Dissertation

submitted to the

Combined Faculties for the Natural Sciences and for Mathematics
of the Ruperto-Carola University of Heidelberg, Germany

for the degree of

Doctor of Natural Sciences

presented by

Mr. Shi-Bin Li

born in: Xingtai, Hebei Province, P.R.China

Oral-examination: July 18th, 2014

D4 Dopamine Receptor-Mediated Modulation of Hippocampal Synaptic Efficacy and Network Activity in Behaving Mice

Referees: Priv.-Doz. Dr. Georg Köhr

Prof. Dr. Andreas Draguhn

Table of Contents

Abbreviations

AC: adenylyl cyclase

ADHD: attention deficit hyperactivity disorder

AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANOVA: analysis of variance

BDNF: brain-derived neurotrophic factor

CA: cornu ammonis

CaMKII: calmodulin-dependent protein-kinase II

CFC: Cross-frequency coupling

CNS: central nervous system

CPP: conditioned place preference

DA: dopamine

DB: diagonal band of Broca nuclei

DBS: deep brain stimulation

DG: dentate gyrus

EC: entorhinal cortex

eCBs: endocannabinoids

EEG: electroencephalography

eLFPs: evoked local field potentials

eNOS: endothelial Nitro oxide synthase

FC: fear conditioning

GABA: gamma-aminobutyric acid

GC: granule cell

Glu: glutamate

GPCRs: G protein-coupled receptors

HFO: high frequency oscillations

HFS: high-frequency stimulation

IA: inhibitory avoidance

IPSPs: inhibitory postsynaptic potentials

ISI: inter-stimulus interval

KO: knockout

L: D4R antagonist L745870

LC: locus coeruleus

LFS: low-frequency stimulation

LTD: long-term depotentiation

LTP: long-term potentiation

ME: median eminence

MH: mediobasal hypothalamus

MS: medial septum

NA: nucleus accumbens

NMDA: N-methyl-D-aspartate

NREM: non-rapid eye movement

NS: normal saline

OR: stratum oriens

PAK: p21-activated kinase

PBS: phosphate buffered saline

PD: D4R agonist PD168077

PFA: paraformaldehyde

PFC: prefrontal cortex

PPF: Paired-pulse facilitation

PPR: paired pulse ratio

PYR: stratum pyramidale

RAD: stratum radiatum

REM: rapid eye movement

SC: Schafer collateral

SEM: standard error of the mean

sLFPs: spontaneous local field potentials

SL: stratum lucidum

SLM: stratum lacunosum and stratum moleculare

SN: substantia nigra

SPW-R: sharp-wave ripple complex

STDP: spike timing dependent plasticity

SUB: subiculum

TM: transmembrane

VDCCs: voltage-dependent Ca^{2+} channels

VTA: ventral tegmental area

List of original publications and manuscript

1. Treviño M, Aguilar-Garnica E, Jendritza P, **Li SB**, Oviedo T, Köhr G & De Marco RJ (2011) Discrimination learning with variable stimulus 'salience'. *International archives of medicine* 4, 26.
2. Treviño M, Oviedo T, Jendritza P, **Li SB**, Köhr G & De Marco RJ (2013) Controlled variations in stimulus similarity during learning determine visual discrimination capacity in freely moving mice. *Sci Rep* 3, 1048.
3. **Shi-Bin Li**, Dan Du, Mazahir T. Hasan, Georg Köhr, D4 receptor activation differentially modulates hippocampal basal and apical dendritic synapses in behaving mice, under review

Conference abstracts

1. Rodrigo J. De Marco, **Shi-Bin Li**, Tatiana Oviedo, Georg Köhr, Mario Treviño, Discrimination learning depends on the arrangement of training stimuli, *Ninth Göttingen Meeting of the German Neuroscience Society,* March 23-27, 2011, Göttingen, Germany
2. Mario Treviño, Tatiana Oviedo, **Shi-Bin Li**, Georg Köhr and Rodrigo J. De Marco, Visual discrimination learning with variable salience in the mouse, *American Neuroscience Meeting 2011*, November 12-16, 2011, Washington DC, USA
3. **Shi-Bin Li,** Georg Köhr, Dopaminergic modulation of hippocampal synaptic efficacy and oscillations in freely behaving mice, *IZN Retreat 2012*, July 1-2, 2012, Kloster Schöntal, Germany
4. **Shi-Bin Li,** Georg Köhr, Dopaminergic modulation of local field potentials in the hippocampus of freely behaving mice, *German Physiology Society 92nd Annual*

Meeting, March 2-5, 2013, Heidelberg, Germany

5. **Shi-Bin Li,** Georg Köhr, D4 dopaminergic modulation of hippocampal synaptic efficacy and neural-network activity in freely behaving mice, *SFB 636 Autumn School*, October 17-18, 2013, Speyer, Germany
6. **Shi-Bin Li,** Georg Köhr, D4 Dopamine receptors modulate synaptic plasticity and neural network activity in the hippocampus of freely behaving mice, *American Neuroscience Meeting 2013*, November 8-13, 2013, San Diego, California, USA
7. **Shi-Bin Li,** Georg Köhr, D4 dopamine receptor activation modulates hippocampal synaptic plasticity and network activity but not contextual fear memory in awake mice, *Federation of European Neuroscience Societies (FENS) Spring Conference: Controlling Neurons, Circuits and Behaviour*, April 20-23, 2014, Rungstedgaard, Copenhagen, Denmark
8. **Shi-Bin Li**, Miriam A. Vogt, Peter Gass, Georg Köhr, Hippocampal oscillations during contextual fear conditioning in mice, *Central Institute of Mental Health Retreat*, May 5-6, 2014, Bad Dürkheim, Germany
9. **Shi-Bin Li**, Dan Du, Mazahir T. Hasan and Georg Köhr, D4 Receptor Activation Differentially Modulates Hippocampal Synapses in Behaving Mice, *Central Institute of Mental Health Retreat*, May 5-6, 2014, Bad Dürkheim, Germany

Abstract

Dopamine (DA) is the predominant catecholamine neurotransmitter in the mammalian brain, where DA governs a variety of functions including locomotor activity, cognition, emotion, synaptic plasticity, learning and memory formation. In the hippocampus, activation of D1/5 receptors (D1/5Rs) is known to stabilize memory formation and late, protein-dependent long-term potentiation (LTP) in the stratum radiatum (RAD), whereas activation of D4 receptors (D4Rs) inhibits early LTP in the stratum oriens (OR) and depotentiates LTP in RAD. Interestingly, novelty exploration rescues memory impairment caused by blockade of D1/5Rs in the hippocampus. However, the function of D4Rs in synaptic plasticity, neural network activity and memory-associated behaviors remained to be elucidated *in vivo*. I implanted two recording electrodes targeting OR and RAD together with a bipolar stimulation electrode placed either in OR or RAD while monitoring depth profiles of stimulus-evoked local field potentials (eLFPs). At least one week later, eLFPs and spontaneous oscillatory activities (sLFPs) were recorded in the hippocampus of freely behaving mice to investigate the role of D4Rs under physiological condition. My results show that systemic, intraperitoneal treatment with the D4R agonist PD 168077 (PD) slightly decreased eLFPs both in OR and RAD and in parallel increased the paired-pulse ratio (PPR) between two eLFPs, indicating presynaptic mechanisms were involved in this modulation. PD treatment postponed the rapid eye movement (REM) sleep and, during REM onset, the theta peak frequency was shifted to lower band, the gamma band power was reduced and the strength of theta-gamma coupling was attenuated. Furthermore, D4R agonist treatment impaired late LTP (4 hours) both in OR and RAD, while early LTP (30 min) was reduced only in OR. When mice were transferred from their home cage to a fear box, band power of fast gamma increased in that novel environment, in particular after receiving an electric footshock on day 1 and during context exposure on day 2, and these increases persisted when the mice were returned to home cage immediately after. These changing patterns of oscillatory activities were not affected by PD treatment, and therefore the D4R-mediated layer-specific modulation of synaptic plasticity in the hippocampus is unlikely implicated in learning and memory during novelty exploration or fear conditioning.

Zusammenfassung

Dopamin (DA) ist der vorherrschende Katecholamin-Neurotransmitter im Säugerhirn, wo DA vielfältige Funktionen hat wie Lokomotion, Kognition, Emotion, synaptische Plastizität, Lernen und Gedächtnis. Aktivierung von D1/5 Rezeptoren (D1/5R) im Hippokampus stabilisiert bekanntermaßen Gedächtnis und die späte, Protein-abhängige Langzeitpotenzierung (LTP) im Stratum Radiatum (RAD), wohingegen die Aktivierung von D4 Rezeptoren (D4R) frühes LTP im Stratum Oriens (OR) reduziert und LTP im RAD depotenziert. Interessanterweise verbessern Neuheiten eine Gedächtniseinschränkung, die durch Antagonismus von D1/5R im Hippokampus verursacht wurde. Dagegen wurde die Funktion von D4R bei synaptischer Plastizität, neuronaler Netzwerkaktivität und Gedächtnis-assoziiertem Verhalten nur selten *in vivo* untersucht. Ich implantierte Meßelektroden in OR and RAD und eine bipolare Stimulationselektrode in OR oder RAD und registrierte Tiefenprofile Reiz-evozierter lokaler Feldpotentiale (eLFP). Nach mindestens einer Woche wurden eLFP und spontane Oszillationen (sLFP) im Hippokampus frei beweglicher Mäuse registriert, um die Rolle von D4 Rezeptoren unter physiologischen Bedingungen zu untersuchen. Meine Ergebnisse zeigen, daß systemische, intraperitoneale Behandlung mit dem D4R Agonisten PD 168077 (PD) eLFPs in OR also auch in RAD reduziert und gleichzeitig das Verhältnis Doppelpuls-evozierter LFPs erhöht, weshalb an dieser Modulation vermutlich präsynaptische Mechanismen involviert sind. PD Injektion verzögerte den REM Schlaf, und während des REM Beginns war der Theta-Peak zu einer niedrigeren Frequenz verschoben, die Power im Gamma-Frequenzband reduziert und die Theta-Gamma Kopplung abgeschwächt. Weiterhin beeinträchtigte PD Injektion späte LTP (4 Stunden) in OR und RAD, während frühes LTP (30 min) nur in OR abnahm. Wurden Mäuse aus dem Haltungskäfig in eine neue Umgebung gebracht, dann nahm die Power im fast Gamma-Frequenzband zu, besonders wenn sie dort einen elektrischen Fußschock ausgesetzt waren. Diese Gamma-Zunahme wiederholte sich am darauffolgenden Tag auch ohne Fußschock und blieb erhöht nachdem die Mäuse in ihren Haltungskäfig zurückgesetzt wurden. Diese sich ändernden oszillatorischen Aktivitäten traten auch in Gegenwart von PD auf und deshalb ist die D4R-vermittelte, Schicht-spezifische Modulation der synaptischen Plastizität im Hippokampus unwahrscheinlich involviert beim Lernen und der Gedächtnisbildung während Neuigkeitserfahrungen oder Furchtkonditionierung.

Chapter I

Introduction

1.1 The hippocampus

1.1.1 Anatomy

1.1.1.1 Location of the hippocampus

The hippocampus (named after its resemblance to the seahorse, from the Greek hippos meaning “horse” and kampos meaning “sea monster”) is a major component of the brains of humans and other vertebrates. It belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation (Scoville & Milner, 1957; Lee et al., 2005; Bannerman et al., 2012; Cohen et al., 2013; Ramirez et al., 2013). Humans and other mammals have two hippocampi, one in each side of the brain, forming a chiral configuration. The hippocampus is located under the cerebral cortex; in primates it is located in the medial temporal lobe, underneath the cortical surface. It contains two main semicircle-shaped interlocking parts: Cornu Ammonis horn and the dentate gyrus.

1.1.1.2 Laminar structure

More than hundred years ago, Santiago Ramón y Cajal had depicted the well laminar structured transverse section of hippocampus formation under light microscope (Ramón y Cajal, 1893). The hippocampus comprises multiple subfields. The bigger semicircle is frequently used with the nomenclature Cornu Ammonis

(abbreviated as CA) and the smaller counterpart is termed as dentate gyrus (abbreviated as DG). Starting from the dorsal part close to subiculum, CA is differentiated into fields CA1, CA2, CA3, with CA4 ending up inside DG. The dentate gyrus contains the fascia dentata and the hilus. The principal neurons along the longitudinal axis in CA are pyramidal neurons, while the smaller principal neurons sitting in the smaller interlocking DG are known as granule cells. The CA regions are structured in clearly defined strata (or layers) from outside to inside as: alveus, stratum oriens (OR), stratum pyramidale (pyramidal cell layer, PYR), stratum lucidum (SL, only in CA3), stratum radiatum (RAD), stratum lacunosum and stratum moleculare (SLM). The DG is structured with three layers: stratum moleculare, stratum granulosum (granule cell layer), polymorphic layer (Amaral et al., 2007; El Falougy et al., 2008) (Figure 1).

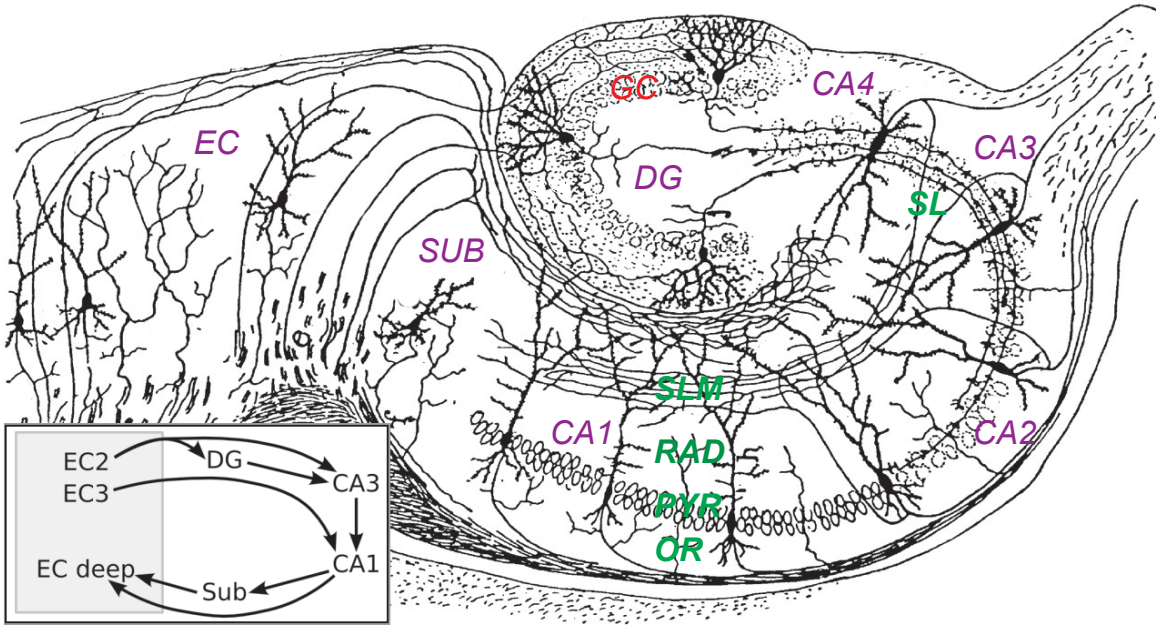


Figure 1. Basic circuits of the hippocampus, drawn by Santiago Ramón y Cajal (Histologie du Systeme Nerveux de l'Homme et des Vertebretes, Vols. 1 and 2. A. Maloine. Paris. 1911). Letters in purple colour indicate different areas of hippocampus and parahippocampal entorhinal cortex (EC) and subiculum (SUB). Letters in green are the laminar structure within the dorsal hippocampus.

1.1.1.3 Cell types

The vast majority of hippocampal neurons are glutamatergic principal cells and a smaller proportion of them are GABAergic interneurons. The somata of pyramidal cells in CA and granule cells in DG are known as principal cells. All pyramidal and granule cells are excitatory because they use glutamate as their primary neurotransmitter. There are various types of interneurons, which are classified as inhibitory neurons whose main neurotransmitter is GABA. Different classification standards are adopted to differentiate various interneurons. Based on the morphological appearance, about 20 different types of interneuron are distinguished. This terminology is quite useful, because it give intuitive insights to the striking features of a verity of interneurons. For example, the “stellate” cell indicates the typical morphology of “star with shining light” shape, and “axo-axonic cells” highlights the fact that they innervate the axon initial segment of the postsynaptic cells. The development of immunohistochemistry supplements the nomenclature based on the peptides the interneurons contain, such as parvalbumin positive interneurons. Given the difference of physiological and synaptic properties of interneurons, they were named after the distinct electrical activity signature, such as fast spiking interneurons (Maccaferri & Lacaille, 2003; Klausberger & Somogyi, 2008; Jinno, 2009).

1.1.1.4 Neural circuitry within the hippocampal formation

1.1.1.4.1 Circuitry within the ipsilateral site

Within the ipsilateral hippocampus, there is a trisynaptic wiring diagram loop (circuit) indicating a relay of synaptic transmission (Figure 1). The three major cell groups: granule cells, CA3 pyramidal neurons, and CA1 pyramidal cells are the three nodes of the trisynaptic circuit. The axons originating from the entorhinal cortex layer

II project to the dendrites of granule cells in DG, making up the perforant-DG pathway. The axons (known as mossy fibers) of DG granule cells project to the proximal apical dendrites of CA3 pyramidal neurons. The axons of CA3 pyramidal neurons project to ipsilateral CA1 pyramidal cells through Schaffer collaterals, known as SC-CA1 pathway. The SC-CA1 pathway is frequently used to test synaptic efficiency (Laurberg, 1979; Dong et al., 2008; Neves et al., 2008).

1.1.1.4.2 Inputs from the contralateral hippocampus

CA3 pyramidal neurons not only project to ipsilateral CA1 pyramidal cells via SC-CA1 pathway, but also project to contralateral CA3 and CA1 pyramidal cells basal dendrites through commissural connections (Dong et al., 2008; Neves et al., 2008).

1.1.1.5 Connections with parahippocampal structures

1.1.1.5.1 Entorhinal cortex-hippocampus

The entorhinal cortex (EC) has long been considered as a relay station that provides the major source of afferent inputs to the hippocampus based on neuroanatomical studies (Jones, 1993). Layer III of EC mainly project to CA1 and subiculum, and EC layer II to CA3 and DG. Using a transgenic mouse whose layer III inputs of the EC to the hippocampus are silenced, Suh and colleagues found significant impairments in mutant mice performing spatial working-memory tasks and in the encoding phase of trace fear-conditioning (Suh et al., 2011). Köhler found the layer IV of EC also projects to DG following the perforant-DG pathway and layer VI of EC innervates the outer two-thirds of the molecular layer, the subgranular zone and the deep part of the hilus of the area dentate, using retrograde fluorescent tracing (Köhler, 1985). In turn, the ventral two thirds of field CA1 give rise to a prominent

projection to EC mainly in layer IV. From dorsal CA1, there is a projection terminating not only in the deep layers but also prominently in layer I of perirhinal cortex, and appearing to be strictly ipsilateral (Witter et al., 1989).

1.1.1.5.2 Septum-hippocampus

As a subcortical structure, the septum nucleus belongs to the limbic formation and composes two major areas: lateral (lateral septal nuclei) and medial (medial septum/diagonal band of Broca nuclei (MS/DB), each having different neuronal populations. Reciprocal pathways between the hippocampus and the septum nucleus have been suggested based on anatomical studies (Okada & Okaichi, 2010). The lateral septum receives a strong glutamatergic input from the hippocampus and a GABAergic input from the MS/DB complex, and in turn, the medial the septum innervates the hippocampus via cholinergic and GABAergic inputs (Khakpai et al., 2013).

1.1.1.5.3 Other regions

Besides the strong reciprocal connections between hippocampus and EC, septum, there is also evidence showing connections between the hippocampus and other brain structures. Compared to the strong connections between the hippocampus and EC, the perirhinal and postrhinal cortices project weakly and exlusively to CA1 and the subiculum. The postrhinal cortex preferentially targets the dorsal CA1 and subiculum, whereas the perirhinal cortex targets the ventral subiculum, and in turn, the perirhinal cortex receives more input from ventral hippocampal formation structures and the postrhinal cortex receives more input from dorsal hippocampal structures (Agster & Burwell, 2013). After lesions of the olfactory tubercle, by tracing the coarse

degenerating fibers, Cragg found direct degenerated fibers connected to the hippocampus, which indicates that the olfactory tubercle plays a relay role in olfactory impulses to the hippocampus (Cragg, 1961). Earlier publications demonstrated that dopaminergic fibers originating from the ventral tegmental area/substantia nigra (VTA/SN) innervate the hippocampus (Gasbarri et al., 1994a; Gasbarri et al., 1994b). Smith and Greene found that the noradrenergic inputs originating from locus coeruleus (LC) project to the dorsal hippocampus (Smith & Greene, 2012). The hippocampal formation and parahippocampal areas receive heavy inputs from lateral, basal, accessory basal, and posterior cortical nuclei of amygdala, while the substantial inputs to amygdala originate from the temporal end of the CA1 subfield and subiculum of the hippocampal formation, as reviewed by Pitkänen and colleagues (Pitkänen et al., 2000).

1.1.2 Synaptic plasticity

1.1.2.1 Plasticity of synaptic efficacy

1.1.2.1.1 Long-Term Potentiation (LTP)

One hundred years ago, Cajal originally hypothesized that information storage relies on the changes in the strength of synaptic connections between neurons that fire coherently. The idea was refined by Hebb as (1949) as: "When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes place in firing it, some growth process or metabolic change takes place in one or both cells, such that A's efficiency, as one of the cells firing B, is increased", which is known as the Hebbian learning rule. LTP describes a phenomenon in which a sustained increase in synaptic strength that is elicited by brief high frequency stimulation of excitatory

afferents (Malenka, 1994). Bliss and Colleagues demonstrated for the first time that high-frequency stimulation induced sustained increase of the synaptic transmission in the perforant-DG pathway both in anaesthetized and unanaesthetized rabbits (Bliss & Gardner-Medwin, 1973; Bliss & Lømo, 1973). LTP is characterized by three basic properties: cooperativity, associativity and input-specificity, see review (Bliss & Collingridge, 1993). Cooperativity describes the existence of an intensity threshold for induction, that LTP can only be triggered if the tetanic stimulation is strong enough to activate sufficient afferent fibres. LTP is associative in the sense that a "weak" input can be potentiated if it is active at the same time as a strong tetanus to a separate but convergent input. Input-specificity describes a condition in which other inputs that are not active at the time of the tetanus do not share in the potentiation induced in the tetanized pathway. There are NMDAR-dependent and presynaptic forms of LTP exist (Kauer & Malenka, 2007).

