signaling during exploration.

The lateral septum receives massive excitatory projections from the hippocampus and projects to locomotor regions such as the hypothalamus. We found that when the main subcortical output of the hippocampus, to the lateral septum, was suppressed, running speed increased, possibly via release from inhibition of locomotor regions.

4.3.6. Hippocampal theta oscillations in various species

I suggest that data reported here can be translated to other mammals, including rats, and maybe humans. Theta rhythm is an ancient common feature of hippocampal operation and at the computational level its function is likely to be homologous across species. Theta occurs in the hippocampus of all species so far investigated, including rats, birds, reptiles, monkeys and humans (Vanderwolf, 1969; Crowne *et al*, 1972; Cantero *et al*, 2003; Ekstrom *et al*, 2005; Siegel *et al*, 2005; Shein-Idelson *et al*, 2016). Macroscopic features of theta in the mouse and rat are similar, including

spatial and regional distribution of voltage changes, frequency bands of oscillatory patterns, as well as relation between LFP and unit activity and behaviour (Buzsaki *et al*, 2003). This indicates that underlying physiological mechanisms are similar as well (Buzsaki *et al*, 2003). Human hippocampal theta amplitude varies much more during behaviour, while in rodents it is generally more stable over time (Jacobs, 2014; Buzsaki, 2002). The hippocampal computational framework which enables mice to navigate in real space may be similar to the framework that allows humans to navigate in memory space (Buzsaki and Moser, 2013; Eichenbaum, 2017a). This connection became very evident in a case report of a mnemonist, who travelled in an imaginary environment, the landmarks of which guided him during encoding and retrieval of semantic memories (Luria, 1987). Interestingly, the mnemonist had instead dramatic deficits in future planning and goal directed behaviour. Physical activity in humans is known to improve cognitive functions including memory performance and can increase hippocampal size (Erickson *et al*, 2011). I assume that gaining insights about hippocampal computations in rodents during navigation can support understanding of mechanisms underlying cognitive processes, such as memory flow, in humans.

4.4. The hippocampus to lateral septum pathway

4.4.1. Methodological considerations

For inhibition of the hippocampus to lateral septum pathway eNpHR or hM4Di-DREADDs, were expressed under the CaMkinase II alpha promoter to confine expression to pyramidal cells, which project to the lateral septum, but not to the medial septum. Therefore light which was targeted to the lateral septum could not affect medial septum cells.

For optogenetic inhibition yellow light for eNpHR activation was briefly (<1 min) applied to the lateral septum, in order to prevent change of reversal potential of $GABA_A$ receptors in response to intracellular chloride accumulation (Yizhar *et al*, 2011b; Raimondo *et al*, 2012). To control for effects of tissue heating when using yellow light, in additional control experiments light was delivered in the same manner to the lateral septum of animals expressing only the fluorophore but not the opsin. Optic fibers or guide cannulas were implanted bilaterally above the lateral septum to ensure inhibition of bilateral projections.

Pharmacogenetic effects using DREADDs last for the total course of our experiments (Alexander *et al*, 2009), thus enabled us to study effects on average running speed. CNO activates DREADDs upon intracranially applied micromolar concentrations, as used in our study, but not at lower concentrations (Gomez *et al*, 2017). CNO has been described in various studies as an biologically inert ligand which only activates DREADDs (Armbruster *et al*, 2007; Ji *et al*, 2016). According to a study published just recently, however, CNO can potentially bind to histamine (H1), serotonine (5-HT_{2A}), muscarinic (M1,M3,M4) and dopamine (D1,D2) receptors (Gomez *et al*, 2017, discussed below), activation of which in the lateral septum has not been associated with increased locomotor activity (Zarrindast *et al*, 2008;

Chee and Menard, 2013; Viana Mde *et al.*, 2008).

ChR2 was expressed in medial septum GABAergic cells for optogenetic theta entrainment in the same animals. ChR2 expression did not spread to the lateral septum, which was confirmed by histology. It was confined to the medial parts of the medial septum, where GABA cells are located (see Kiss *et al*, 1990). Hippocampal theta could be successfully entrained during inhibition of the hippocampus to lateral septum pathway. That is in line with a previous study which reported intact hippocampal theta upon lateral septum lesions (Rawlins *et al.*, 1979).

4.4.2. The role of the lateral septum in mediating hippocampal locomotor speed control

The main subcortical output pathway of the hippocampus is to the lateral septum (Risold and Swanson, 1996). This pathway has long been under-emphasized (Hartley *et al*, 2014). The lateral septum is a key element in circuits adjusting innate behaviour to environmental context (Sheehan *et al*, 2004; Luo *et al*, 2011).

High coherence of the hippocampus and lateral septum in the theta frequency band indicates that information transfer is facilitated during the theta state (Bender *et al*, 2015). Moreover, in accordance with a previous study in urethane-anesthetized rats (Pedemonte *et al*, 1998), one third of lateral septum cells fired phase locked to local theta during active behaviour in our study. Theta rhythmic firing of lateral septum cells has previously been suggested to reflect hippocampal activity (McLennan and Miller, 1974b) and to gate hippocampal input to the lateral septum (McLennan and Miller, 1976). Single pulse electrical stimulation of the fimbria results typically in an activation-inhibition sequences. The period of inhibition lasts between 100-800 ms (McLennan and Miller, 1974b; McLennan and Miller, 1976), hence one to several theta cycles. Instead, during theta rhythmic (7-12 Hz) stimulation the inhibitory component is completely eliminated (McLennan and Miller, 1976). The inhibitory component probably derives from a recurrent collateral inhibitory system within the lateral septum (McLennan and Miller, 1974a) and remained upon lower than theta frequency fimbria stimulation (McLennan and Miller, 1976). Lateral septum cells can exert place-, direction- and speed-related activity (Zhou *et al*, 1999; Leutgeb and Mizumori, 2002).

Inhibition of the hippocampus to lateral septum pathway prevented modulation of running speed by hippocampal network dynamics. We found that hippocampal theta entrainment reduced running speed and speed variability when the hippocampus to lateral septum pathway was intact. When the hippocampus to lateral septum pathway was inhibited, running speed and speed variability was not changed during theta entrainment, indicating an essential role of the hippocampus to lateral septum pathway in transmitting hippocampal activity to subcortical regions for rapid adjustments of locomotor speed. Main efferents of the lateral septum are subcortical (Leranth *et al*, 1992; Staiger and Nurnberger, 1991b; Gulyas *et al*, 1991) and the majority of lateral septum cells are GABAergic cells (Risold and Swanson, 1996; Colom, 2006). A main target of the lateral septum is the lateral hypothalamus, which

plays an important role in locomotor control (Grastyan *et al*, 1965; Grillner *et al*, 2008; Sinnamon, 1993) and arousal in general (Herrera *et al*, 2016). The dorsal hippocampus projects to lateral septum areas which project to the lateral hypothalamus (Risold and Swanson, 1996). Lesions of the lateral septum lead to hyperactivity (Sheehan *et al*, 2004). Optogenetic stimulation of the lateral septum to lateral hypothalamus pathway at constant theta frequency resulted in similar behavioural effects- a reduction in running speed (Bender *et al*, 2015). Therefore, hippocampal activity may be directly relayed via the lateral septum to the lateral hypothalamus during the theta state to exert control of ongoing locomotor activity according to hippocampal computations. Hippocampal areas CA1-CA3 project to the subiculum, the ventral part of which sends also direct projections to the lateral hypothalamus (Swanson and Cowan, 1977) and striatum (Groenewegen *et al*, 1987). Still, the main hippocampal subcortical output is relayed via the lateral septum (Risold and Swanson, 1996). The lateral septum integrates incoming signals aside from the hippocampus from various brain regions relevant for behavioural control, such as the amygdala (Sheehan *et al*, 2004), or mPFC (Carus-Cadavieco *et al*, 2017). Dynamic and flexible frequency and pathway specific synchronization of the lateral septum may be required to adjust behavioural output rapidly in accordance with current demands. In summary, we show evidence that hippocampal steady theta rhythmic activity guides slow and steady locomotion via subcortical output through the lateral septum. Consistent theta frequency input from the hippocampus to the lateral septum may prevent autoinhibition in the lateral septum, i.e. repeated excitatory input from the hippocampus could overwrite inhibitory inputs from the local axon collateral system and reset the system. The lateral septum may then more consistently inhibit downstream locomotor regions, including the lateral hypothalamus. Coordinated theta rhythmicity within the hippocampus may further ensure consistent and persistent drive to the lateral septum, as well as promote the theta state in the lateral septum and downstream regions, such as the lateral hypothalamus.

4.5. Role of mPFC to lateral septum gamma signaling during goal directed behaviour

Both hippocampus and mPFC project to the lateral septum (Carus-Cadavieco *et al*, 2017; Sheehan *et al*, 2004). While coherence of hippocampus and lateral septum is highest at theta frequencies (Bender *et al*, 2015), coherence of the mPFC and lateral septum is highest at gamma frequencies (Carus-Cadavieco *et al*, 2017), suggesting that information transfer from the mPFC to the lateral septum is facilitated during gamma epochs. The data further suggest that mPFC to lateral septum signaling at gamma frequencies enhances decision making in a spatial learning task.

Improved associative recognition memory in an object-in-place task has been demonstrated upon optogenetic activation of mPFC glutamatergic pyramidal cells (Benn *et al*, 2016). Furthermore, increase in gamma occurrence and synchronization of gamma across cortical brain regions has been previously reported before mice were turning into the reward arm during a spatial working memory T-maze task in correct but not incorrect test trials (Yamamoto *et al*, 2014). The authors suggested that the observed

increase in high frequency gamma during the decision making process reflected information recall in working memory. In contrast, optogenetic gamma inhibition decreased the number of correct trials. Furthermore, gamma activity in the mPFC has been linked to goal-driven attentional processing (Kim *et al*, 2016). The present study revealed the importance of gamma synchronization across cortical-subcortical structures for working memory (Carus-Cadavieco *et al*, 2017).