NMDAR-dependent LTP requires the activation of NMDARs by presynaptically released glutamate when the postsynaptic membrane is significantly depolarized. This depolarization relieves the voltage-dependent blockade of NMDAR by Mg^{2+}, allowing Ca^{2+} to enter postsynaptic dendritic spines. The crucial rise in postsynaptic Ca^{2+} concentration leads to the activation of complex intracellular signaling cascades including AMPAR trafficking to the postsynaptic plasma membrane, that account for the strengthened synaptic transmission after LTP induction (as illustrated in Figure 2).

Presynaptic LTP was found at synapses of the mossy fibres of DG, hippocampal CA3 pyramidal neurons, neocortex and cerebellum. This type of LTP likely does not require NMDARs or postsynaptic factors (still remains controversial). Presynaptic LTP appears to be initiated by an activity-dependent rise in intracellular Ca^{2+} within presynaptic terminals, which eventually leads to a persistent increase of glutamate

release from the presynaptic site (Figure 2).

Based on the expression time course of potentiation, LTP can be classified as early LTP (E-LTP), which is independent of gene expression and the synthesis of new protein, and late LTP (L-LTP), which is gene expression- and protein synthesis-dependent. The persistence of LTP depends on the intensity of induction protocols. Widely used protocols include: high-frequency stimulation (HFS), the physiology mimicking theta bursts, and low-frequency stimulation paired with postsynaptic depolarization. In addition, learning can also potentiate basal synaptic transmission in the SC-CA1 pathway in Long Evans rats in an inhibitory avoidance (IA) training task, which is known as learning-induced LTP (Whitlock et al., 2006).

In animals of different ages, LTP induction exhibits distinct properties. Although aged animals potentiated to the same degree as young ones, they do not retain the potentiated synaptic response for longer durations and lose it much more rapidly than young ones (Geinisman et al., 1994).

1.1.2.1.2 Long-Term Depression (LTD)

As the counterpart of LTP, LTD depicts sustained synaptic strength decrease after prolonged low-frequency stimulation (LFS) or treatment of receptor ligands (Collingridge et al., 2010). LTD shares several features with LTP, such as input-specificity and the requirement of activation of NMDARs, as well as an increase of intracellular Ca^{2+} at the postsynaptic site (Malenka, 1994). There are several types of LTD (see reviews) (Kauer & Malenka, 2007; Collingridge et al., 2010). NMDAR-dependent LTD is induced by weak activation of NMDARs, during which a different subset of Ca^{2+}-dependent intracellular signaling is triggered, which eventually leads to the removal of synaptic AMPARs via dynamin- and clathrin-dependent endocytosis

(Figure 2). Metabotropic glutamate receptor-dependent LTD is similarly mediated by the clathrin-dependent endocytosis of synaptic AMPARs, but following activation of postsynaptic mGluRs. Strong postsynaptic Ca^{2+} influx also triggers the synthesis of endocannabinoids (eCBs), which travel retrogradely across the synapse to bind to presynaptic CB1 receptors and transiently depress neurotransmitter release and thus weaken synaptic transmission. This type of LTD is defined as endocannabinoid-mediated LTD. Notably, the behavioral perception of novelty in animals also induces LTD in the dorsal hippocampus (Manahan-Vaughan & Braunewell, 1999).

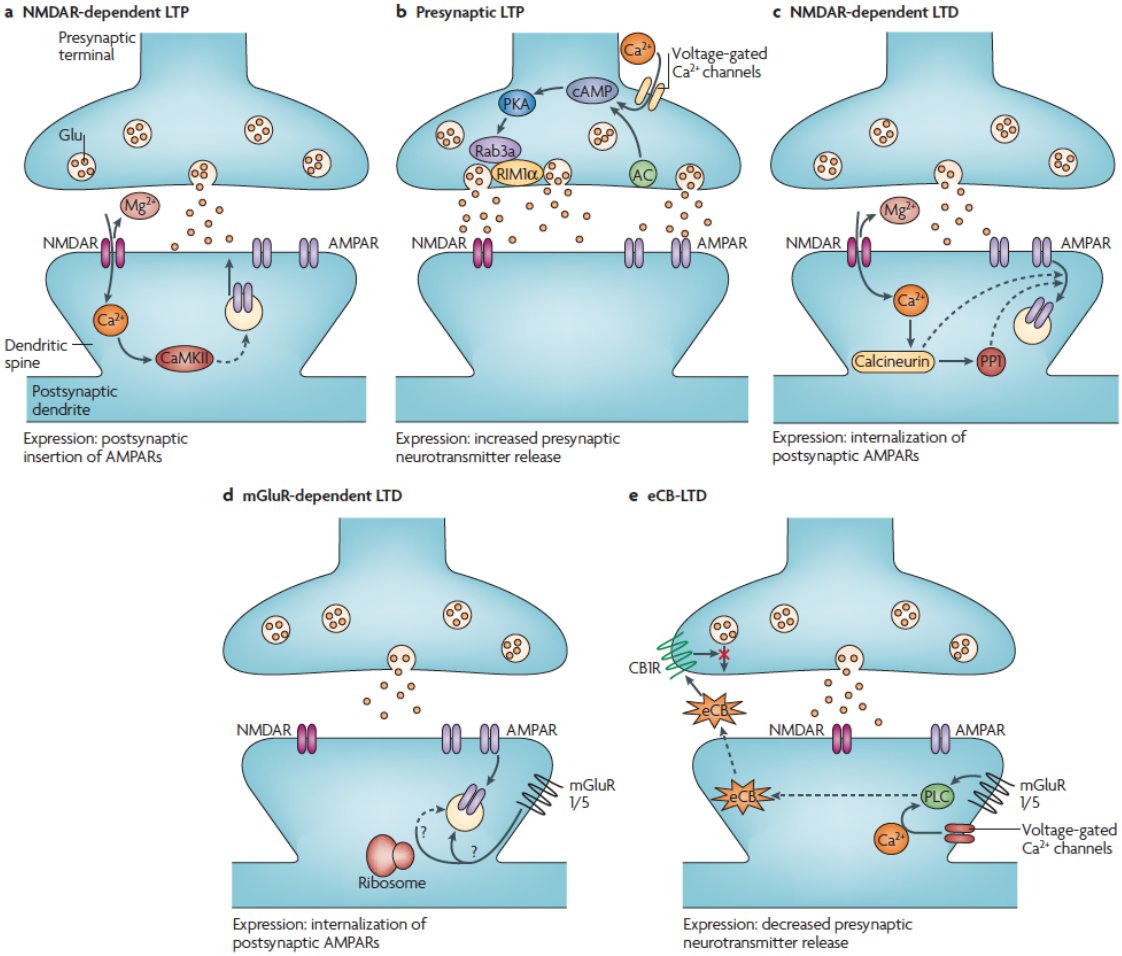


Figure 2. Well-described mechanisms of LTP and LTD. Highly simplified diagrams of the induction and expression of synaptic plasticity observed in the rodent brain. **a** | n-methyl-d-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) has been observed in many different brain regions

and is dependent on postsynaptic NMDAR activation and calcium/calmodulin-dependent protein-kinase II (CaMKII) for its initiation. The voltage-dependent relief of the magnesium block of the NMDAR channel allows the synapse to detect coincident presynaptic release of glutamate (Glu) and postsynaptic depolarization. α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) insertion into the postsynaptic membrane is a major mechanism underlying LTP expression. **b** | Presynaptic LTP has been best characterized at mossy fibre-CA3 hippocampal synapses as well as at parallel fibre-Purkinje cell cerebellar synapses. Repetitive synaptic activity leads to the entry of presynaptic Ca^{2+}, which activates a Ca^{2+}-sensitive adenylate cyclase (AC) leading to a rise in cAMP and the activation of cyclic AMP-dependent protein kinase A (PKA). This in turn modifies the functions of Rab3a and RIM1α leading to a long-lasting increase in glutamate release. Involvement of postsynaptic signalling molecules (not shown) has also been reported. **c** | NMDAR-dependent long-term depression (LTD) is triggered by Ca^{2+} entry through postsynaptic NMDAR channels, leading to increases in the activity of the protein phosphatases calcineurin and protein phosphatase 1 (PP1). The primary expression mechanism involves internalization of postsynaptic AMPARs and a downregulation of NMDARs by an unknown mechanism. **d** | Metabotropic glutamate receptor (mGluR)-dependent LTD has been best characterized at cerebellar parallel fibre-purkinje cell synapses and hippocampal synapses. Activation of postsynaptic mGluR1/5 triggers the internalization of postsynaptic AMPARs, a process that under some conditions appears to require protein synthesis. **e** | Endocannabinoid-LTD is the most recently discovered form of LTD, and has been observed in many brain regions. Either mGluR1/5 activation, leading to activation of phospholipase C (PLC) or an increase of intracellular Ca^{2+} (or both), in the postsynaptic neuron initiates the synthesis of an endocannabinoid (eCB). The eCB is subsequently released from the postsynaptic neuron, travels retrogradely to bind to presynaptic cannabinoid 1 receptors (CB1R) and this prolonged activation of CB1Rs depresses neurotransmitter release via unknown mechanisms (figure taken from (Kauer & Malenka, 2007)).

1.1.2.1.3 Spike timing dependent plasticity (STDP)

When presynaptic spiking precedes postsynaptic spiking ("pre-post") within a window of several tens of milliseconds, LTP is induced, whereas spiking of the reverse order ("post-pre") leads to LTD. This form of activity-dependent LTP/LTD is now referred to as spike timing-dependent plasticity (STDP) (Dan & Poo, 2006). Given the conventional Ca^{2+} based model of LTP/LTD, Dan and Poo summarized that pre-post spiking leads to a brief high-level Ca^{2+} influx, due to effective activation of NMDARs by

postsynaptic spiking, and this yields activation of the kinase pathway and eventually induces LTP. In contrast, spiking in the opposite order leads to a low-level Ca^{2+} rise due to the limited extent of NMDAR activation by the afterdepolarization associated with the postsynaptic action potential (AP), and this preferentially activates the phosphate pathway and leads to LTD (Dan & Poo, 2006). Further evidence from fluorescence Ca^{2+} imaging studies has indeed demonstrated that Ca^{2+} influx through NMDARs and voltage-dependent Ca^{2+} channels (VDCCs) exhibits supralinear summation with pre-post spiking and sublinear summation with post-pre spiking (Koester & Sakmann, 1998; Nevian & Sakmann, 2004). In support of this Ca^{2+} model for STDP, pre-post spiking under partial inactivation of NMDARs in CA1 of the hippocampus leads to the induction of LTD instead of LTP (Nishiyama et al., 2000). Blockade of supralinear calcium signals in basal dendrites during AP bursts by low concentrations of the T- and R-type calcium channel antagonist nickel can also occlude LTP and NMDA channel activation, which provides direct evidence for the importance of dendritic calcium spikes for the induction of STDP (Kampa et al., 2006). Together with postsynaptic intracellular Ca^{2+} and dendritic calcium spikes, McCabe and colleagues proposed that interneuron-mediated inhibitory function also markedly shapes LTD, which alters the classical STDP curve apparently (MaCabe et al., 2007). Morphological changes are also observed during synaptic plasticity, as summarized as below.

1.1.2.2 Synaptic morphology plasticity

1.1.2.2.1 Morphological changes during LTP

At the level of single synaptic contacts, modifications of neuronal architecture have been observed in association with LTP, including a mean area increase in dendritic spines, a larger postsynaptic density, and more negative synaptic curvatures characterized by concavity of postsynaptic sites relative to the presynaptic terminals (Agnihotri et al.,

1998; Matsuzaki et al., 2004). Electrophysiological results support the idea that more synapses are formed after LTP induction. Bolshakov and colleagues found that synapses between a single presynaptic CA3 neuron and a single postsynaptic CA1 neuron normally release only a single quantum of transmitter; however, higher release probabilities are observed during early phase LTP, and increased quanta release occurs together with late LTP, which is possibly due to new synapses formation (Bolshakov et al., 1997). These two key hypotheses regarding the structural basis of LTP are strengthened by observing both enhanced connectivity and enlarged synapses following LTP induction in immature rat hippocampal slices (Harris et al., 2003). It is also well known that activity-driven changes in synaptic efficacy modulate spine morphology, due to alterations in the underlying actin cytoskeleton/filaments, which dynamically traffic actin-binding proteins underlying synaptic function (Bosch & Hayashi, 2012; Fortin et al., 2012).

1.1.2.2.2 Morphology changes during LTD

In contrast to LTP-associated spine enlargement, LTD is normally coupled with spine shrinkage, reducing synaptic strength (Bosch & Hayashi, 2012). The induction of LTD by either electrical (Zhou et al., 2004) or chemical stimulation (He et al., 2011) induces shrinkage or loss of dendritic spines. Becker and colleagues investigated presynaptic morphological changes during LTD induction with time lapse two-photon laser scanning microscopy and extracellular field recordings. In their work, LTD induction dramatically increased the turnover of presynaptic boutons, while decreasing the number of putative synaptic contacts between Schaffer collateral boutons and spines of CA1 pyramidal cells (Becker et al., 2008). In slice cultures, following the LTD induction protocol, the number of spines decreased 3hr after the stimulation. Spines in slices from older animals are generally more stable after low frequency stimulation (LFS) (Nagerl et al., 2004). In the meantime, there is

evidence that shows a dissociation between physiological and morphological expression of LTD. Wang and colleagues observed that spine shrinkage and LTD can occur independently of each other using combined two photon time-lapse imaging with patch-clamp recording in acute hippocampal slices (Wang et al., 2007). An actin depolymerization inhibitor blocked the expression of LTD, suggesting that morphologically silent actin remodeling may be involved in the physiological expression of LTD and different subpopulations of actin filaments undergo changes during LTD (Wang et al., 2007).

1.1.2.3 Functional changes during synaptic plasticity

Convergent lines of evidence, including results from wild-type animals (Federmeier et al., 2002) and genetically-manipulated animals (Hayashi et al., 2004), indicate that changes in the number or structure of synapses may represent a substrate of memory formation following learning. The morphological changes established by new synaptic connections or remodeling of existing synapses lead to higher synaptic efficacy, which is believed to correlate with better behavioral performance (Geinisman, 2000). Genetic manipulation in animals has also demonstrated a decrease in spine formation that is negatively coupled to hippocampus-dependent memory. For example, forebrain-specific dominant-negative p21-activated kinase (PAK) transgenic mice, which have fewer dendritic spines relative to wild-type controls, showed normal acquisition but impaired consolidation/retention in spatial memory and contextual fear conditioning paradigms (Hayashi et al., 2004). Using unbiased electron microscopy, Bloss and colleagues found the density of large synapses in the prefrontal cortex (PFC) remained unchanged, but an approximate 50% decrease in the density of small synapses in the PFC following aging, which may account for aging-related impairments in executive functions (Bloss et al., 2013). Together with the aforementioned LTP/LTD-associated

morphological changes, functional plasticity occurs when animals adjust their strategies to accommodate a dynamically changing environment.

1.1.3 Oscillatory activities

1.1.3.1 Theta oscillations

Theta rhythm is a spontaneous oscillatory pattern in local field potential (LFP) signals recorded from either inside the brain or from electrodes glued to the scalp. It is characterized with 4-12 Hz frequency. Two types of theta rhythm have been described. "Hippocampal theta rhythm" is a strong oscillation that can be observed in the hippocampus and other brain structures, including the subicular complex, entorhinal cortex, perirhinal cortex, cingulated cortex, and amygdala in numerous species of mammals, including rodents, rabbits, dogs, cats, bats, and marsupials (Buzsáki, 2002). "Cortical theta rhythms" are low-frequency components of scalp LFPs, usually recorded from humans, also known as electroencephalography (EEG).

Because of the fine laminar structure and important role in learning and memory formation, as well as the characteristic EEG depth profile, the hippocampus draws the most attention for oscillatory activity research. In rodents, hippocampal theta is seen mainly in two conditions: first, when an animal is running, walking, or in some other way actively interacting with its surroundings, and second, during rapid-eye movement sleep (REM) (Buzsáki, 2002; Buzsáki et al., 2003; Scheffzük et al., 2011).

A couple of subcortical nuclei have been postulated to be critically involved in theta rhythm generation. Afferents from structures reciprocally connected to hippocampus (see the hippocampus anatomy part) release neurotransmitters that may have a “permissive” action on network oscillations or work as “pacemakers”,

providing a coherent theta frequency output (Buzsáki, 2002). The medial septum (MS) is regarded as the ultimate theta rhythm generator, as lesioning or inactivating the MS disrupts theta waves in the hippocampus and many other cortical targets (Buzsáki, 2002; Colgin, 2013). GABAergic inhibitory interneurons expressing the hyperpolarization-activated and cyclic nucleotide-gated nonselective cation channels (HCN channels) in the MS are believed to be theta pacemaker cells (Toth et al., 1993; Toth et al., 1997; Varga et al., 2008), as these interneurons fire rhythmically at theta frequencies and are phase-locked to theta rhythms in the medial septum (Hangya et al., 2009; Colgin, 2013). By contrast, cholinergic neurons, another main type of neurons in the MS, do not fire rhythmically at theta frequencies and thus are unlikely to act as theta pacemakers, but may instead modulate the excitability of other neurons in a way that promotes their theta rhythmic firing (Colgin, 2013). The reciprocal connections via hippocampal-MS glutamatergic excitatory and GABAergic inhibitory backprojections may be important in maintaining the coupling between the two regions (Khakpai et al., 2013).

Small medial septum lesions (Winson, 1978) or inactivation (Asaka et al., 2002) not only eliminates theta in the hippocampus, but also produces severe spatial memory deficits in mammals, indicating the important role of theta rhythm in learning and memory formation. The prevailing LTP induction protocol of “theta bursts” is believed to act as a “natural tetanizer” in the modification of hippocampal activity (Vertes et al., 2004).

1.1.3.2 Gamma oscillations

Gamma oscillations are characterized by the frequency band 30-90 Hz, with a peak frequency of approximately 40 Hz. Normally, gamma rhythms occur with theta

rhythms when animals actively engage in voluntary behaviors or during REM sleep, known as “awake gamma” and “sleep gamma”, respectively. However, differences exist between the “awake gamma” and “sleep gamma”. The band power of gamma is lower during REM than during active waking (Scheffzük et al., 2011). Regarding the mechanisms for gamma generation, two concepts have been proposed. The first is that gamma rhythmic patterns occur via the interaction of excitatory principal cells and fast basket cell inhibitory interneurons acting on fast gamma-amino butyric acid ($GABA_A$) receptors (Nyhus & Curran, 2010). Secondly, a network of mutually connected inhibitory interneurons is also believed to be a major generator of gamma oscillation, as the gamma activity relies predominantly on $GABA_A$ receptor (Bartos et al., 2007).

Based on these concepts, computational models have been constructed to explain the mechanisms for gamma generation, as recently summarized (Buzsáki & Wang, 2012). The excitatory-inhibitory (E-I) neuron model illustrates the synchronization by an excitatory-inhibitory loop, a model based primarily on the reciprocal interaction between pyramidal neurons and interneurons. This model is supported by the fact that the delay between the timing of pyramidal cell and interneuron spikes is a prominent feature of gamma oscillations and that knockout of AMPA receptors in fast spiking interneurons reduces the amplitude of gamma oscillations. The inhibitory-inhibitory (I-I) neuron model depicts synchronization via mutual inhibition between interneurons. In this model, the frequency of gamma oscillations is determined mainly by the kinetics of inhibitory postsynaptic potentials (IPSPs) and net excitation of interneurons. Based on where gamma rhythms are sampled, the E-I and I-I hybrid gamma networks may work together to generate gamma frequency oscillations. Compared to the long-range synchronization of neuronal activity by theta rhythms,

gamma oscillations are prone to synchronize neuronal networks locally. In slice preparation, the work done by Gloveli and colleagues indicates an orthogonal arrangement of gamma and theta rhythm-generating microcircuits in hippocampal area CA3. Theta frequency oscillations are the dominant network activity along the long axis of the hippocampus interconnected through CA3 pyramidal cells and O-LM interneurons. In contrast, gamma-band oscillations are observed predominantly within the transverse axis of the hippocampus (Gloveli et al., 2005).

1.1.3.3 Sharp-wave ripple complex (SPW-R)

Unlike theta and gamma rhythms, that have regular frequencies, sharp wave-ripples (SPW-R) are irregular population oscillatory patterns in hippocampal local field potentials (LFPs) that occur when the animal has minimal or no interaction with its environment such as immobility, consummatory behavior or slow wave sleep (Buzsáki, 2006). SPW-R is also involved in memory consolidation and the replay of wakefulness-acquired memory (Buzsáki & Silva, 2012). The SPW-R complex is composed of low frequency large amplitude sharp waves in LFPs and fast field oscillations (around 200Hz) known as ripples (Ylinen et al., 1995). Sharp wave bursts are induced by a cooperative discharge of CA3 pyramidal cells and are believed to be the most synchronous physiological patterns in the hippocampus (Buzsáki & Peyrache, 2013). In conjunction with sharp wave bursts, CA1 pyramidal cells display a prominent high-frequency (200Hz) pattern (ripples) generated by fast perisomatic inhibition (Maier et al., 2003; Cutsuridis & Taxidis, 2013) and gap junction-mediated effects (Draguhn et al., 1998), and the large SPW field also exerts ephaptic entrainment of neurons (Anastassiou et al., 2010). Among those, the synchronously discharging pyramidal cells responsible for the negative peaks of the ripples (‘mini

populations spikes') and synchronous IPSCs in nearby pyramidal cells brought about by basket neurons are the main generators for LFP ripples (Buzsáki & Silva, 2012).

SWP-R has also been shown to be important for learning, memory and cognitive functions. In wild-type rats, the observation that selectively suppressing the ripples after learning disrupts the memory consolidation supplies direct evidence for a causal role of SPW-R in memory processing. CA1 ripples were detected bilaterally in the CA1 pyramidal layer and aborted by simultaneous electric stimulation delivered to the hippocampal commissure during post-learning sleep. Such targeted interference did not distort other aspects of sleep but impaired the daily performance in a hippocampus-dependent reference memory task (Girardeau et al., 2009).