The hippocampus and mPFC have different but complementary functions (Eichenbaum, 2017b). While the hippocampus modulates rapid adjustments of ongoing behaviours, the mPFC supports planning of actions, predictions on the basis of previously stored memories and decision making processes.

4.6. Conclusions

1. A distinct brain rhythm - hippocampal theta oscillations - could be entrained with a high temporal precision in behaving mice by a frequency-specific optogenetic stimulation of hippocampal afferents from the medial septum.

2. Simultaneous electrophysiological recording of theta oscillations enables monitoring of the fidelity of the optogenetic entrainment – an emergent feature of physiological oscillations control, which varies dynamically within as well as between experimental subjects.

3. The frequency of optogenetically entrained theta rhythm is dissociated from instantaneous changes of running speed, in agreement with the model of hippocampal theta frequency regulation by ascending afferents.

4. Temporal regularity of hippocampal neuronal activity during theta oscillations causally determines slower and more regular running speed.

5. The regularizing impact of hippocampal theta-rhythmic output on locomotion is mediated by efferents to the lateral septum.

6. Gamma-rhythmic input to the lateral septum from mPFC supports spatial working memory-dependent goal directed behaviour. Thus frequency- and input- specific information processing in the lateral septum is causally involved in distinct aspects of spatial behaviour.

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6. Appendix

6.1. List of abbreviations

AAV	Adeno-associated viral vector
ACh	Acetylcholine
ARAS	Ascending reticular activating system
CA	Cornu ammonis
CAMKII	Calcium/calmodulin-dependent protein kinase II
ChAT-Cre	Choline acetyltransferase-Cre knock-in
ChR2	Channelrhodopsin-2
CNO	Clozapine-N-oxide
CPG	Central pattern generator
CV	Coefficient of variation
DMSO	Dimethyl sulfoxide
DREADD	Designer receptors exclusively activated by designer drugs
EC	Entorhinal cortex
EEG	Electroencephalogram
EMG	Electromyogram
EPSP	Excitatory postsynaptic potential
eNpHR	Natronomonas halorhodopsin enhanced
GABA	Gamma-aminobutyric acid
Glu	Glutamate
hM4Di	human M4 muscarinic (hM4) modified DREADD receptor activating G_i signaling pathway
LH	Lateral hypothalamus
LIA	Large irregular activity
LS	Lateral septum
LTP	Long-term potentiation
HC	Hippocampus
HCN	Hyperpolarization-activated cyclic nucleotide-gated
Ih	Hyperpolarization-activated current
IPSP	Inhibitory postsynaptic potential
LFP	Local field potential
MS	Medial septum
mPFC	Medial prefrontal cortex
NREM sleep	Non rapid eye movement sleep
PH	Posterior hypothalamus
PV	Parvalbumin
PV-Cre	Parvalbumin-Cre knock-in
REM sleep	Rapid-eye moment sleep
RF	Reticular formation
SOM	Somatostatin
Str. L-M	Stratum lacunosum-moleculare
Str. or.	Stratum oriens
Str. pyr.	Stratum pyramidale
Str. rad.	Stratum radiatum
SUM	Supramammillary nucleus
WT	Wildtype

6.2. Statement of contributions

The study addressing the role of hippocampal theta oscillations was designed by my PhD supervisors, Dr. Alexey Ponomarenko and Dr. Tatiana Korotkova, and me. The experiment regarding the mPFC to LS signaling pathway were designed by my PhD supervisors Dr. Alexey Ponomarenko and Dr. Tatiana Korotkova.

Some of the data presented here were acquired with the help of other people. Data analysis was performed in collaboration with Alexey Ponomarenko and Maria Gorbati.

In the following I state the contribution of other people to the data presented:

Emmanouela Volitaki, who was under my supervision as a Master student from January 2016 to September 2016, performed a part of hippocampal theta entrainment experiments during sleep.

Ania Chrzanowska, who was under my supervision as an exchange student from October 2016 to February 2017, performed a part of hippocampal theta entrainment experiments during sleep.

Yubin Hu, who was a rotation student from January to February 2015 in the lab under my supervision performed a part of the T-maze experiments.

In addition to the data presented here, I conducted many experiments for one published (Carus-Cadavieco *et al.*, 2017) and two unpublished studies. I conducted further pilot experiments that are not shown in this thesis.

6.5. Eidesstattliche Erklärung

Ich, Franziska Bender, versichere hiermit eidesstattlich, dass ich die vorgelegte Dissertation mit dem Thema: "Regulation of locomotion by hippocampal theta oscillations revealed by optogenetic entrainment" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe. Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.

Berlin, den 6.11.2017