In a schizophrenia mouse model that has a forebrain-specific knockout of the synaptic plasticity-mediating phosphatase calcineurin, Suh and colleagues observed that calcineurin knockout (KO) mice exhibited a 2.5-fold increase in the abundance of SPW-R events during awake resting periods and single units in KO were also increased during SPW-R events. Furthermore, the sequential reactivation of place cells during SPW-R events was completely abolished in KO and associated with impaired information processing (Suh et al., 2013). As temporal spike sequences of ripples in SWP-R correlate with behavioral experience (Diba & Buzsáki, 2007), these findings provide a potential mechanism underlying abnormal brain activity that may be implicated in cognitive impairments associated with schizophrenia.

1.1.3.4 Oscillations at other frequency bands

Other oscillation frequency bands are delta (1-4 Hz), beta (13-30 Hz), ultra fast (200-600 Hz) (Buzsáki & Draguhn, 2004). Alpha oscillations serve to protect working memory maintenance against anticipated distracters via phase adjustment (Bonnefond

& Jensen, 2012). The observation of prevalent synchronized neuronal oscillation at beta frequencies in the human motor system suggests that beta bands are relevant to voluntary motor control (Jenkinson & Brown, 2011; Davis et al., 2012). Ultrafast bands are hypothesized to be relevant to local dendritic computation (Ozaki & Hashimoto, 2005).

1.1.3.5 Cross-frequency coupling (CFC)

The oscillatory activities conventionally assigned to different frequency bands are not completely independent. One type of interaction is cross-frequency coupling (CFC), which describes a phenomenon in which the amplitude and/or phase of higher frequency oscillations can be modulated by the amplitude and/or phase of low-frequency rhythms (Canolty & Knight, 2010; Belluscio et al., 2012). The phase of low frequency theta rhythm modulates the amplitude of high gamma with stronger modulation occurring at higher theta band power. Different behavioral tasks evoke distinct CFC patterns in either human brain (Canolty et al., 2006) or brain of rodents even with crossing different brain structures coupling (Tort et al., 2008). As high-frequency brain activity reflects local domains of cortical processing, low-frequency brain rhythms are dynamically entrained across distributed brain regions by both external sensory input and internal cognitive events. CFC might thus serve as a mechanism to transfer information from large-scale brain networks operating at behavioral timescales to the fast, local cortical processing required for effective computation and synaptic modification, thus integrating functional systems across multiple spatiotemporal scales, see review (Canolty & Knight, 2010).

1.1.4 Sleep architecture

Sleep is characterized by the cyclic occurrence of rapid eye movement (REM) sleep and non-REM (NREM) sleep, which includes slow wave sleep (SWS, stage 3 and 4) and lighter sleep stage 1 and 2. In humans, the first part of the night (early sleep) is dominated by a high amount of SWS, whereas REM sleep prevails during the second half (late sleep) (Figure 3) (Diekelmann & Born, 2010; Rasch & Born, 2013). Given the reasonable speculation that the sleeping animal is relatively susceptible to predators, changing levels of vigilance might have biological utility. In general, rodents have a day-night inverted circadian cycle, sleeping during the daytime, while behaving actively during the night time (Twyver, 1967; Campbell & Tobler, 1984; Adamantidis et al., 2008). There are characteristic activities during different phases of sleeping, as discussed above. Briefly, REM sleep is theta oscillation-dominated, while the SPW-R complex only appears during NREM.

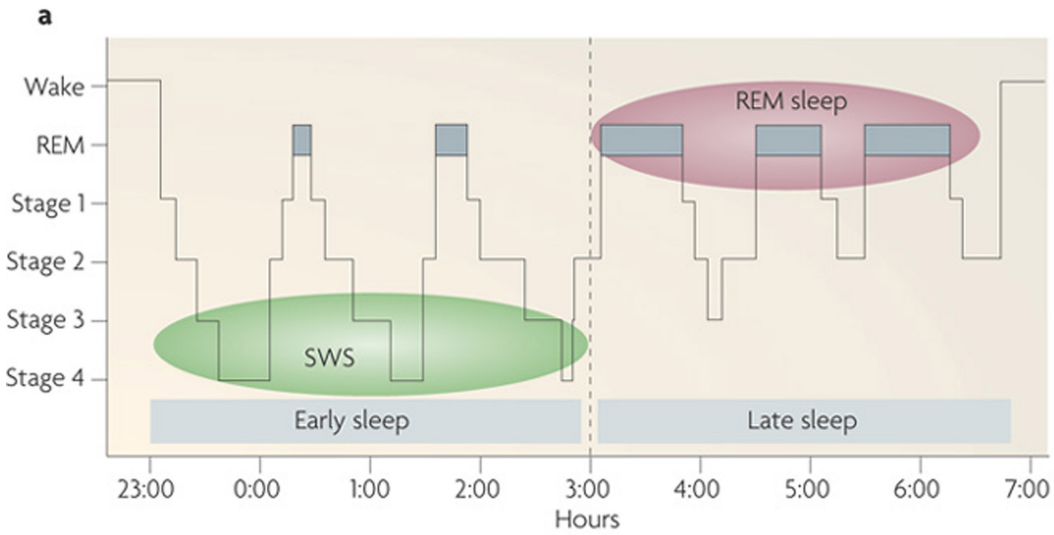


Figure 3. Human sleep consists of repeated SWS and REM sleep. SWS appears more in the early sleep and REM sleep dominates the late sleep. Episodes of SWS sleep is getting shorter and REM sleep is getting longer over the whole sleeping in general (figure taken from (Diekelmann & Born, 2010)).

1.1.5 Relevance of hippocampus to learning and memory

Direct evidence form human clinical research indicates that the hippocampus is important for memory formation. Bilateral hippocampal resection in man results in a persistent impairment of recent memory, but early memories and technical skills remain intact, explicitly indicating the important role of hippocampus in declarative memory formation (Scoville & Milner, 1957). In rodents, with transgenic, pharmacological, anatomical and optogenetic techniques, the hippocampus has been examined in various behavior paradigms for its role in novelty detection/memory, spatial memory, non-spatial memory (Lee et al., 2005; Bannerman et al., 2012; Cohen et al., 2013; Ramirez et al., 2013). Notably, in the inhibitory avoidance task, Rossato and colleagues showed that weak footshock-induced short-term memory can be transformed into long-term memory via local administration of D1/5R agonist to the dorsal hippocampus, while strong footshock-induced long-term memory can be abolished in the presence of D1/5R antagonist (Rossato et al., 2009). This work clearly indicates that dopamine plays an important role for memory storage in the hippocampus.

1.2 The central dopaminergic system

1.2.1 Dopamine receptors

1.2.1.1 Dopamine receptor classification

The physiological actions of dopamine are mediated by five distinct but closely related G protein-coupled receptors (GPCRs). Based on the original biochemical observations showing that dopamine is able to modulate adenylyl cyclase (AC) activity, dopamine receptors are classified into two families, D1-like and the D2-like receptors. D1-like receptors activate the $G\alpha_{s/olf}$ family of G proteins to stimulate cAMP production via AC. The D1-like subfamily includes D1 and D5 dopamine

receptor subtypes. D2-like dopamine receptors are coupled to the $G\alpha_{i/o}$ family of G proteins and thus induce inhibition of AC. The D2-like subfamily includes D2, D3 and D4 dopamine receptor subtypes and also see table 1 (Missale *et al.*, 1998; Beaulieu & Gainetdinov, 2011; Strange & Neve, 2013).

1.2.1.2 Structure of dopamine receptors

DA receptors belong to the seven transmembrane (TM) domain G protein-coupled receptor family and most of their structural characteristics are homogeneous. The amino acid sequences within the TM domains are considerably conservative. Members of the same subfamily have high homology, 80% identity of the TM domains are shared by D1 and D5 receptors, and D2 and D3, D2 and D4 receptors share 75% and 53% identity in the TM domains. The NH_2-terminal stretch shares similar amino acids in all the subtypes but with variable number of consensus N-glycosylation sites. The D1 and D5 receptors possess two such sites with one in the NH_2 terminal and the other in the second extracellular loop. The D2, D3, D4 show variability, and they own four, three and one N-glycosylation sites, respectively. Regarding the COOH terminal, the D1-like is characterized by about seven times longer COOH terminal tail and a smaller third intracellular loop where the site interacts with G proteins. The highly conserved residues characterizing TM domains are in the core position of the protein and believed to form a narrow binding pocket as the agonist biding site (details reviewed by (Missale et al., 1998) and also see Table 1).

1.2.1.3 Dopamine receptor distribution

Using different methods, including autoradiographic localization, sequence-

specific antibody staining, immunofluorescence, mRNA *in situ* hybridization, the distribution of different subtypes of dopamine receptors has been addressed (Table 1, as reviewed by (Strange & Neve, 2013)).

	D_1-like		D_2-like		
	D_1	D_5	$D_{2S/L}$	D_3	D_4
Amino Acids	446 (human, rat)	477 (human) 475 (rat)	414/443 (human) 415/444 (rat)	400 (human) 446 (rat)	387 (human*, rat)
Homology					
with D_1	100%	82%	44%	44%	42%
with D_2 (short)	44%	49%	100%	76%	54%
Localization	Caudate/putamen, nucleus accumbens, olfactory tubercle, hypothalamus, thalamus, frontal cortex	Hippocampus, thalamus, lateral mamillary nucleus, striatum, cerebral cortex (all low)	Caudate/putamen, nucleus accumbens, olfactory tubercle, cerebral cortex (low)	Nucleus accumbens, olfactory tubercle, islands of Calleja, putamen (low), cerebral cortex (low)	Frontal cortex, midbrain, amygdala, hippocampus, hypothalamus, medulla (all low), retina
Response	Adenylyl cyclase↑	Adenylyl cyclase↑	Adenylyl cyclase↓	Adenylyl cyclase↓	Adenylyl cyclase↓
Introns in Gene	None	None	Yes	Yes	Yes
Organization of Amino Acid Sequence					
Third intracellular loop	Short	Short	Long	Long	Long
Carboxyl terminal tail	Long	Long	Short	Short	Short

Table 1: The properties of the principal dopamine receptor subtypes identified by gene cloning are shown. They are divided into 'D_1-like' and 'D_2-like' groups to reflect amino acid homology, functional similarity, structural similarity, and pharmacological properties. This grouping conforms a previous classification based on pharmacological and biochemical properties. D_{2S} and D_{2L} refer to different alternatively spliced forms of the D_2 receptor gene. The homology values are for the transmembrane-spanning regions. The localizations and relative expression levels shown are the principal ones known at present from in-situ hybridization and use of the polymerase chain reaction (table taken from (Strange & Neve, 2013)).

1.2.2 Dopaminergic pathways

Four major dopaminergic pathways have been identified in the mammalian brain: the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular systems that originate from the A9 (nigrostriatal), A10 (mesolimbic and mesocortical, often collectively termed the mesocorticolimbic pathway), and A8 (tuberoinfundibular) groups of dopamine-containing cells (See Figure 4, adapted from (Money & Stanwood, 2013)). These neurons are critically involved in various vital central

nervous system functions, including voluntary movement, feeding, affect, reward, sleep, attention, working memory, and learning.

The mesolimbic pathway transmits dopamine from the VTA to the limbic system via the nucleus accumbens (NA). The VTA is located in the midbrain, and the nucleus accumbens is in the ventral striatum. Dysfunction of this pathway is implicated in schizophrenia.

The mesocortical pathway transmits dopamine from the VTA to the frontal cortex. Given the VTA in the midbrain, the "cortical" refers to the cortex. Abnormal function of this pathway is also related to schizophrenia.

The nigrostriatal pathway transmits dopamine from the substantia nigra (SN) to the striatum. This pathway is associated with motor control, and dysfunction of this pathway is implicated in the Pakinson's desease and chorea.

The tuberoinfundibular pathway transmits dopamine from the hypothalamus to the pituitary gland. This pathway influences the secretion of some hormones, including prolactin. Dysfunction of this pathway is involved in hyperprolactinemia.

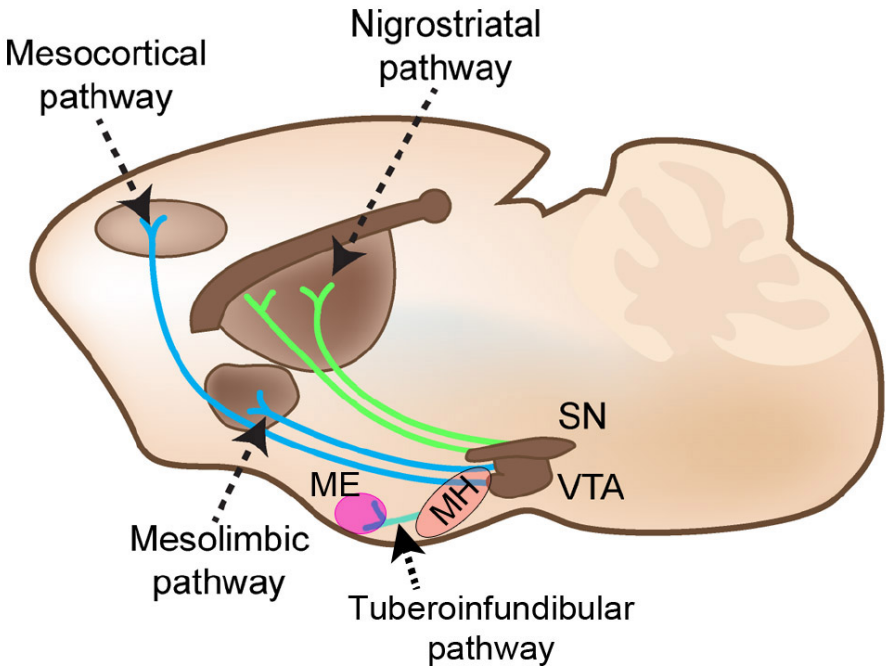


Figure 4. Nigrostriatal, mesocortical, mesolimbic and tuberoinfundibular pathways cartooned in an adult mouse brain in the sagittal plane. SN projects to GABAergic projection neurons in the dorsal striatum. The VTA projects to both subcortical limbic areas and to the medialfrontal cortex. The arcuate nucleus of the mediobasal hypothalamus (MH) (the 'tuberal region') projects to the median eminence (ME) (the 'infundibular region') (figure adapted from (Money & Stanwood, 2013)).

1.2.3 Dopaminergic implication in synaptic plasticity and network activity

1.2.3.1 Dopaminergic modulation of synaptic transmission

Dopamine (DA) is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine functions (Missale et al., 1998; Beaulieu & Gainetdinov, 2011). In recent years, more and more studies provide evidence that DA receptors are involved in synaptic plasticity, learning and memory (Moncada & Viola, 2007; Stramiello & Wagner, 2008; Rossato et al., 2009; Wang et al., 2010). Many experiments have addressed the dopaminergic modulation of the synaptic transmission of Schaffer collateral-CA1 (SC-CA1) pathway. Briefly, activation of D1/D5 receptors by agonists enhances the long-term potentiation (LTP) in stratum radiatum (RAD) of the hippocampus. This enhancement likely contributes to the late, protein synthesis-dependent component of LTP (Huang & Kandel, 1995; Duffy & Nguyen, 2003; Sajikumar & Frey, 2004; Navakkode et al., 2007). The DA transporter specific blocker GBR 12,935 or activation of D3 receptor has been shown to augment early LTP in the RAD layer of rat hippocampal slices (Swant & Wagner, 2006). Evidence from immunocyto-chemistry studies with site-directed polyclonal antibody staining showed that D4 receptor is widely distributed in the rat central nervous system (CNS), showing higher labelling in the hippocampus (CA1, CA2, CA3 and dentate gyrus), frontal cortex,

entorhinal cortex, caudate putamen, nucleus accumbens, olfactory tubercle, cerebellum, supraoptic nucleus and sustancia nigra pars compacta (Defagot et al., 1997). Recent work from our group in slices has demonstrated that in the presence of exogenously applied DA receptor agonists, which activated D4 receptors, early LTP was strongly reduced in stratum oriens (OR), but barely affected in RAD (Herwerth et al., 2012).

D4 receptors are hypothesized to be relevant to the cognitive deficit symptoms including schizophrenia, attention-deficit hyperactivity disorder (ADHD), and autism, based on the altered EEG patterns of the subjects suffering mental disorders (Furth et al., 2013). In a novel object recognition task, Sood and colleagues showed PD improved the discrimination ability of non-phencyclidine-treated rats, and restored the differentiation ability of cognition deficits caused by phencyclidine treatment in rats (Sood et al., 2011). In addition, dopamine D4 receptor null mice exhibited reduced exploration of novel stimuli (Dulawa et al., 1999). These studies indicate D4 receptors are implicated in novelty detection/recognition. PD also showed promnesic effect in C57BL/6J mice in the inhibitory avoidance training system during the memory trace is susceptible to modulation, namely PD was administrated to the animals shortly after training (Bernaerts & Tirelli, 2003).

Learning potentiates basal synaptic transmission in the SC-CA1 pathway in Long Evans rats in an inhibitory avoidance (IA) training task, which is known as learning induced LTP (Whitlock et al., 2006) even though it is not as strong as the potentiation induced by classical HFS or theta bursts. It is clear that behavior stimuli can exert a similar enhancing effect on basal synaptic transmission with some neuromodulators, such as augmented basal synaptic transmission, which was observed in the presence of D1/5 agonist in slices (Huang & Kandel, 1995; Wang et al., 2010). In vivo work

has shown that basal synaptic transmission in the medial perforant path input to the granule cell layer in dentate gyrus (DG) was dose-dependently impaired by loading D2-like receptor agonists quinpirole and noraporphine to the ipsilateral cerebral ventricle locally in rats (Manahan-Vaughan & Kulla, 2003). This evidence is in line with the classification of DA receptors based on their correlation with AC activity.

1.2.3.2 Dopaminergic modulation of neuronal oscillations and relevance to pathology

Beta oscillatory activity is modulated by net dopamine levels at sites of cortical input to the basal ganglia and assumed to be relevant to voluntary motor activities (Jenkinson & Brown, 2011). The core pathology of Pakinson's disease is the degeneration of midbrain dopaminergic neurons in the substantia nigra (SN) pars compacta that project to the striatum and other basal ganglia nuclei (Weinberger & Dostrovsky, 2011). Pathophysiological studies of Parkinson's disease demonstrate that abnormal local field potential (LFP) oscillations (increased beta and decreased gamma) within cortico-basal ganglia circuits appear together with behavioral deficits, and L-DOPA as well as deep brain stimulation (DBS) are able to restore these dysfunctions in humans (Levy et al., 2002; Weinberger et al., 2006; Weinberger & Dostrovsky, 2011) and in rat models (Lemaire et al., 2012). In schizophrenia research, data from animal models have suggested that alterations in the dopaminergic and cholinergic systems may contribute to abnormal oscillations, yet remains to be fully explored (Lisman et al., 2008; Uhlhaas & Singer, 2010). Research in vitro also shows that the importance of endogenous dopamine maintains synchronous oscillation of intracellular calcium in primary cultured-mouse midbrain dopaminergic neurons, and this effect is abolished in the presence of an NMDA receptor antagonist (Yasumoto et al., 2004). Moreover, activation of D4 receptors increased kainate-induced gamma

oscillation in hippocampal slices via enhancing synchronization of fast-spiking interneurons (Andersson et al., 2012). With another D4 receptor selective agonist A-412997, Kocsis and colleagues showed enhanced gamma activity with short latency and long-lasting (2h) effect during natural behavior in both freely moving rats and a methylazoxymethanol-induced neurodevelopmental model of schizophrenia (Kocsis et al., 2013).

1.2.3.3 Implications of dopaminergic modulation to learning and memory formation

Since 1957, when Dr. Arvid Carlsson identified dopamine as a distinct neurotransmitter in the brain, rather than only working as the precursor of norepinephrine, it has been extensively investigated in different levels for its role in brain functions (Iversen & Iversen, 2007; Yeragani et al., 2010). As the predominant neurotransmitter in the mammalian brain, it controls a variety of functions, including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine functions (Missale et al., 1998; Beaulieu & Gainetdinov, 2011). Here, I will concentrate on the function of dopamine in learning and memory.

1.2.3.3.1 Working memory

Working memory is a component of short-term memory that is defined as the ability to maintain or hold a temporarily active representation of information for further processing. It is widely assumed to be essential for acquisition and subsequent long-term memory formation (El-Ghundi et al., 2007). The interaction of dopamine with working memory formation has been well documented for the prefrontal cortex (PFC), which is believed to play a central role in working memory (Surmeier, 2007). Local administration of a D1 receptor antagonist to the PFC of rhesus monkeys

induces errors and increases latency in performance on an oculomotor task that requires memory-guided saccades in a dose- and delay period duration-dependent manner, but has no effect on performance in a control task requiring visually-guided saccades (Sawaguchi & Goldmanrakic, 1991). In the radial maze task, local infusion of D1 receptor agonist to the medial PFC of rats either 30 min or 12 hr prior to the test phase improved memory retrieval after the 12-hr delay but disrupted performance is at the 30 min delay, indicating D1 receptors exert differential effects over PFC depending on memory trace strength (Floresco & Phillips, 2001). Mice lacking D2/3Rs exhibit obvious spatial working memory deficits in the spatial delayed alteration task (Glickstein et al., 2002). In addition, low doses of D2-like agonists have been shown to impair working memory whereas higher doses increase memory performance in monkeys on a delayed response memory task, indicating D2Rs in working memory processing (Arnsten et al., 1995). Testing based on the baseline working memory, low doses of a D4R antagonist have no effect but interruption by high doses with good baseline memory, while low doses of a D4R antagonist improves working memory but have no effect with high doses for testing poor baseline memory rats (Zhang et al., 2004).

1.2.3.3.2 Spatial learning and memory

Convergent evidence shows that spatial memory is hippocampus-dependent (Eichenbaum et al., 1999), this concept is strengthened in particular by the findings of hippocampal place cells, which fire more frequent when a rat is at a particular location in its environment (O'Keefe & Conway, 1978). Aging is thought to be relevant to hippocampal LTP impairment and memory loss, including spatial memory, which can be attenuated in the presence of a D1/5R agonist administrated

systemically (Bach et al., 1999). In the water maze task, using local infusion of the D1/5R antagonist SCH23390 to the dorsal hippocampal CA1 immediately after training, the long-term spatial memory formation is hindered, while post-training infusion of the D1/5R agonist SKF38393 enhances retention and facilitates the spontaneous recovery of the original spatial preference after reversal learning (da Silva et al., 2012). Similarly, in the Morris water maze, D3R gene knockout mice exhibit apparent normal learning ability; by contrast, D1R gene knockout mice do not acquire spatial memory (Xing et al., 2010). Using local administration of a D2R agonist to ventral hippocampus, SD rats show a dose-dependent improvement in choice accuracy in the radial-arm task, while the effect of a D1R agonist in ventral hippocampus is less compelling (Wilkerson & Levin, 1999). Yet, the role of D2R and D3R in spatial working memory remains controversial, as mice lacking D2R and D3R have been shown to have spatial working memory deficits in the T-maze task (Glickstein et al., 2002). D4 knockout mice perform better in an alternation T-maze for spatial working memory testing and similarly with wild-type animals in the Morris water maze for spatial learning ability evaluation (Falzone et al., 2001). Taken together, the dopaminergic system is implicated in spatial memory processing, although the functions of some subtypes of dopamine receptors remain to be fully explored.

1.2.3.3.3 Aversive and reward-related incentive learning

Aversive learning is that an aversion is created toward a targeted behavior by pairing it with an unpleasant stimulus, and contextual fear conditioning and passive avoidance learning are regarded to belong to this paradigm (El-Ghundi et al., 2007). Strong evidence shows D1/5Rs are relevant to the hippocampal memory formation. In

the inhibitory avoidance (IA) traning paradigm, memory persistence linearly correlates with the intensity of the electric footshock. Using hippocampal local administration of a D1/5R agonist, weak footshock-induced short-term memory can be transformed in to a long one, whereas strong footshock induced long-term memory can be attenuated to a short one by administrating a D1/5R antagonist into the dorsal hippocampus (Rossato et al., 2009). In a mouse model, activation of dopamine neurons in response to an aversive stimulus is attenuated by conditional genetic inactivation of functional NMDA receptors on dopaminergic neurons. With this model, Zweifel and colleagues found that altering the magnitude of excitatory responses by dopamine neurons in response to an aversive stimulus is associated with impaired conditioning to a cue that predicts an aversive outcome (Zweifel et al., 2011). Systemic blockade of D2-like receptors facilitates the extinction of conditioned fear in mice, thus suggesting a role for D2 antagonists as a potential therapy for human anxiety diaorders, including panic disorder, obsessive-compulsive disorder, and post-traumatic stress disorder (Ponnusamy et al., 2005). Regarding the D4Rs, in the IA training system, the performance of mice can be improved in a dose-dependent manner by systemic appilcation of the D4 agonist PD168077 (Bernaerts & Tirelli, 2003).

Incentive learning in animals is defined as the the ability of previous neutral stimuli to control behavior when associated with a rewarding experience (Jentsch & Taylor, 1999; El-Ghundi et al., 2007). As one of the four dopaminergic pathways in the mamalian brain, the dopaminergic neurons projecting from ventral mesencephalic neuclei to forebrain targets play a critical role in reward-rlated incentive learning (Beninger & Miller, 1998). Optogentic tools allow researchers to selectively control the main source of dopaminergic pathways to investigate the behavioral phenotype

caused by dopamine release. Tsai and colleagues showed that phasic optical activation of dopaminergic neurons in the VTA is sufficient for behavioral conditioning in transgenic mice using the conditioned place preference (CPP) paradigm (Tsai et al., 2009). Furthermore, dopamine receptor-mediated effects within the hippocampal formation are also implicated in the reward incentive learning. In an "everyday appetitive" behavioral paradigm, Wang and colleagues showed that encoding of low reward-induced place memory decays over 24 h, whereas spatial memory encoded by using strong food reward persists for periods longer than 24 h unless encoding occurred under hippocampal D1/5 receptor blockade (Wang et al., 2010).

1.2.3.3.4 Dopaminergic implications in cognitive functions

Different kinds of brain disorders including Parkinson's disease, schizophrenia, attention deficit hyperactivity disorder (ADHD) and drug addiction are associated with deficits in the ability to regulate behavior in response to changing environmental demands, leading to inflexibility, impulsivity and/or compulsivity (Cools & Robbins, 2004). Here, I focus on dopaminergic modulation of novelty perception.

As previously mentioned, in the "everyday appetitive" behavioral model, low food reward only induces place memory lasting for less than 24 h, but this memory can be strengthened to longer than 24 h if the animals are allowed to experience "novel paradigms" 30 min after the weak food reward. And this novelty-memory facilitation can be abolished if D1/5R antagonists are locally administrated to the dorsal hippocampus 15 min after the "novelty exploration" trial, indicating the dopaminergic involvement in novelty perception (Wang et al., 2010).

Novelty acquisition is not only involved in the manipulation of a behavioral

phenotype, but also associated with synaptic plasticity. Weak high frequency stimulation (HFS) is used to induce early LTP (E-LTP) and weak low frequency stimulation (LFS) is adopted to induce early LTD (E-LTD). Both E-LTP and E-LTD can be facilitated into the long counterparts if the animals are simultaneously experiencing holeboards, which are novelty stimuli for animals, and this facilitation can be hindered by the presence of D1/5R antagonists in the hippocampus (Lemon & Manahan-Vaughan, 2006).

1.3 Aim and hypothesis

The hippocampus is highly relevant to learning and memory formation, as bilateral hippocampal lesions in human brain resulted in a loss of recent memory. Studies in rodents demonstrated the function of hippocampus in spatial and non-spatial memory, associative and non-associative memory, and novelty recognition memory. In particular, dopamine D1/5Rs have been shown to be involved in the modulation of synaptic efficacy, learning and memory formation. By contrast, the function of D4Rs in synaptic efficacy, learning and memory formation remains to be elucidated *in vivo*, except for improved cognitive performance in the presence of the D4R agonist PD 168077.

The aim of this thesis was to establish a system to monitor extracellular local field potentials (LFPs) in behaving animals, and eventually record and analyze electrical signals and behavioral patterns in parallel to dissect mechanisms that underlie behavioral tasks. Central to this were D4Rs whose activation by agonists in acute hippocampal slices demonstrated a layer-specific modulation of early LTP in area CA1 without affecting basal synaptic transmission. Late LTP has not been investigated. Furthermore in slices, D4Rs mediated an increase in kainate-induced gamma oscillations. Based on these *in vitro* observations, the working hypothesis was

that D4Rs under physiological conditions in behaving mice modulate hippocampal synaptic efficacy, neural network activity and potentially learning and memory.

The specific objectives of this study were:

1. Test the effects by systemically applied D4R ligands on basal synaptic transmission and network activity in dorsal hippocampus (evoked LFPs and spontaneous EEG).
2. Test the effects by systemically applied D4R ligands on early and late LTP in basal and apical CA1 dendrites.
3. Examine whether D4R-mediated modulation of either synaptic efficacy and/or neural network activities are relevant to behavioral performance during novelty exploration and during fear conditioning.

Chapter II

Materials and methods

2.1 Evoked local field potential (eLFP)

2.1.1 Establishing a system for electrical signal recording in behaving mice

Seven-week old C57BL/6N mice were deeply anaesthetized with a mixture of Ketamine and Xylazine (K/X mixture, Ketamine: 65mg/kg, Xylazine: 14mg/kg). After injection, mice were positioned in a stereotaxic frame. The distance (D) between bregma and lambda was measured after opening the scalp and removing the soft tissue with 15% H_2O_2. Two mini-screws were fixed above the cerebellum and served as reference and ground wires, respectively. The positions for a bipolar stimulation electrode and two recording electrodes were marked on the same hemisphere according to the stereotaxic coordinates (Franklin & Paxinos, 1997). Due to differences in D, the position of the stimulation (S) electrode anterior-posterior (AP) to bregma was obtained by $AP_S = 2.0 \times D/4.2$ mm, and the middle-lateral (ML) distance was obtained by $ML_S = 2.3 \times D/4.2$ mm (for recording (R) electrodes: $AP_R = (2.0 \times D/4.2) - 0.05$ mm; $ML_R = 1.7 \times D/4.2$ mm). Two 0.5 mm holes in diameter were drilled through the marked points. For stimulation, two insulated tungsten wires (same length, single wire diameter: 52 μm) with 80 - 100 μm distance between the tips were lowered by a motorized manipulator (Luigs & Neumann, Ratingen, Germany).

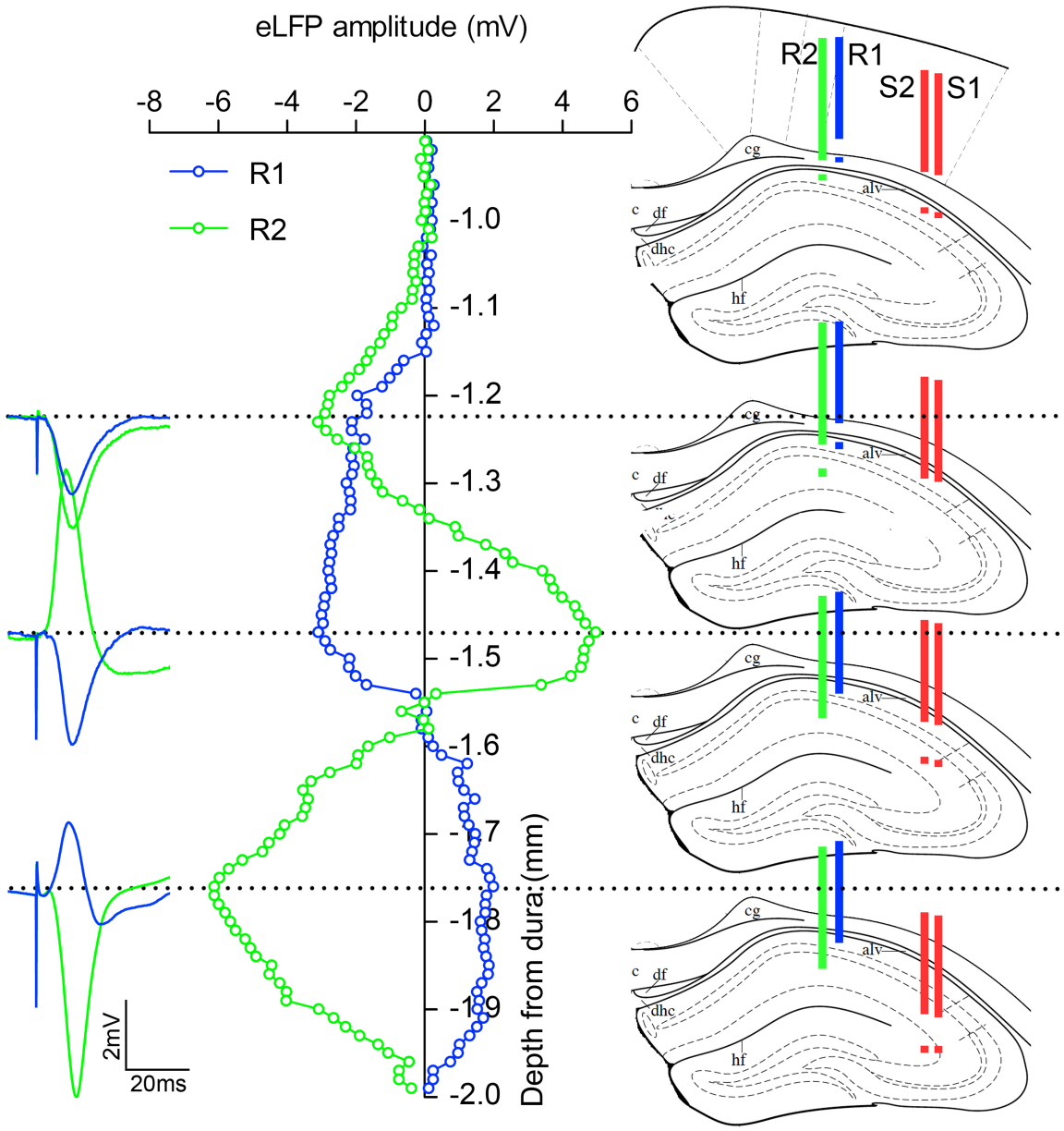


Figure 5. Depth profiles during electrode implantation. Left panel: Typical traces at the given depths aligned to the corresponding depth in the middle panel. Middle panel: Changes of peak amplitudes of bipolar stimulation electrode (S1 & S2) evoked local field potentials (eLFPs) recorded by two recording electrodes (shorter: R1; longer: R2). Note the direction of deflections are getting smaller and inverted when the electrodes are passing through the pyramidal cell layer. Right panel: Schema of the electrode implantation procedure. The schematic illustration of the gradually changing positions of the stimulation and recording electrodes in the given depth range. The tips of long color coded electrodes indicate the starting points of the given depth range, the same color dots indicate the ending positions of the electrodes with in the range. The amplitudes of the evoked eLFPs are dynamically changing with the movements of electrodes. Note both stimulation and recording electrodes are lowered only in the top

section isolated by dashed lines, only the stimulation or the recording electrodes are lowered in the other sections.

For recording, two tungsten wires with one protruding (~250 μm) were placed in OR and RAD. The final depths for both stimulation and recording electrodes were determined by online monitoring depth profiles of evoked local field potentials (LFPs) (negative response in OR, positive response in RAD when stimulation electrode was implanted in OR, and reversed deflections when stimulation electrode was implanted in RAD (Figure 5), consistent with the depth profiles obtained during electrode implantation in rats (Kaibara & Leung, 1993; Shires et al., 2012). Subsequently, a full range of input-output (IO) was generated to further confirm the correct positions. After that, craniotomy holes were filled with a small amount of dental acrylic to fix the electrodes permanently (about 0.5 g including pins). Surgical wounds were sutured, and animals were supplied with softened, wet food during the recovery period and housed singly. With access to food and water *ad libitum*, the animals recovered for at least one week before recording.

2.1.2 Data acquisition and analysis

In the evening before the experimental day, animals were put in the recording chamber (50 cm diameter round arena, 50 cm high) for habituation. On the experimental day, animals were briefly exposed to 95% O_2 containing 4% isoflurane to alleviate the stress when connecting the pins to the miniature headstage (1 g, npi electronic GmbH, Tamm, Germany) for differential measurement of evoked LFPs via two extracellular amplifiers (EXT-02F, npi electronic GmbH). The miniature headstage also allowed extracellular stimulation with an isolated stimulator (A365,

WPI, Berlin, Germany) as well as acquisition of movements via a 3-dimension accelerometers (ADXL327, Analog Devices). Signals were filtered at 1 to 500 Hz, digitized at 10 kHz (ITC-16, Patchmaster, HEKA Elektronik, Lambrecht, Germany), and noise at 50 Hz was filtered by Hum Bug Noise Eliminators (AutoMate Scientific, Inc., Berkeley, CA). Evoked LFPs were stored on PCs, and their slopes were analyzed based on the middle one third of the rising phase (Fitmaster, HEKA Elektronik). At the beginning of each recording, two IO curves per mouse were generated, applying stimulation voltages with both polarities. To evaluate changes in synaptic efficacy a stimulus strength eliciting 35-40% of maximum slope was used as test pulse given every 30 sec. Towards the end of each 30 min period, 10 paired pulses with 50 ms inter-stimulus interval (ISI) were generated to examine the paired pulse ratio (PPR). For LTP induction, two trains of high frequency stimulation (HFS, 50 ×, 100 Hz, 100 μs pulse width, same intensity as test pulse) separated by 5 min were used.

2.1.3 Drug preparation and injection

Normal saline (NS, 0.9% NaCl) was used as vehicle for the control group. D4 receptor agonist PD 168077 maleate (PD, Biotrend chemicals AG, Switzerland) was dissolved in NS to reach 1mg/ml concentration. Both D4 receptor antagonist L745870 trihydrochloride stock solution (L, Tocris Bioscience, UK) and D1 agonist SKF38393 hydrochloride (SKF, Biotrend chemicals AG, Switzerland) stock solution were made similarly to 1mg/ml concentration. After IO curve acquisition, a stable baseline was obtained (> 60 min). The control group received NS (0.1ml/10g) intraperitoneally (i.p.), and the other groups received drugs (0.1ml/10g, i.p.). After injection and/or two trains of HFS (50 ×, 100Hz, 100μs pulse width per pulse), the recording continued for

4 more hours. For the contextual fear conditioning and novelty exploration tasks, the animals received injection of PD (10mg/kg) 30min before the fear/novelty box session on the first experimental day.

2.2 Spontaneous local field potential (sLFP, EEG)

2.2.1 Acquisition of EEG signals

At the meantime of eLFP signal acquisition, the continuous sLFPs (EEG signals) were also filtered at 0.3 to 500 Hz, digitized at 2kHz and stored in another PC for later analysis. The simultaneous 3-dimension accelerometer signals were also acquired for assisting the EEG signal analyzing.

2.2.2 Analysis of EEG signals

The EEG signals were exported from the Fitmaster software (HEKA Elektronik, Lambrecht, Germany), and imported to a customer written Matlab based EEGProcessing program (courtesy of Prof. Dr. Andreas Draguhn's lab). Briefly, based on the simultaneous accelerometer signals, the EEG signals were staged according to the signature characteristics of different oscillation types.

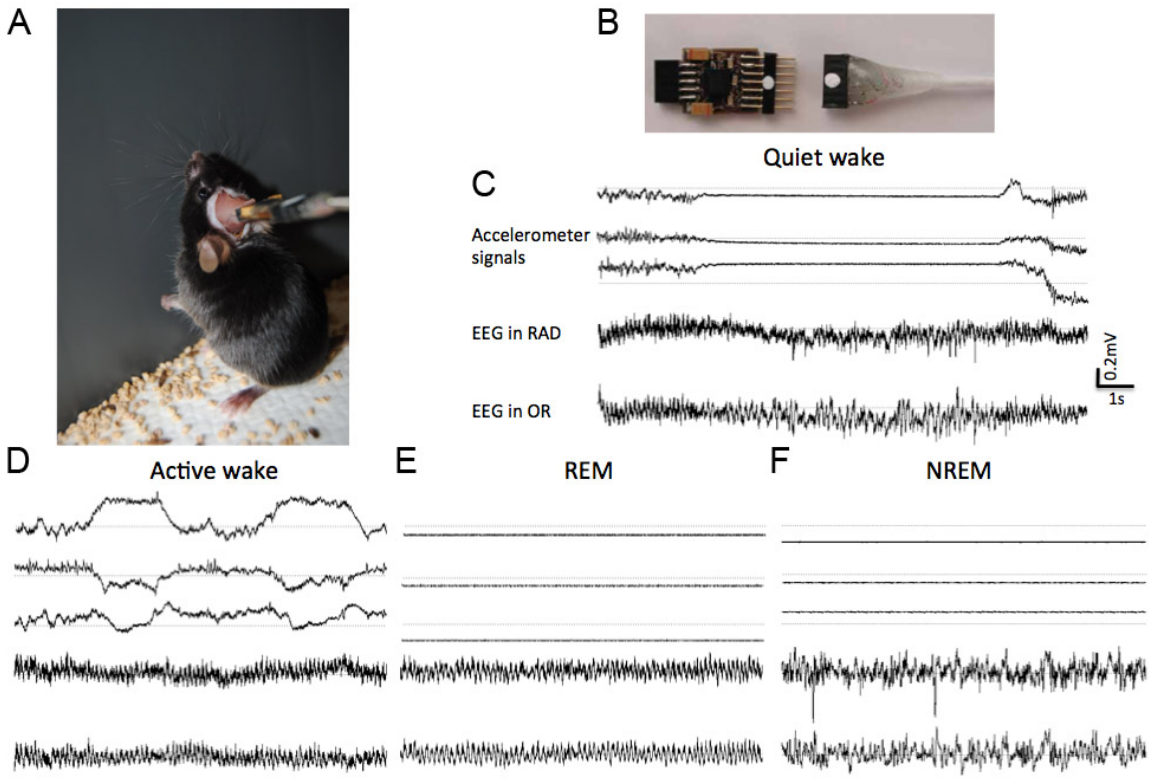


Figure 6. Continuous spontaneous EEG recording associated with simultaneous accelerometer signal acquisition. A. A freely behaving mouse during electrical signal recording. B. 1 g miniature headstage equipped with EEG signals recording channels and stimulation channels, 3-dimension accelerometer as well as the attached signal preamplifier to reduce cable movement artifacts. C-F. Examples of spontaneous hippocampal activities associated with 3- dimension accelerometer signals. (Top three traces: 3- dimension accelerometer signals; fourth trace: EEG in RAD; fifth trace: EEG in OR; the scaling bar is to the right of panel C)

2.2.3 Histology

After recording, animal were deeply anesthetized with K/X mixture, electrical lesions were induced twice (20 μA, 10 s) for each single tungsten wire separately. Subsequently, the mice were perfused with phosphate buffered saline (PBS) followed by 4% Paraformaldehyde (PFA). The brains of the mice were collected and incubated in a mixture of PBS and 4% PFA (1:1) over night. On the following day, the brains

were sliced coronally at 80 μm thickness and mounted on gelatinized glass slides and classical Nissl staining was carried out to verify electrode locations.

2.3 Behavioral paradigms

2.3.1 Inhibitory avoidance training

Memory in an inhibitory avoidance (IA) task is inferred from the delay of a response that was readily made before the training (Cahill & McGaugh, 1998; Izquierdo et al., 1999). There are two types of IA training systems widely used, the step through paradigm and the step down paradigm. In the step through paradigm, the experiments are conducted in a two-compartment behavioral apparatus, where one compartment is designed to be naturally preferred (dark side) by the animal (see Figure 7). During training, the animal is placed in the less-preferred compartment and the latency to enter the preferred compartment is noted. Upon completely entering the preferred compartment, the animal receives one or more inescapable foot shocks of a specified intensity and duration. The information the animal gathers during the training is fear learning. At a retention test, conducted hours, days, or months later, the animal is returned to the previously less-preferred compartment and the latency to enter the shock compartment, which at this point is not electrified, is measured. This measure (retention latency) is used to infer the animal's memory for the fearful experience, the longer the retention latency indicates the better the memory. A long retention latency indicates a significant modification in the animal's behavior, as it contrasts with the animal's low initial entrance latency displayed before the training. For the step down paradigm, in a given chamber equipped with metallic floor connected to electricity, there is an elevated platform on the metal floor for

withstanding the animals. Similarly, the latency of rodents to step down is used as the index to evaluate the memory performance of the animals on the testing day.

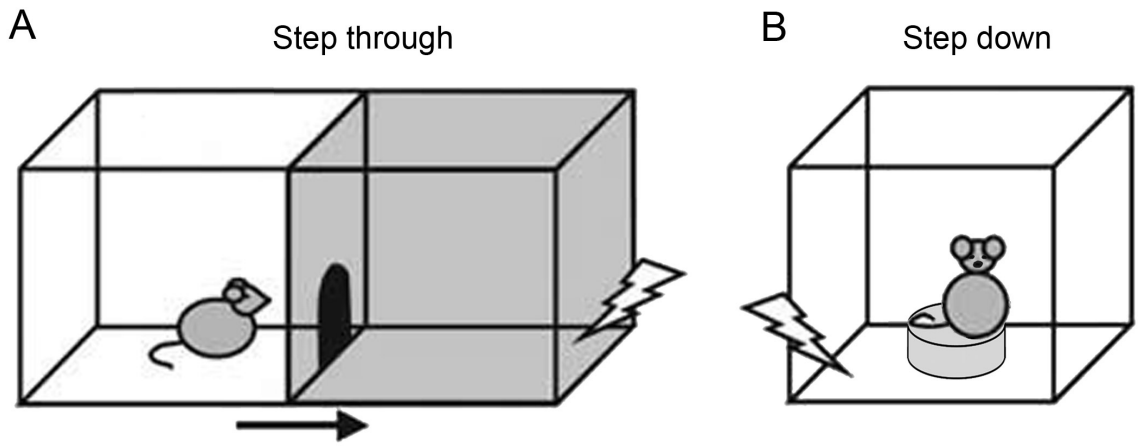


Figure 7. Inhibitory avoidance (IA) training systems. A. Step through paradigm, after the training experience, the latency for the animals to step through the door is used to evaluate the memory persistence. B. Step down paradigm, similarly, the latency for the animals to step down from the platform is used to evaluate the memory persistence on the testing day.

2.3.2 Contextual fear conditioning

Contextual fear conditioning is the most basic of the conditioning procedures. It involves taking an animal and placing it in a novel environment, providing an aversive stimulus, and then removing it. When the animal is returned to the same environment, it generally will demonstrate a freezing response if it remembers and associates that environment with the aversive stimulus. Freezing is a species-specific response to fear, which has been defined as "absence of movement except for respiration." This may last for seconds to minutes depending on the strength of the aversive stimulus, the number of presentations, and the degree of learning achieved by the subject. The experimental procedure was illustrated by the flow chart below. The continuous EEG signals were also acquired during different experimental sessions (Figure 8).

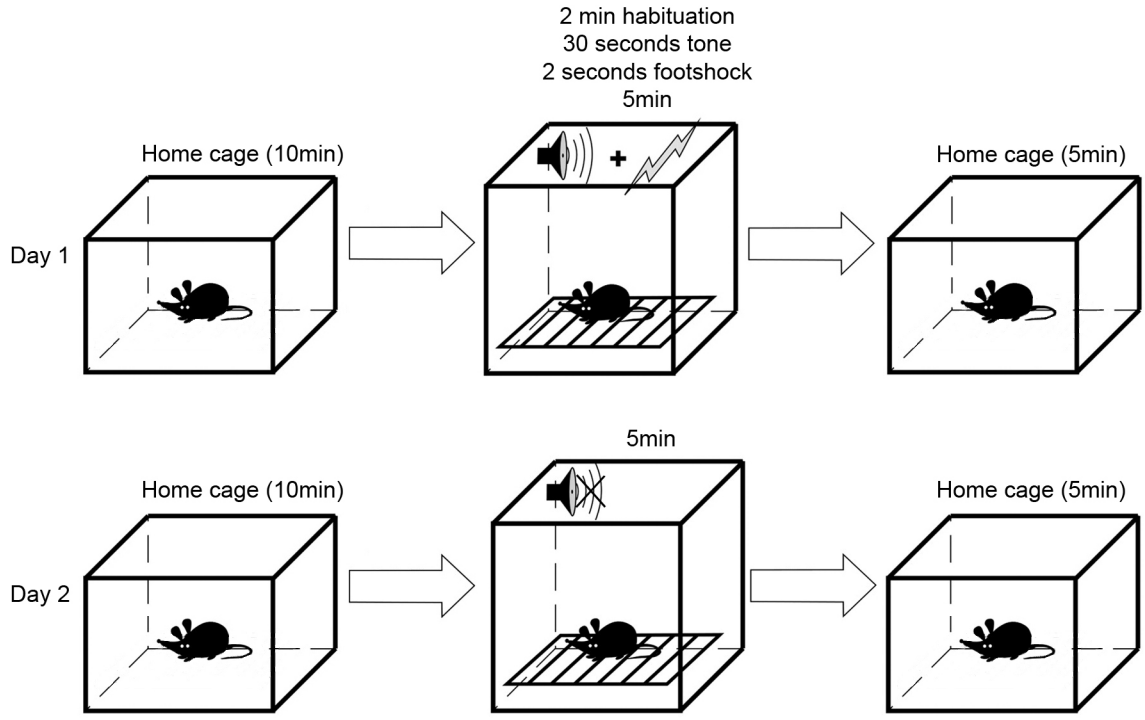


Figure 8. Contextual fear conditioning paradigm. The implanted electrodes were connected to the electrical signal acquisition setup before commencement of the experiment. On day 1 (conditioning day), the animals were firstly put in the home cage for 10 min, and then transferred to the conditioning chamber to experience the events listed above the fear conditioning chamber, and then back to home cage for 5 min recording. On day 2, the similar procedure was applied to the animals except for no footshock in the part of the fear conditioning box as illustrated in the figure. The behavioral states were determined based on the accelerometer signals. The EEG signals were also staged for analyzing (figure adapted from (Warthen et al., 2011)).

2.3.3 Novel context recognition

There are many paradigms to detect the ability of the animals for novelty recognition, including the novel object recognition, novel place recognition and novel context recognition. To match the fear conditioning data, I used the novel context recognition task here.

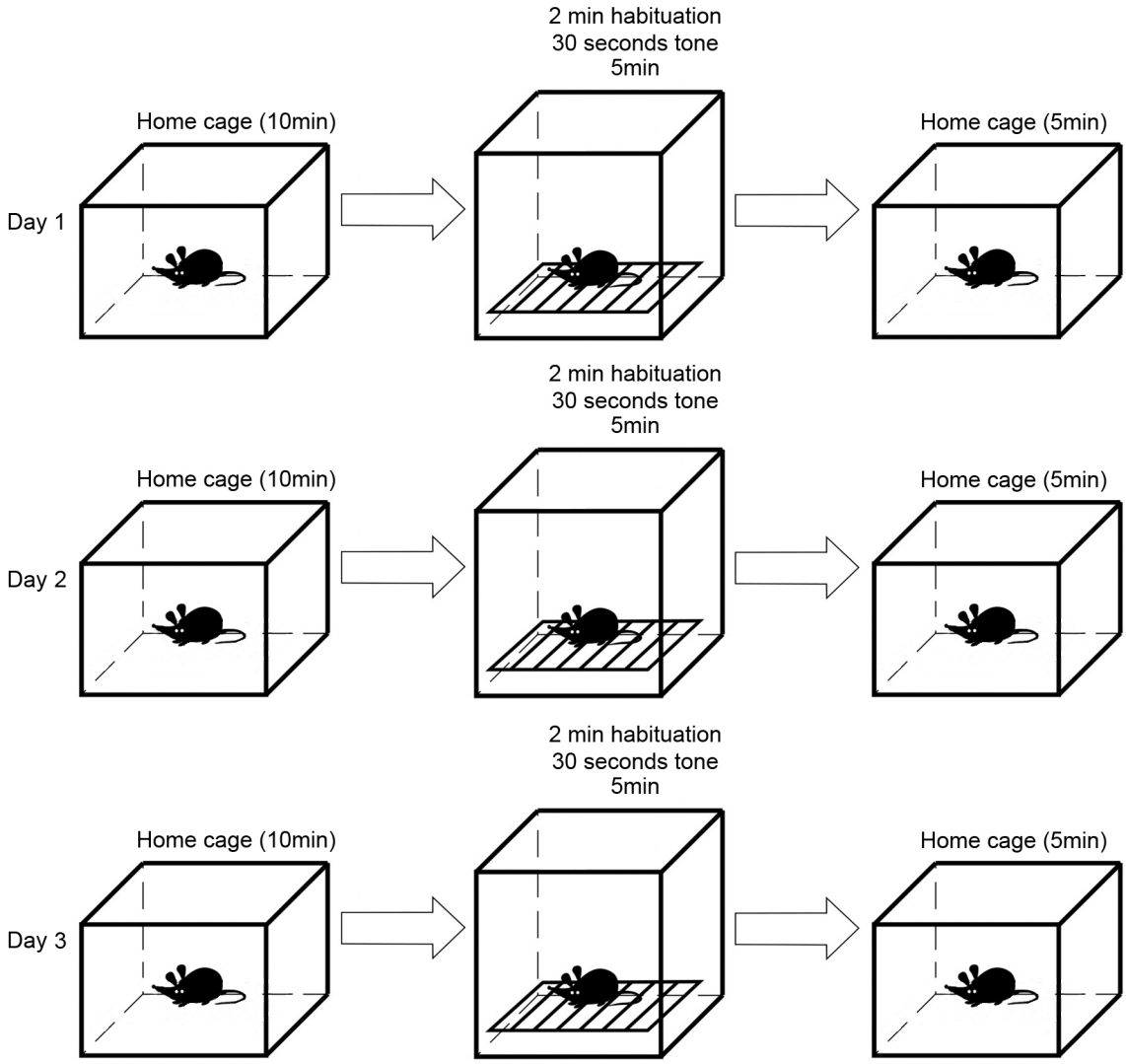


Figure 9. Novel context recognition. The implanted electrodes were connected to the electrical signal acquisition setup before commencement of the experiment. There are three days of work continuously carried out as depicted in the schematic figure. The only difference is that the control group received injection of NS, while the other group received PD (10 mg/kg) 30 min before the animals were exposed to the novel context (fear conditioning box, but no footshock here). The EEG signals were also staged for analyzing (figure adapted from (Warthen et al., 2011)).

2.4 Statistics

Two sample Kolmogorov-Smirnov test (K-S test) was used to test for differences in the IO efficiency. Repeated measures analysis of variance (ANOVA) was used to compare the slope of LFPs after injection/HFS for NS versus PD group, and post hoc t-test to compare slopes at distinct time points. Two tailed paired t-test was used to

compare the PPR after injection and/or HFS with control (before injection). Two tailed unpaired t-test was adopted to compare the difference of PPR after normalization to before injection between NS and PD group. One way ANOVA followed by paired t-test was used to compare difference at different time points for oscillation peak frequencies, band power and CFC strength for EEG signals. Errors shown in the figures indicate standard error of the mean (SEM). The level of significance was set to $P < 0.05$, 0.01 or 0.001.

Chapter III

Results

3.1 Evoked local field potentials

3.1.1 Higher IO efficiency in RAD than in OR

Here, I recorded evoked LFPs in freely moving mice with two tungsten electrodes in OR and RAD while stimulating with one bipolar electrode in OR or RAD. With this configuration (Figure 10 A, D), I recorded negative evoked responses on the site of stimulation and opposite deflections on the other site of stratum pyramidale (Figure 10 B, E). When supra-threshold stimulus intensities were applied either in OR or RAD (Figure 10 C or F), the output (absolute slope of the LFP) was stronger in RAD than in OR (green vs. blue in Figure 10 C and F; K-S test, $P < 0.01$). This output difference was more pronounced for the pathway in RAD than in OR (Figure 10 F vs C; K-S test, $P < 0.01$). Also, the input/output relationship was steeper for RAD than OR (Figure 10 C, F; K-S test, $P < 0.01$), and consequently the stimulus intensity to saturate evoked LFPs was lower in RAD than in OR (Figure 10 C, F*;* 9.6V vs 12V). Finally, the slope of maximally evoked LFPs were about 2-fold in RAD (Figure 10 F, green) compared to OR (Figure 10 C, blue; K-S test, $P < 0.01$; OR: n = 42, RAD: n = 40).

These observations indicate a lamina-specific synaptic efficiency difference in hippocampal CA1.

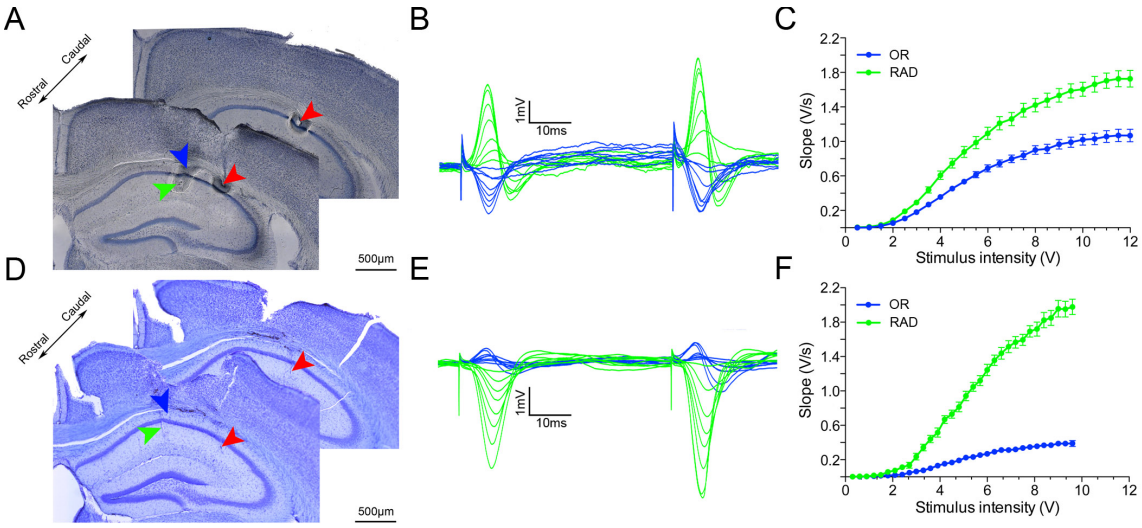


Figure 10. Placement of electrodes, representative traces of evoked LFPs and IO curves. (A, D) Nissl staining following electrical lesions illustrated the locations of bipolar stimulation electrode (red arrow heads, A, OR; D, RAD), recording electrode in OR (blue arrow head) and recording electrode in RAD (green arrow head). (B, E) Representative, colour-coded traces of evoked responses during acquisition of the IO when the stimulation electrode placed in OR (B) and RAD (E). (C, F) IO curves based on absolute values of slope against stimulus intensity when stimulating in OR (C, n = 42) and (F, n =40) (two-sample K-S test indicates significant difference existed between the compared groups).

3.1.2 D4R agonist's modulation of basal synaptic transmission and PPR

To assess whether i.p. injection of the D4 agonist PD 168077 (PD, 10 mg/kg) can affect synaptic transmission in awake mice in apical and/or basal CA1 dendrites, I evoked LFPs by single-pulse stimulation for one hour (baseline) before injecting PD or NS. Relative to baseline, PD reduced the slope of LFPs in both pathways, stronger in OR than in RAD (Figure 11 C; maximal effects: OR, ~15% at 45 min; RAD, ~10% at 20 min; $^{*}P < 0.05$; NS, n = 6; PD, n = 7). 30 min after injection, the robust PD's effect on the slope of the LFP in OR shifted the peak of the LFP to the right in OR, which was not similarly obvious for the LFPs in RAD (Figure 11 A). Also, PD's effect lasted longer in OR (more than 1 hour) than in RAD (15 min).

To consider whether short-term effects were involved in the reduction of LFPs by PD, I determined the PPR (50 ms ISI) every half hour during the 5-hour recording sessions. Before i.p. injection, PPR was similar in OR and RAD (OR, 1.55 ± 0.05, n = 13; RAD, 1.67 ± 0.07, n = 13). In both pathways, PPR remained stable in the NS group (n = 6) but increased in the PD group as long as the slope of LFPs was decreased (Figure 11 B; $^{\#}P < 0.05$; OR, n = 6; RAD, n =7). Consistently, a transient but significant PPR difference was found between NS and PD (Figure 11 B; $^{*}P < 0.05$).

Overall, the D4 agonist reduced evoked LFPs and increased PPR stronger in OR than in RAD. In both pathways, these PD's effects were reversible.

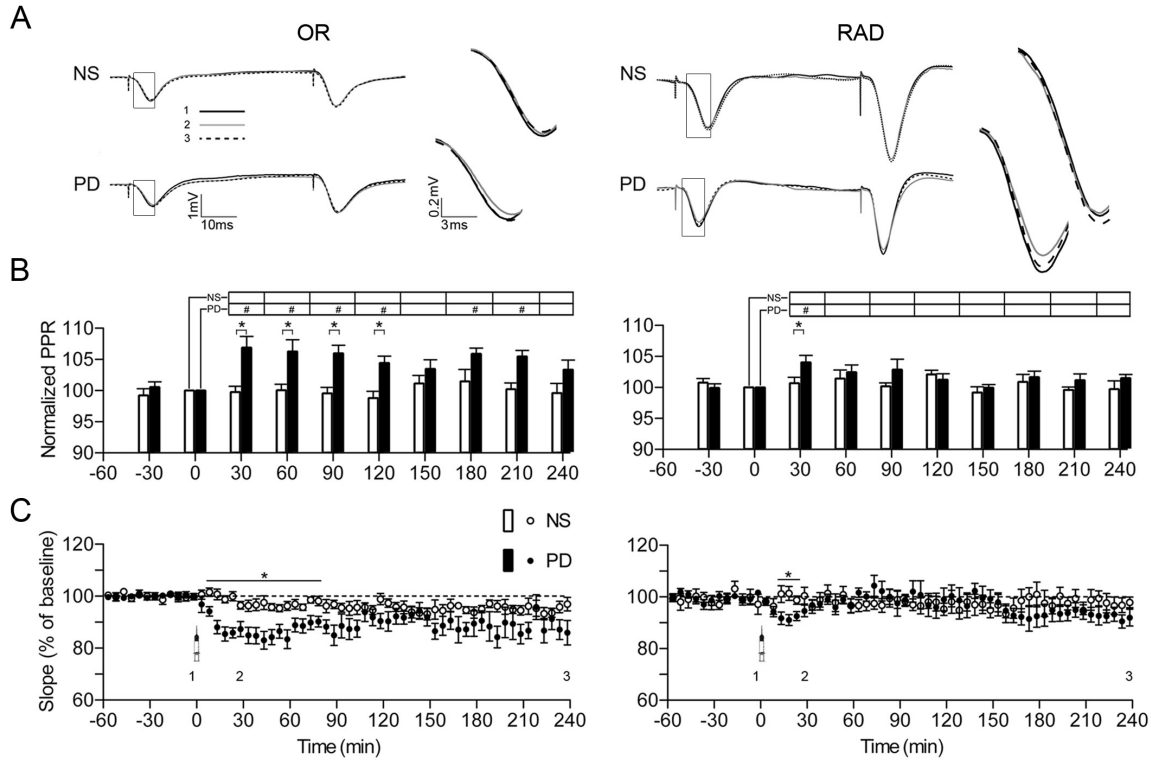


Figure 11. D4R agonist transiently reduced basal synaptic transmission both in basal (OR, left) and apical (RAD, right) dendrites. (A) Averaged (10 ×), representative, traces acquired before injection (1) and 30 min (2) / 240 min (3) after injection of NS or PD. Insets show magnification of boxed regions. (B) PPRs at time points corresponding to the time course in (C) and normalized to values obtained before injection of NS or PD. (B, C) NS, n = 6; PD, n = 7 for both OR and RAD ($^{*}P < 0.05$, differences between NS and PD; $^{\#}P < 0.05$, different from PPR before injection). (C) Syringe indicates

time of injection, bar indicates duration of significant difference (*P < 0.05, repeated measures ANOVA, post-hoc t test).

3.1.3 LTP differed in basal versus apical dendrites

In awake rats, LTP is easier induced in basal dendrites (OR) than in apical dendrites (RAD) (Leung & Shen, 1995). Consistently, stronger LTP was induced in OR than in RAD of awake mice using two trains of high frequency stimulation (HFS) at 100 Hz 30 min after NS injection (Figure 12 C, P < 0.01, OR, n = 6; RAD, n = 6). In particular, the potentiation during the first 30 min after induction (early LTP) was stronger in OR than RAD (~180% vs ~145% relative to baseline; P < 0.01). Early LTP in OR did not decline and persisted for 4 hours (late LTP, Figure 12 C), while early LTP in RAD gradually declined within 90 min before stabilizing at a potentiated level (Figure 12 C; P < 0.01). In NS and following LTP induction, the PPR was reduced for up to 4 hours after induction in both pathways (Figure 12 B, $^{\#}P$ < 0.05; OR, n = 6; RAD, n = 6). Notably, the PPR changes were not stronger in OR than in RAD (Figure 12 B; e.g. at 270 min, P > 0.05; OR, n = 6; RAD, n = 6) even though stronger LTP was induced in the OR pathway.

Thus, in OR early and late LTP had comparable magnitudes, whereas in RAD early LTP was higher than late LTP. In both pathways, the PPR similarly remained reduced throughout LTP expression. This is in line with previous findings.

3.1.4 D4R agonist's modulation of synaptic plasticity and PPR

In hippocampal slices, PD modulates early LTP (40 min) in OR but not in RAD (Herwerth et al., 2012).

Consistently in awake mice, PD reduced early LTP in OR without modulating early LTP in RAD (Figure 12 C, $^{*}P < 0.05$; OR, n = 6; RAD, n = 6). During the subsequent 3-4 hours of recording, LTP also decreased in RAD in presence of PD but a late LTP remained ($P < 0.01$, relative to baseline), while the extent of LTP reduction remained constant in OR. As before (Figure 12 C), PD increased the PPR in OR and RAD but LTP induction strongly interfered and PPR decreased (Figure 12 B; $^{\#}P < 0.05$). This PPR reduction in PD was less pronounced and shorter lasting (120 min) as compared to NS (270 min), leading to differences in PPR between NS and PD during LTP expression (Figure 12 B; $^{*}P < 0.05$). In OR, that difference was noticeable throughout LTP expression and therefore correlated with weaker early and weaker late LTP. By contrast in RAD, the PPR difference between NS and PD was exclusively significant towards the end of the recording (180 - 270 min) when LTP was reduced (Figure 12 B, right panel, $^{*}P < 0.05$).

In summary, PD decreased early and late LTP in basal dendrites but exclusively late LTP in apical dendrites. These effects involved in part the modulation of PPR.

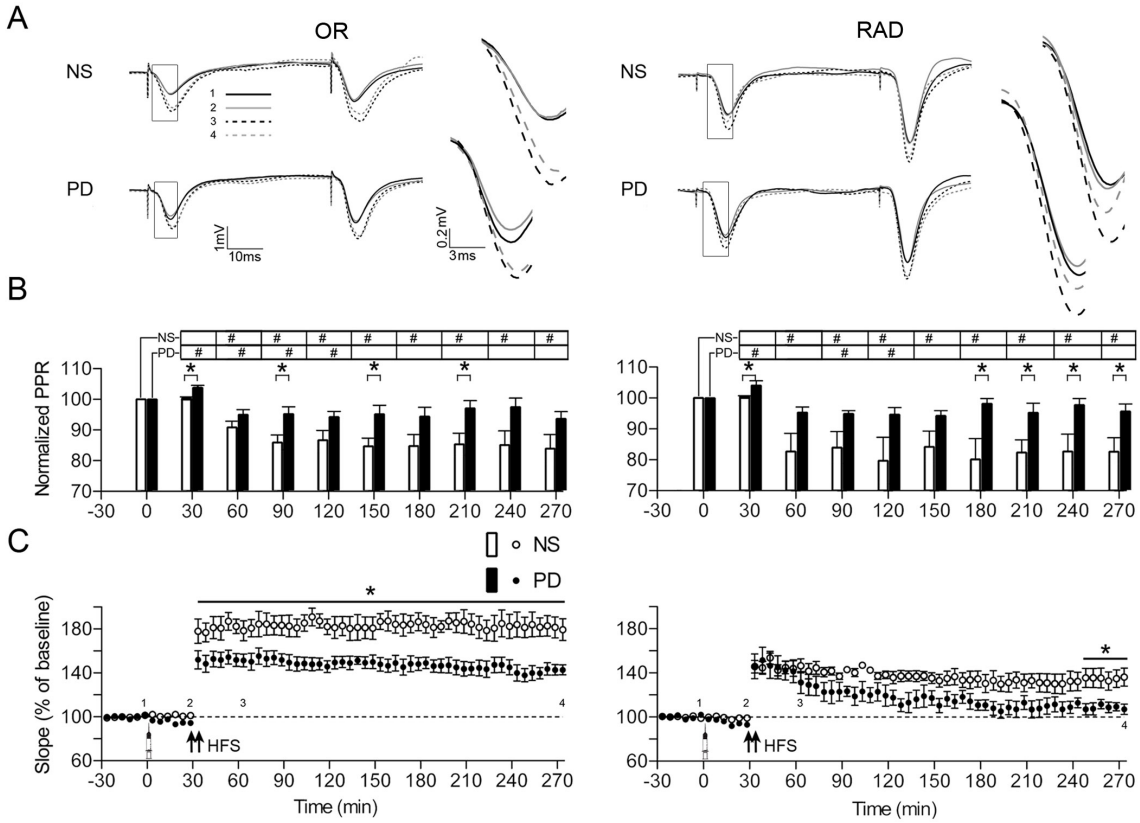


Figure 12. D4R agonist differentially modulated LTP in basal (OR, left) and apical (RAD, right) dendrites. (A) Averaged (10 ×), representative traces acquired before injection (1), before HFS (2), and 30 min (3) / 240 min (4) after injection of NS or PD. Insets show magnification of boxed regions. (B) PPRs at time points corresponding to the time course in (C) and normalized to values obtained before injection of NS or PD. (B, C) NS, n = 6 both for OR and RAD; PD, n = 7 for OR and n = 6 for RAD (*P < 0.05, unpaired t test, differences between NS and PD; $^{\#}P$ < 0.05, unpaired t test, different from PPR before injection). (C) Syringe indicates time of injection, arrows indicate two trains of HFS, separated by 5 min, bar indicates duration of significant difference (*P < 0.05, repeated measures ANOVA, post-hoc t test).

3.1.5 D4R antagonist had no effect on basal synaptic transmission and PPR

To examine whether D4 antagonist can affect basal synaptic transmission or not, I also checked the basal synaptic transmission in the presence of D4 antagonist L745870 (L). Unlikely D4 agonist, L had neglectable effect right after the injection. The PPR was comparable with the control group (Figure 13).

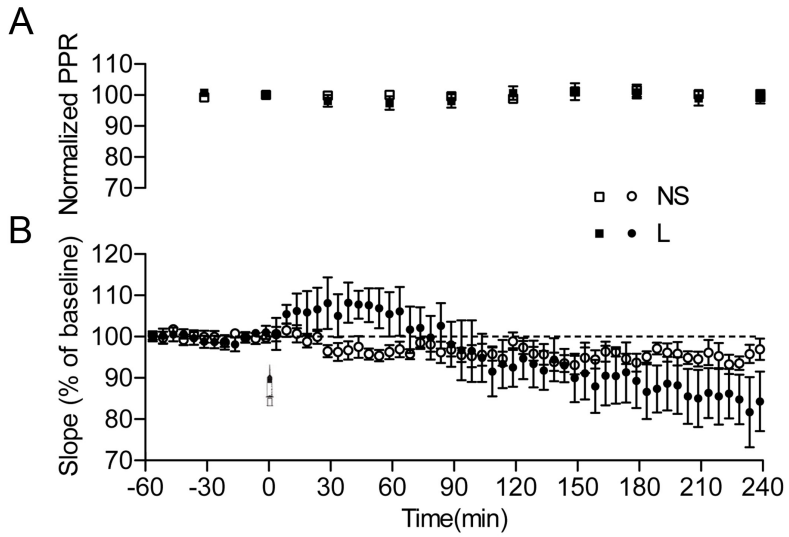


Figure 13. D4R antagonist L had no obvious effect on basal synaptic transminssion in OR. (A) PPRs at time points corresponding to the time course in (B) and normalized to values obtained before injection of NS or PD. (B) L does not change the basal synaptic transmission to a significantly different level. Syringe (B) indicates time of injection ($P > 0.05$, repeated measures ANOVA for comparison of basal synaptic transmission after the injection n = 6 for NS, and n = 7 for L).

3.1.6 D4R antagonist had no effect on synaptic plasticity and PPR

Since significant difference was found for LTP induction in the presence of D4 agonist PD168077 (Figure 12), it will be also interesting to find out whether D4 antagonist L can modulate LTP induction or not. Similarly, the LTP induction protocol was applied to the animals 30 min after the injection of L (10 mg/kg). After LTP induction, the LFPs were similarly potentiated with the NS group, and the PPR significantly declined with the same time course, which is also similar to the PPR in

control group (Figure 14, *P < 0.01, for the details see figure legend). These results indicated that the D4 antagonist (10 mg/kg) has no obvious effect on the modulation of LTP induction.

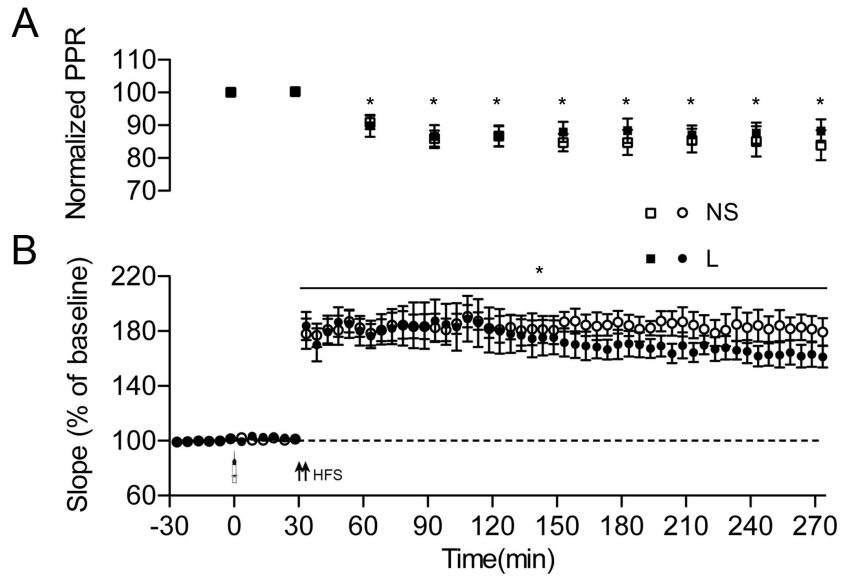


Figure 14. D4R antagonist had no obvious effect on LTP in basal hippocampal dendrites. (A) PPRs at time points corresponding to the time course in (B) and normalized to values obtained before injection of NS or PD. Note that after LTP induction, both groups were strongly potentiated independent of injection of NS/L (B). At the meantime, the PPR were significantly reduced (A). At the given time points, there was no significant difference found between the NS and L group (*P < 0.01, paired t-test revealed significant PPR difference between before and after LTP induction for both groups, and unpaired t-test showed no difference after LTP induction between the NS and L group). Syringe (B) indicates time of injection, and arrows indicate two trains of HFS, separated by 5 min (*P < 0.01, repeated measures ANOVA for comparison of LTP induction with before induction, followed by post-hoc t test for the distinct time points, repeated measures ANOVA revealed no difference between the two groups after LTP induction, n = 6 for both NS and L).

3.2 Behavioral tasks

3.2.1 Inhibitory avoidance training

C57BL/6J mice are frequently used in the IA training task, since they can perform the task very well (Bernaerts & Tirelli, 2003; Baarendse et al., 2008; Dubrovina & Red'kina, 2012). Since I used C57BL/6N mice for the electrophysiology experiment, I also used C57BL/6N mice for IA training to match the electrophysiology data. To establish the system, 0.25 mA and 0.5 mA footshock were used in the conditioning phase (step-down paradigm). Surprisingly, there was no significant difference found compared to its own control condition for the footshock intensities, which have been checked (Figure 15).

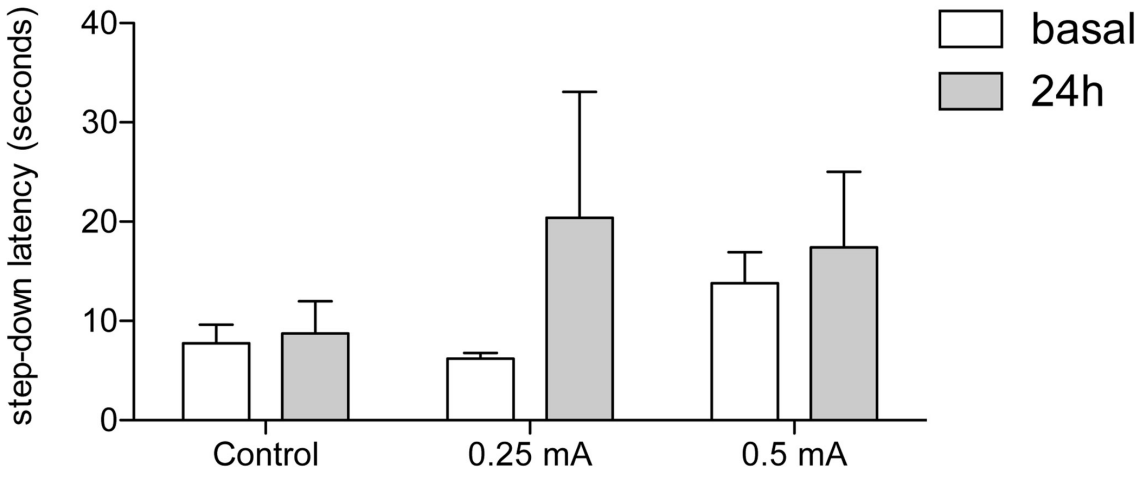


Figure 15. Inhibitory avoidance training couldn't be successfully established with C57BL/6N mice in the step down paradigm with the footshock intensities we have tested. In the control group (n = 4, *P* > 0.05 by paired t-test), similar step down latencies were recorded on two sequential days. With the 0.25 mA and 0.5 mA footshock intensities, the latency to step down from the elevated platform 24 h later was indistinguishable compared to its own control condition (n = 6, *P* > 0.05 by paired t-test for both 0.25 mA and 0.5 mA) (data collected by Miriam A. Vogt and analyzed by Shi-Bin Li).

3.2.2 Contextual fear conditioning

Since it was not clear whether the passive IA training system worked well or not with C57BL/6N mice based on our preliminary data, another widely used behavioral

paradigm (the classical contextual fear conditioning) was adopted to evaluate the memory formation in the presence of D4 receptor agonist PD. The animals were injected with NS or PD (10 mg/kg) 30min before training on day 1. Based on the accelerometer signals, the immobility percentage was quantified. The immobility percentage was comparable right after the footshock (day 1) and also indistinguishable 24 h later (day 2) when the animals were exposed to the context. The animals showed obvious increased immobility on day 2 for both groups (Figure 16).

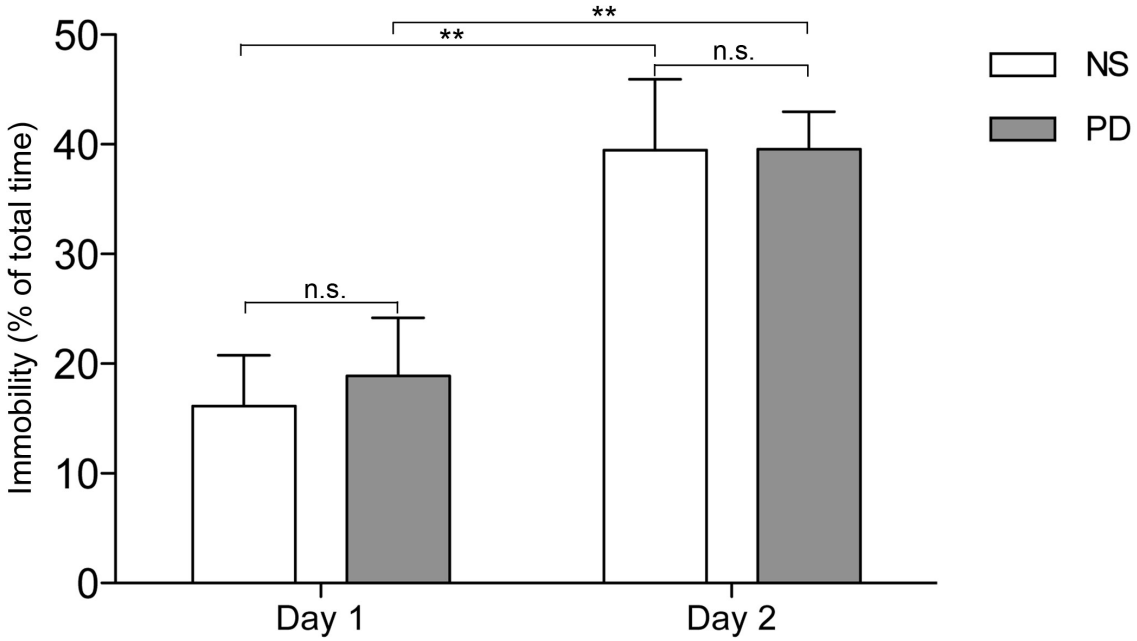


Figure 16. D4 agonist did not change the behavioral performance of the mice in the contextual fear conditioning task. Injection of D4 agonist PD (10 mg/kg) had neither effect on the freezing behavior right after the footshock (Day 1) nor effect on memory formation which was reflected by freezing behavior during the context exposure 24 h later (NS: n = 14; PD: n = 16; n.s., unpaired t test for the comparison between groups; $^{**}P < 0.01$, paired t test for comparison within groups of NS or PD) (data collected by Shi-Bin Li, Miriam A. Vogt, and analyzed by Shi-Bin Li).

3.2.3 Novel context recognition

With the D4R knockout mice during the active phase, Dulawa and colleagues showed D4R-/- mice react much less to novelty than the D4R+/+ controls. The largest

phenotypic differences were observed in the novel object test, which maximizes approach behavior, and the smaller differences were found in the open field test, which maximizes avoidance behavior (Dulawa et al., 1999). In the classical contextual fear conditioning task, the immobile behavior of animals is not only affected by the electric footshock, but also impacted by the effect of novelty when they are exposed to the new environment (fear box). Hence, I also checked whether the behavioral performance was altered or not in the presence of D4 agonist when the animals are exposed to the novel environment during the inactive phase of the animals. The animals received injection of NS or PD (10 mg/kg, i.p.) 30 min before the training on day 1. The two groups showed similar explorative behavior on the same days in the task (Figure 17). Comparing to the contextual fear conditioning task, the immobility percentage was lower for both day 1 and day 2 (Figure 16), indicating the footshock caused significant increase of immobility.

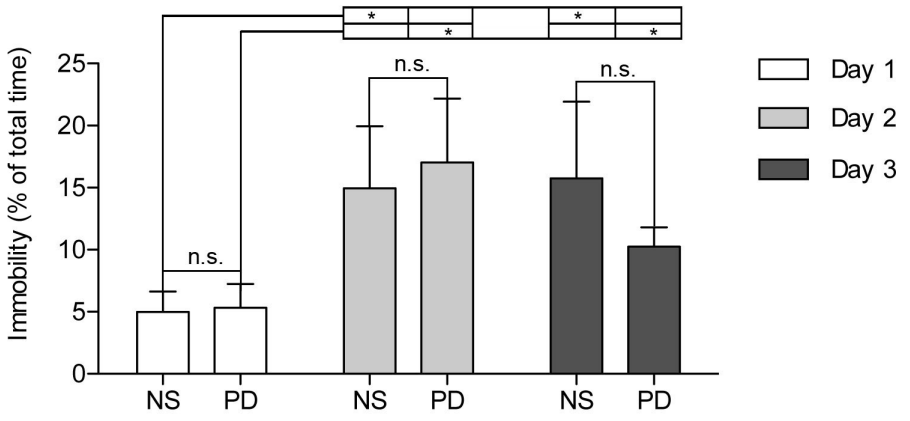


Figure 17. D4 agonist (10 mg/kg) did not change the behavioral performance of the mice in the novel context exploration task. D4 agonist PD (10 mg/kg) had neither effect on the explorative behavior 30 min after the injection on novelty memory formation on day 1 nor effects on day 2 and day 3. Note comparing to the first day, the immobility percentage was significantly increased on day 2 and day 3, but was comparable on the last two days (NS: n = 6; PD: n = 6, unpaired t test for the comparison

between groups, paired t test for comparison within groups of NS or PD) (data collected by Shi-Bin Li, Catarina Luis, and analyzed by Shi-Bin Li).

3.3 Spontaneous local field potentials

3.3.1 D4R mediated modulation of EEG in mice during natural status

3.3.1.1 Manipulation of D4R postponed REM but not NREM

At the meantime of recording evoked LFPs, I also recorded the spontaneous EEG in the hippocampus simultaneously, which allowed me to analyze whether manipulation of D4Rs can affect the spontaneous brain patterns. For the D4R ligands, both D4R agonist and antagonist delayed REM sleep (*P < 0.01), but did not postpone NREM sleep. The delay of REM was more pronounced in the combination of D4 agonist and antagonist (*P < 0.01), yet the NREM latency still remained unchanged in contrast to NS group (Figure 18).

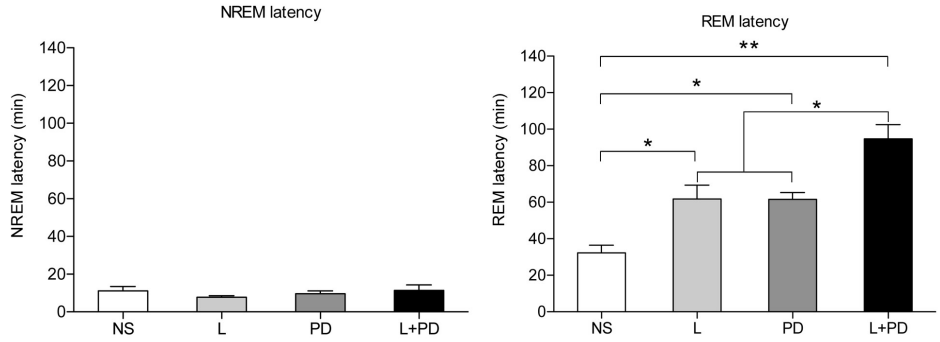


Figure 18. D4R ligands significantly postponed the REM sleep. Left panel: The NREM latency remained unchanged by the treatment of D4R agonist PD, antagonist L or combination of L and PD. Right panel: REM onset was delayed by the treatment of L, PD, and the delay was even more conspicuous by the combination of L and PD (NS: n = 7; L: n = 16; PD: n = 9; L+PD: n = 6; *P < 0.01, **P < 0.001, one way ANOVA followed by unpaired t test).

3.3.1.2 PD modulated the neural network activities in freely behaving mice

Based on the characteristics of the spontaneous brain activity patterns and associated 3-dimension accelerometer signals, the EEG signals were staged into different segments according to behavioral status. Due to the fact that the animals fell NREM sleep ~15 min after the drug treatments (Figure 18), the active waking EEG signals collected between injection and NREM onset was insufficient for analysis. Since the most obvious observation was the delayed onset of REM sleep after the treatment of D4R ligands, I staged the EEG signals of REM sleep from before, after and the 4th hour after the injection of D4R ligands. The band power, peak frequency and cross frequency coupling (CFC) strength were analyzed for the staged data segments.

3.3.1.2.1: PD decreased theta peak frequency and gamma/fast gamma band power during REM onset after injection

During the onset of REM after PD treatment, the peak frequency of theta oscillations (4-12Hz) shifted to lower frequency (Figure 19 C) and this was obvious in the normal scale plotting of band power (Figure 19 A). The band power of gamma (30-90 Hz) and fast gamma (120-160 Hz) oscillations was reduced during the REM onset after administration of PD (Figure 19 D, E). By contrast, injection of NS had no effect on the peak frequency and band power of theta oscillations as well as band power of gamma and fast gamma during REM onset (Figure 19, NS panel).

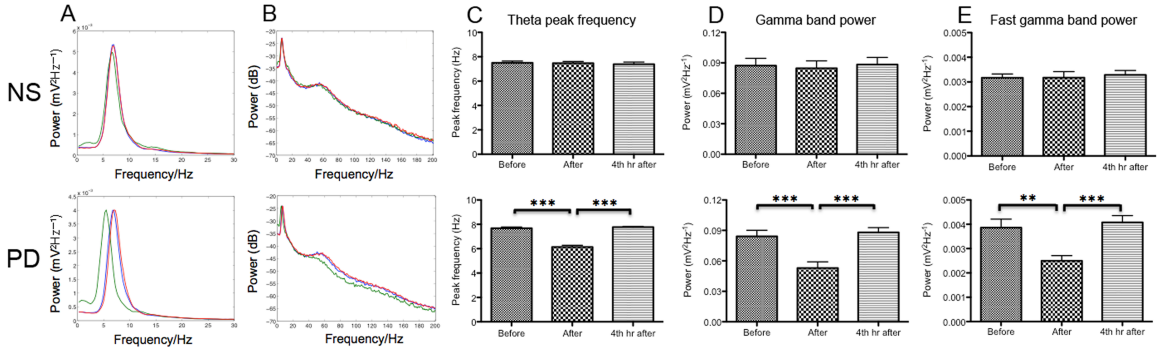
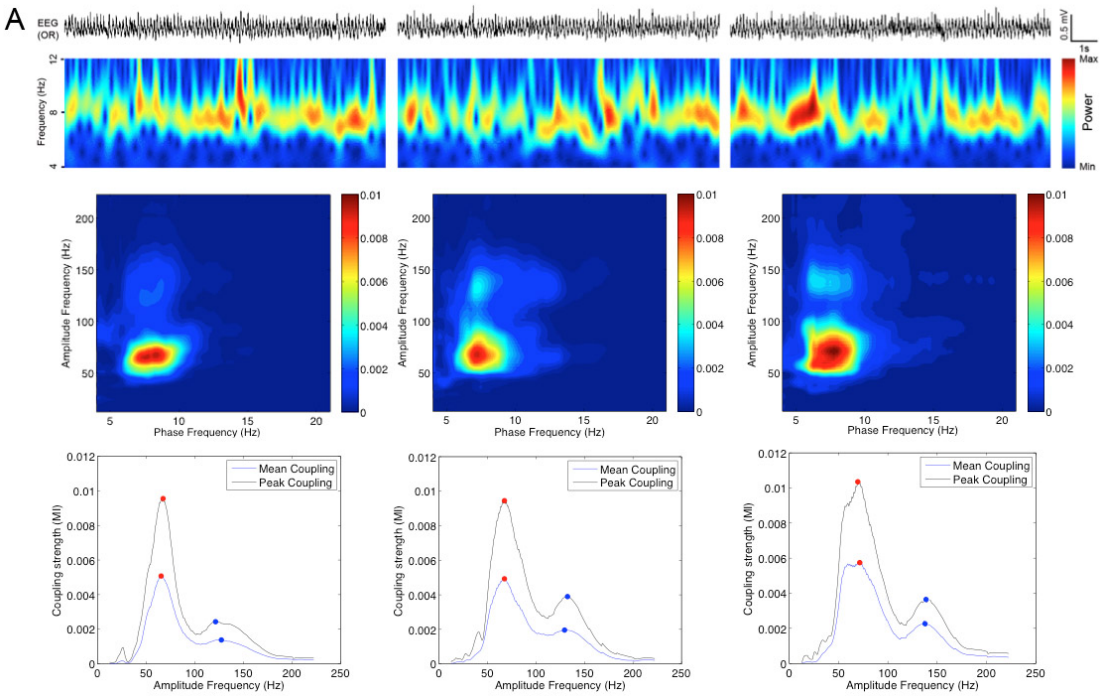


Figure 19. D4 agonist PD modulated brain activity patterns during REM onset. Theta oscillation peak frequency and gamma/fast gamma oscillation band power during REM onset decreased after the injection of PD, and both recovered during the 4th hour after injection (blue, green, red curves represent the time points for REM: before, after, 4th hour after the injection; top left two: normal scale of EEG power spectrum, top right two: log scale of EEG power spectrum; NS: N = 7, PD: n = 9; $^{**}P < 0.01$, $^{***}P < 0.001$, one way ANOVA followed by paired t test).

3.3.1.2.2 PD attenuated theta-high frequency oscillations (HFO) CFC strength during REM onset

The coupling strength of theta-HFO was attenuated after the treatment of PD during REM onset (Figure 20, left to right: before, after, 4th hour after the injection).



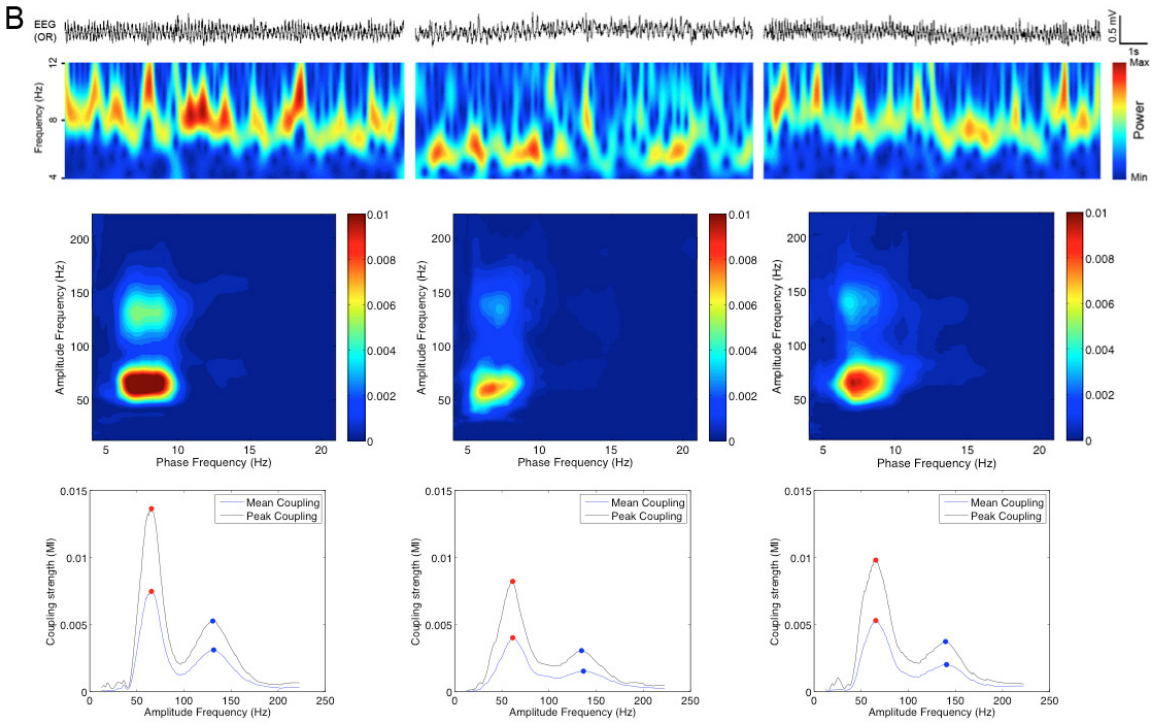


Figure 20. Theta-HFO cross frequency coupling strength among different time points. (A) NS, (B) PD; First and second rows: Original EEG signals recorded in hippocampal OR and continuous wavelet transform. Third row: comodulogram based on EEG signals recorded during REM in hippocampal OR. Fourth row: Theta coupling strength versus amplitude frequency calculated at phase frequencies of maximal coupling were shown for REM at the three indicated time points. Both the mean coupling strength and peak coupling strength were plotted. Compared to before the injection (left), coupling strength between theta-phase and both gamma and fast gamma was significantly reduced during the REM onset after the injection of PD (middle) and recovered during the 4th hr after injection (right) (theta-gamma coupling strength: before vs after: $P < 0.05$, after vs 4th hr after: $P < 0.05$; theta-fast gamma coupling strength: before vs after: $P < 0.05$, after vs 4th hr after: $P < 0.05$, one way ANOVA followed by paired t test).

3.3.1.3 L modulated the neural network activities in freely behaving mice

The data staging was similarly performed as for the processing of PD data (details see above).

3.3.1.3.1 D4 antagonist decreased theta peak frequency but not gamma/fast gamma band power during REM onset after injection

During the onset of REM after D4 antagonist L745870 (L) treatment, the peak frequency of theta oscillations (4-12Hz) shifted to lower frequency (Figure 21 C), which could be easily found in the normal scale plotting of power spectrum (Figure 21 A), while the band power of theta, gamma and fast gamma oscillations remained intact comparing to before the injection. The data for the control group is shown in Figure 19 (NS).

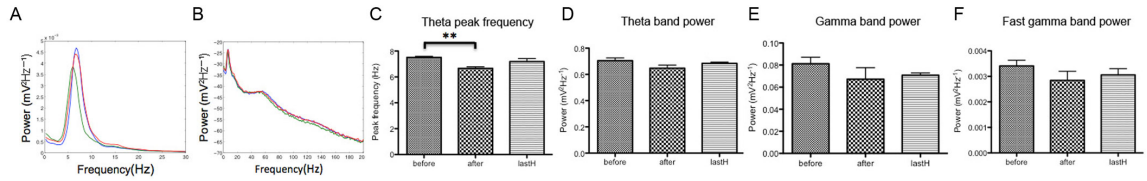


Figure 21. D4 antagonist L decreased peak frequency of theta oscillations during REM onset. L decreased the theta oscillation peak frequency during REM onset after injection in freely behaving mice (**P < 0.01), but had no obvious impact on gamma/fast gamma peak frequency and band power of different frequency oscillations (blue, green, red curves represent the time points for REM: before, after, 4th hour after the injection, paired one way ANOVA followed by t test, n = 6).

3.3.1.3.2 L decreased theta-high frequency oscillations (HFO) coupling strength during REM onset

The cross theta-HFO coupling strength was attenuated after the treatment of L during REM onset (Figure 22). The control data is given in Figure 20 (A).

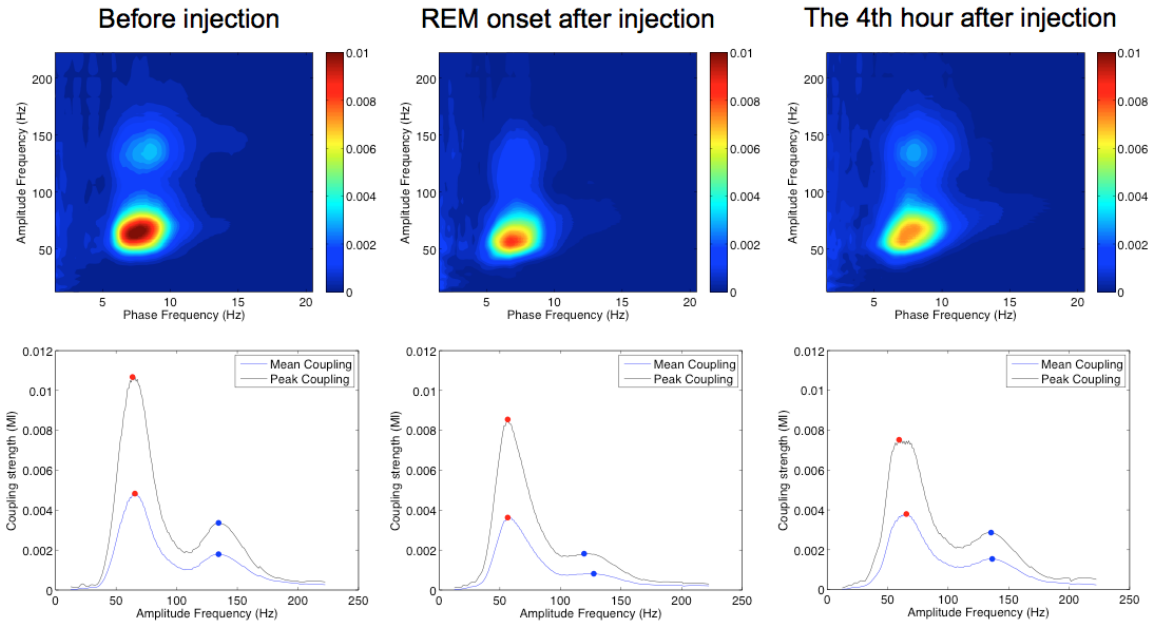


Figure 22. Theta-HFO Cross frequency coupling strength at different time points in the D4 antagonist treated group. Top panels: comodulogram based on EEG signals recorded during REM in hippocampal OR. Bottom panels: theta coupling strength versus amplitude frequency calculated at phase frequencies of maximal coupling are shown for REM at the three indicated time points (n = 6, theta-fast gamma coupling strength: before vs after: $P < 0.05$, after vs 4th hr after: $P < 0.05$, one way ANOVA followed by paired t test).

3.3.1.4 L did not antagonize PD's effect on the neural network activities

Studies on synaptic plasticity showed that, the PD mediated reduction of LTP induction and D4R agonist induced depotentiation of LTP can be occluded by the presence of L (Kwon et al., 2008). I checked whether the PD mediated effect on neural network activities could be abolished by the presence of L or not. In this experimental paradigm, D4R antagonist L was given to the animals 30 min ahead of the administration of D4R agonist PD. The latencies of NREM/REM were defined as the time window between the injection and the onset of NREM/REM.

3.3.1.4.1 Administration of L 30 min before PD did not antagonize PD's effect on theta peak frequency and band power of gamma and fast gamma

Even in the presence of L, PD still shifted the peak frequency of theta oscillation to the lower frequency and decreased the band power of gamma and fast gamma oscillations (Figure 23), which was similar to the case by PD itself (Figure 19).

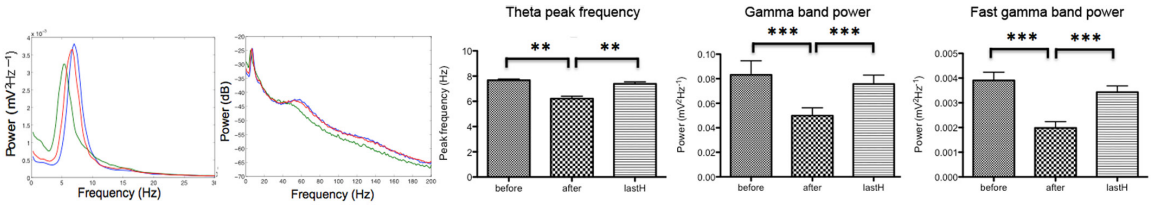


Figure 23. L did not antagonize the effect by PD on neural oscillations. In the presence of D4 antagonist L, D4 agonist PD still exerted a similar effect to that by itself (Figure 19) during REM onset after injection (n = 6; $^{**}P < 0.01$ $^{***}P < 0.001$, blue, green, red curves represent the time points for REM: before, after, 4th hour after the injection, paired t-test)

3.3.1.4.2 Administration of L 30 min before PD did not antagonize PD's effect on the-HFO cross frequency coupling strength

Similar to that the L did not antagonize PD's effect on theta peak frequency and band power of gamma and fast gamma oscillations, the theta-HFO cross frequency coupling strength also showed a similar pattern (Figure 24) to PD's effect on CFC (Figure 20).

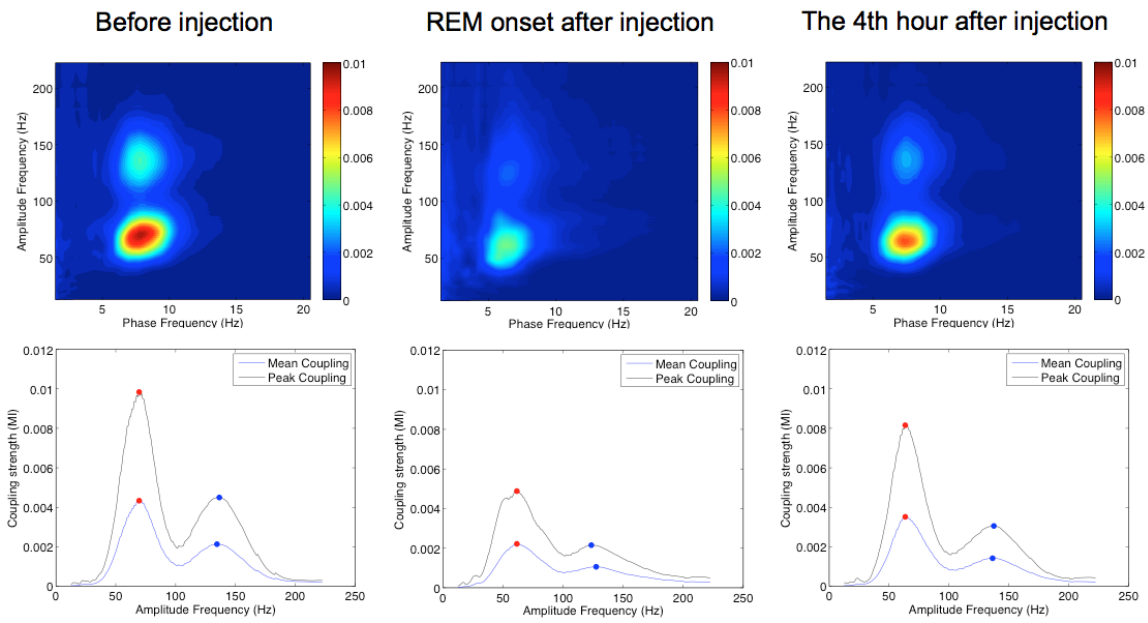


Figure 24. L did not abolish PD's effect on neural oscillations during REM onset. Top panels: comodulogram based on EEG signals recorded during REM in hippocampal OR. Bottom panels: Theta coupling strength versus amplitude frequency calculated at phase frequencies of maximal coupling were shown for REM at the three indicated time points respectively shown in Figure 18 (n = 6, theta-gamma coupling strength: before vs after: $P < 0.01$, after vs 4th hr after: $P < 0.05$; theta-fast gamma coupling strength: before vs after: $P < 0.01$, after vs 4th hr after: $P < 0.01$; one way ANOVA followed by paired t test).

3.3.1.5 Summary of the effects on EEG during REM onset by different treatments

For the dopamine receptor ligands I have checked, the peak frequency, band power of gamma/fast gamma band power as well as the coupling strength between theta and gamma/fast gamma oscillations were reduced by D4 agonist PD during REM onset, and these reduction couldn't be antagonized by D4 antagonist L. By contrast, L reduced the theta peak frequency and coupling strength between theta and gamma/fast gamma oscillations.

group	Theta		Gamma		Fast gamma	
	Peak Frequency	Band power	Band power	Theta-gamma CFC	Band power	Theta-fast gamma CFC
PD168077	↓	–	↓	↓	↓	↓
L745870	↓	–	–	↓	–	↓
L+PD	↓	–	↓	↓	↓	↓

Table 2: The summary of alterations of neural network patterns during REM onset after treatments of D4R ligands. D4R agonist reduced theta peak frequency, band power of gamma/fast gamma as well as the CFC coupling strength of theta-gamma/fast gamma, even in the presence of D4R antagonist L. D4R antagonist L alone reduced the theta band power and the CFC coupling strength of theta-gamma/fast gamma (black arrowheads indicate stronger decrease than the gray arrowheads).

3.3.2 Spontaneous EEG when the animals perform behavioral tasks

3.3.2.1 Band power of the spontaneous EEG in hippocampal OR

As is shown in the methods part, on each day, the task mainly included three sequential sections (home 10 min, fear box, home 5 min), where I also collected the EEG signals in the mouse brain. According to the accelerometer signals together with the characteristics of brain activities as well as the major event (footshock), the EEG signals during active waking (or para-immobility, defined as the episodes surrounding the immobility signals) were staged into different segments in a temporal order (Figure 25). The band power of the EEG signals acquired in hippocampal OR was analyzed when the mice were engaging in different sessions of the task. Surprisingly, when the animals were exposed to the novel environment (fear box), the band power of fast gamma oscillations significantly increased comparing to the previous home cage session (Figure 25). Injection of D4R agonist PD 30 min ahead of commencement of the task did not change the patterns of the band power. Furthermore, in the contextual fear conditioning task, the fast gamma band power further increased after the animals experiencing the electric footshock (Figure 25 A).

The enhancement of fast gamma band power did not decline to the first home cage session level when the animals were returned to the home cage (Figure 25, right panels), indicating the fast gamma oscillations could be implicated in the memory encoding or the initiation phase of memory encoding and the process lasted at least 5 min. The band power of gamma oscillations also increased when the animals were transferred from the home cage to the fear box on day 1 in the contextual fear conditioning task. The theta band power declined after the footshock ("aF" session) on day 1 in the fear conditioning task (Figure 25 A, left panel).

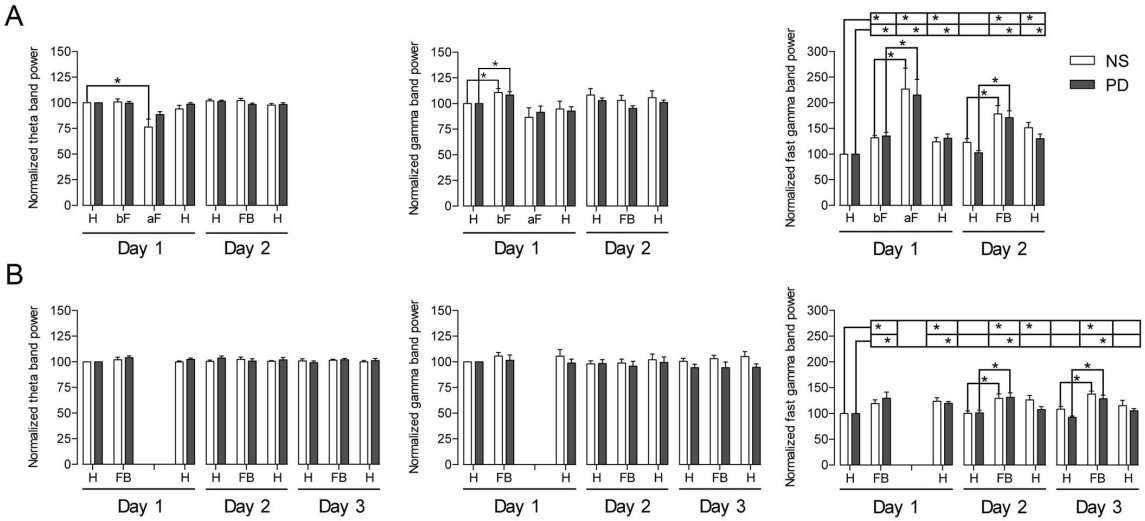


Figure 25. Band power of different frequency oscillations during active waking when the animals were engaging in diverse sessions of contextual fear conditioning (A) and novelty exploration (B). Reduction of theta band power during the "aF" session and increased gamma band power in the "bF" session were found on Day 1 in the fear conditioning task, but not the novelty exploration task (A, B: left and middle panels). The fast gamma band power significantly increased when the animals encountered a new environment (A, B: right panels), which was even more enhanced after the electric footshock (A, right panel). Note that the increment of fast gamma band power did not immediately decline to the level of first home cage session after the novelty experience (A: NS: n = 11, PD: n = 13; B: n = 6, PD: n = 6; *P < 0.05, one way ANOVA followed by paired t test; On the same day, the 1st H indicates home cage 10 min session, the "bF" and "aF" indicate before and after footshock in the fear box, the 2nd H indicates home cage 5 min session, the "FB" indicates fear box).

3.3.2.2 CFC of the spontaneous EEG in hippocampal OR

The theta oscillations not only dynamically modulate the amplitudes of gamma and fast gamma within the striatum and hippocampus but also across the two brain regions during the animals performing the T-maze task (Tort et al., 2008). For the staged EEG signals (active waking), the coupling strength between theta and gamma/fast gamma were similarly calculated. The changes of coupling strength between theta and gamma/fast gamma were inconspicuous except that the coupling strength between theta and gamma was slightly declined after the footshock in the contextual fear conditioning task (Figure 26 A, day 1).

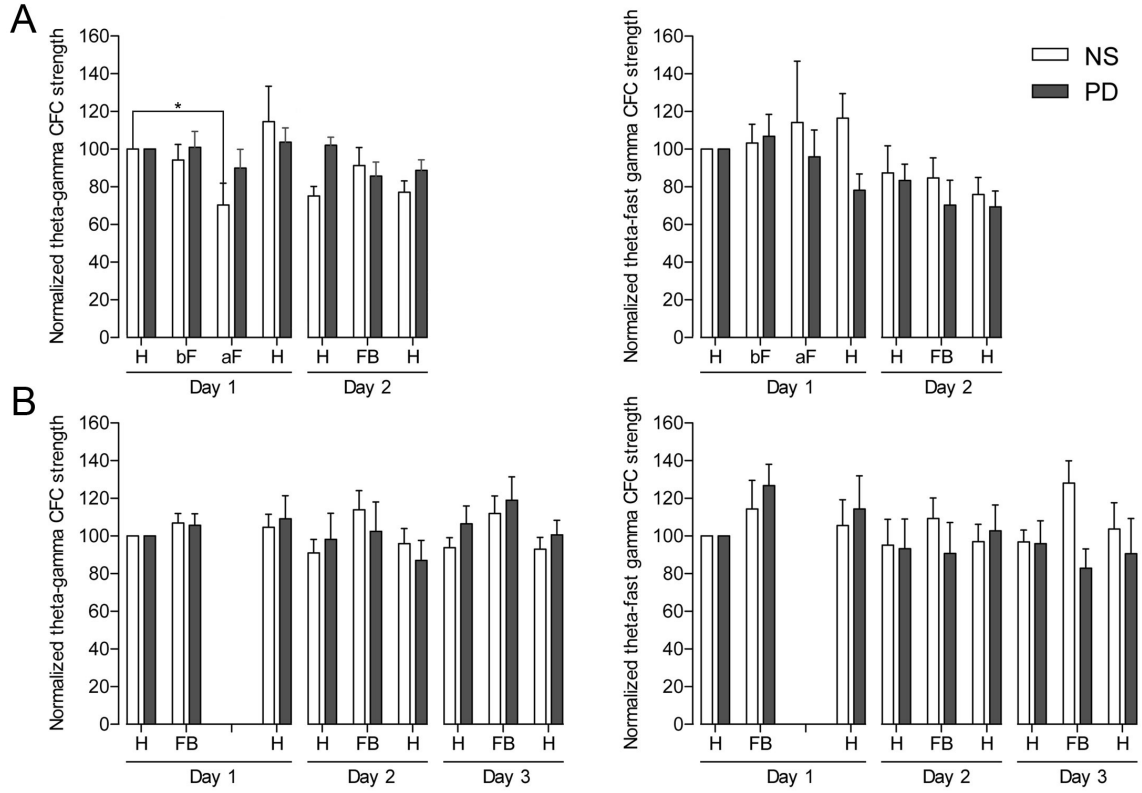


Figure 26. The coupling strength of theta and gamma/fast gamma during active waking when the animals were engaging diverse sessions of fear conditioning task (A) and novelty exploration task (B). The theta-gamma CFC strength declined after the footshock on day 1 in the fear conditioning task. The changes of coupling strength of theta and gamma/fast gamma for other sessions were not significant. Note that the increment of fast gamma band power after footshock (Figure 25 A, right panel) did not lead to enhanced coupling strength between theta and fast gamma oscillations (A: NS: n = 11, PD: n = 13; B: n = 6, PD: n = 6; paired t-test, *$P < 0.05$; On the same day, the 1st H indicates home cage

10 min session, the bF and aF indicate before and after footshock in the fear box, the 2nd H indicates home cage 5 min session, the FB indicates fear box).

3.4 Correlation of memory processing and fast gamma band power

Hippocampal theta and gamma oscillations as well as the amygdalo-hippocampal synchronization are strongly associated with learning and with memory retrieval in the classical Pavlovian fear conditioning (FC) task in rodents (Seidenbecher et al., 2003; Headley & Weinberger, 2013). Normally, the animals encounter the electric footshock in a novel environment, therefore it is not easy to differentiate the effect of the footshock from the effect of spatial novelty, since both stimuli could interfere. With the experimental paradigms I have performed, I quantified the band power of theta, gamma and fast gamma oscillations, which are the most prominent brain activities during active waking to differentiate the impact of footshock from novelty on the brain activities.

3.4.1 Footshock triggered stronger fast gamma band power than novelty

On the training day (day 1), the fast gamma band power significantly increased during the novelty exploration phase ("bF" in fear conditioning task and "FB" in novelty exploration task, see Figure 25), and became even stronger after the electric footshock during the "aF" session in the contextual fear conditioning paradigm. Correlation analysis between the behavior performance and fast gamma band power revealed that the memory formation (behavior phenotype, i.e., immobility percentage) was positively correlated with the band power of fast gamma oscillations during memory encoding phase (para-immobility active waking episodes on day 1) (Figure 27).

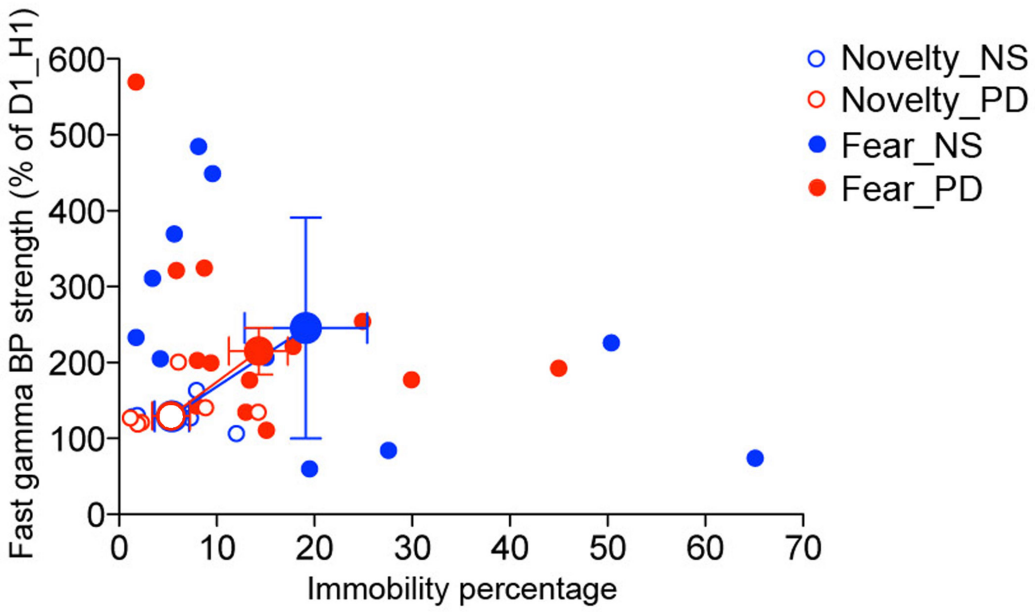


Figure 27. Memory encoding was positively correlated with the band power of fast gamma oscillations (120-160Hz) during the para-immobility active waking episodes. Correlation analysis of behavioral performance (Figure 15 & 16) and band power of fast gamma (Figure 25) in the training session (fear/novel box) on day 1 of the behavioral tasks (Figure 8 & 9) revealed the memory formation was positively coupled with the band power of fast gamma oscillations. The individual open symbol indicates the fast gamma band power during para-immobility active waking for a given animal in the novelty exploration task (day 1) and the individual filled symbol indicates the fast gamma band power during para-immobility (freezing) active waking for a given animal in the training phase of fear conditioning task on day 1, the bigger size symbols indicate the average of the corresponding individuals (Novelty: NS: n = 6, PD: n = 6; Fear: NS: n = 11, PD: n = 13).

3.4.2 Memory retrieval positively correlates with fast gamma band power

During the fear (novelty) box session on the second day of the tasks, I found higher fast gamma band power in the contextual fear conditioning task than in the novelty exploration task for the para-immobility active waking episodes (Figure 28). Correlation analysis of the behavior performance and fast gamma band power revealed that the memory strength (behavior phenotype, i.e., immobility percentage)

was positively correlated with the band power of fast gamma oscillations during memory retrieval (para-immobility active waking episodes) (Figure 28).

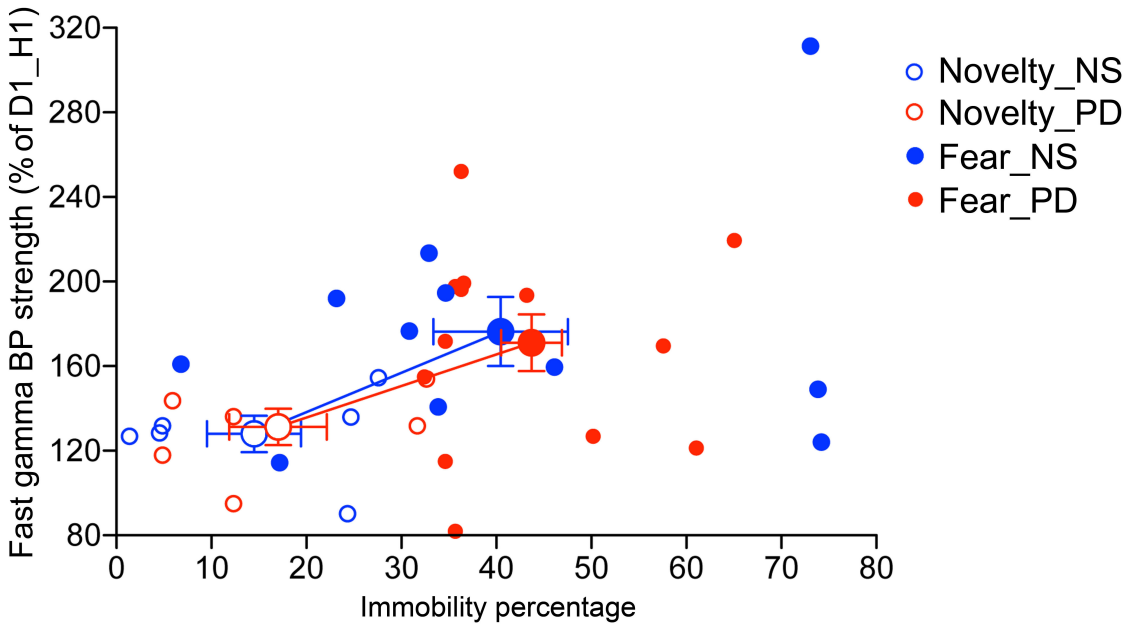


Figure 28. Memory retrieval was positively correlated with the band power of fast gamma oscillations (120-160Hz) during the para-immobility active waking episodes. Correlation analysis of behavioral performance (Figure 15 & 16) and band power of fast gamma (Figure 25) in the contextual exposure session (fear/novel box) on day 2 of the behavioral tasks (Figure 8 & 9) revealed the memory strength was positively coupled with the band power of fast gamma oscillations. The individual open symbol indicates the fast gamma band power during para-immobility active waking for a given animal in the novelty exploration task and the individual filled symbol indicates the fast gamma band power during para-immobility (freezing) active waking for a given animal in the contextual fear conditioning task on day 2, the bigger size symbols indicate the average of the corresponding individuals (Novelty: NS: n = 6, PD: n = 6; Fear: NS: n = 11, PD: n = 13).

Chapter IV

Discussion

4.1 A reliable signal-guided electrode positioning method

Normally if tetrode-acquired unit activities are not required for recording in the hippocampus, people use single recording electrode for extracellular field potential recordings. Even though the IO curves are performed before the formal recording starts, the same deflection direction of the LFPs, when stimulation and recording electrodes were on the same side of the pyramidal cell layer, still distorts the accuracy of the electrodes location. The two recording electrode system, which I used in my experiment, detected the LFPs on both sides of stratum pyramidale, supplying more accurate indices to determine the final positions of the electrodes during implantation (Figure 5 & 10). Furthermore, the higher IO efficacy in RAD than OR ensured the positioning of the electrodes during implantation. These observations are consistent with the depth profiles obtained during electrode implantation in rats (Kaibara & Leung, 1993; Leung & Péloquin, 2010; Shires et al., 2012). The electrical lesion and Nissl staining after the recording demonstrated the precise positions of the electrodes for my recording. And this method is very useful for spontaneous EEG recording, because similar brain activity could be detected from neighbouring brain regions and were even more indistinguishable during electrode implantation in the presence of anesthetics (Buzsáki, 2002; Buzsáki et al., 2003; Caixeta et al., 2013).

4.2 Stronger LTP in basal than in apical synapses of area CA1

Here, in behaving mice, high frequency stimulation induced stronger LTP in basal dendrites (OR) than in apical dendrites (RAD), consistent with results obtained in behaving rats (Leung & Shen, 1995) and in rat slices with intact synaptic inhibition (Arai et al., 1994). Intact inhibition is likely to contribute to the OR/RAD difference in LTP magnitude in rodents, since basal CA1 dendrites of awake rats are less inhibited than apical dendrites (Kaibara & Leung, 1993). Indeed, more interneurons in CA1 project to the apical than to the basal dendrites (Klausberger & Somogyi, 2008). The lower threshold to induce LTP in basal (than in apical) CA1 dendrites (Leung & Shen, 1995) may also be caused by basal dendrites either being more efficient to integrate synaptic excitation to generate an action potential (Georg Köhr and Øivind Hvalby personal communication), or more easily modifiable than apical dendrites as observed for neocortical pyramidal neurons (Nevian et al., 2007), e.g., because of the low density of HCN channels in basal CA1 dendrites (Lörincz et al., 2002).

The LTP underlying mechanisms could be also different. The work in vitro done by Haley and colleagues (1996) suggested that the mechanisms underlying basal dendritic LTP is not dependent on endothelial Nitro oxide synthase (eNOS), whereas the apical LTP is dependent on eNOS, indicating distinct intrinsic properties of basal dendrites versus apical dendrites. Furthermore, the stronger LTP induction was replicated in OR than in RAD in slices with the same protocol for both pathways in their work, and both were blocked by the NMDA receptor antagonist APV (Haley *et al.*, 1996). They summarized the evidence from the anatomical study as follow: the proportion of terminals arising from the contralateral CA4/CA3 cells is higher in OR than in RAD. Thus, one possibility is that the two types of LTP serve to process the inputs arising from the two sides of the brain differently. One hypothesis could be that

afferents from the contralateral possess stronger inherent ability of plasticity to store the information originated from the contralateral side. In contrast, the apical inputs ascending mainly from ipsilateral CA3 region are less prone to be plastic.

Other factors contributing to generate stronger LTP in OR than in RAD could be distinct cellular mechanisms, voltage-gated calcium channels (VDCC) or brain-derived neurotrophic factor (BDNF) (Navakkode et al., 2012). Navakkode and colleagues showed DA induced LTP in basal dendrites is dependent of activation of L-type VDCC, but not in apical dendrites. Furthermore, they also showed BDNF is only required for the induction and maintenance of DA induced LTP in apical dendrites but not basal dendrites, because DA failed to induce LTP in apical dendrites in the presence of the BDNF inhibitor TrKB/Fc (Navakkode et al., 2012).

In my work, the inhibitory inputs remained intact. Thus, similarly I recorded stronger LTP in basal dendrites (OR) than in apical dendrites (RAD) under the very same conditions in freely behaving mice. In other words, I need to increase the HFS intensity to obtain a similar apical synaptic potentiation with basal synaptic potentiation because there is a higher induction threshold for apical LTP, and even this will not guarantee that I will get the same potentiation strength, because intrinsic maximal potentiation of apical pathway may be lower than basal pathway in the CA1 region of the dorsal hippocampus.

4.3 D4Rs' modulation of basal saynaptic transmission and LTP

4.3.1 Differential modulation of basal synaptic transmission in OR and RAD

In freely moving rats, D2-like DA receptor agonists at high concentrations reduce basal synaptic transmission in perforant path-dentate gyrus granule cell synapses,

whereas lower concentrations have receptor priming effects regulating synaptic plasticity (Manahan-Vaughan & Kulla, 2003). Similarly, regarding D4Rs and observations in slices, the D4R agonist PD at μM concentrations (e.g., 20-40 μM) reduces AMPA responses in PFC pyramidal neurons and interneurons (Yuen & Yan, 2009; Yuen et al., 2010), whereas PD at nM concentrations (e.g., 100 nM) has no effect during basal synaptic transmission (e.g., before LTP induction) on synaptic AMPA responses or AMPAR internalization in hippocampal neurons (Kwon et al., 2008; Herwerth et al., 2012), including fast-spiking interneurons (Andersson et al., 2012).

Here, in awake mice, I chose a concentration of PD (10 mg/kg) that is known to improve memory performance, in contrast to lower concentrations (Bernaerts & Tirelli, 2003; Sood et al., 2011). At the concentration of 10 mg/kg, PD induced a transient reduction in basal transmission that was more pronounced in OR than in RAD as observed for both LFP changes (slope and PPR). On the other hand, D1/5R agonists, which can induce late LTP, have no immediate effect on basal AMPAR-mediated transmission neither in slices (Otmakhova & Lisman, 1996; Mockett et al., 2004; Herwerth et al., 2012) nor in behaving rats (Lemon & Manahan-Vaughan, 2006; Lemon & Manahan-Vaughan, 2012). As DA activates D1-like DARs upon phasic DA input and D2-like DARs under tonic stimulation by ambient low levels of DA, acute effects on basal synaptic transmission in the hippocampus are likely dominated by D2-like DA receptors including D4Rs.

D4Rs were also shown to exert homeostatic regulation of glutamatergic transmission in prefrontal cortex (PFC) pyramidal neurons. This dual modulation mechanisms are as follow: at high activity state generated with $GABA_A$ receptors blockade, D4 suppresses AMPAR responses by disrupting the kinesin motor-based

transport of GluR2 along microtubules via reducing the microtubule stability through a mechanism dependent on CaMKII inhibition; on the other hand, at the low activity state, D4 potentiates AMPAR responses by facilitating synaptic targeting of GluR1 via the scaffold protein SAP97 through stimulating CaMKII (Yuen et al., 2010; Yuen & Yan, 2011).

4.3.2 Differential modulation of LTP by D4R agonist in OR and RAD

In the presence of the D4R agonist PD, I observed reduced early LTP in OR but not in RAD in single CA1 neurons (Herwerth et al., 2012). Here, I confirmed this pathway-specific PD effect for LFP responses in awake mice. Fortunately, these in vivo recordings could be maintained for longer periods than our previous whole-cell recordings in slices and unexpectedly exhibited reduced late LTP in RAD in presence of PD. This LTP reduction in RAD developed gradually over about two hours, whereas the reduction in OR occurred immediatedly following LTP induction and persisted to the same extent at least up to four hours.

The fast PD effect in OR in vivo is consistent with our observations in vitro (Herwerth et al., 2012), which suggested a G protein-independent, calcium-dependent reduction of NMDA receptor activity. Similar fast reductions of NMDAR currents were obtained in prefrontal and hippocampal pyramidal neurons via D2-like receptor agonists including PD, involving protein kinase A as well as receptor tyrosine kinases (Kotecha et al., 2002; Wang et al., 2003).

The slower action of PD to reduce LTP in RAD (compared to OR) may be the consequence of pathway-specific induction and/or expression of LTP and/or different mechanisms of PD action are existed in OR vs. RAD. D4Rs belong to the D2-type DA receptors which are negatively coupled to the formation of cAMP by inhibiting

adenylyl cyclase (Missale et al., 1998; Beaulieu & Gainetdinov, 2011). Therefore, PD-mediated cAMP-dependent mechanisms could contribute to reduce LTP, since PKA gates hippocampal early LTP in slices (Otmakhova & Lisman, 1996) and in vivo (Lemon & Manahan-Vaughan, 2006). In addition, it will be interesting to find out whether D4R activation will also be involved in depotentiation of LTP in vivo as observed in apical dendritic synapses in vitro (Kwon et al., 2008).

4.3.3 Implications of presynaptic mechanisms of D4Rs

Paired-pulse facilitation (PPF) describes a phenomenon in which an increase in a second field excitatory postsynaptic potential (EPSP) occurs when it is elicited shortly after a first (Schulz et al, 1994). The ratio of the the second evoked EPSP versus the first one is known as PPR. In their study in slices, they found that PPR changed in association with LTP and the change was inversely related to initial PPR magnitude so that a larger initial PPR was associated with a decrease in PPR while a smaller initial PPR was associated with an increase. In my recording, I used 35-40% of the intensity, which induced the strongest evoked fEPSP in basal dendritic inputs, so I had relative strong PPR before LTP induction. After LTP induction, I saw a roughly 20% reduction of PPR in my recording (Figure 12 B). The proposed PPR underlying mechanism is the residual calcium hypothesis (Katz & Miledi, 1968), and the detailed mechanism has been described previously (Zucker, 1989; Zucker & Regehr, 2002).

LTP induction in the absence of the D4 agonist caused a decrease in PPR in my experiments, which was robust and persisted as previously observed in rats (Madroñal et al., 2009). In attempting to understand the mechanisms underlying the effect of PD on LTP, I examined the PPR during basal synaptic transmission as well as following LTP induction. During basal synaptic transmission in OR and RAD, PD transiently

reduced evoked LFPs and concurrently increased PPRs, suggesting the involvement of presynaptic mechanisms, likely decreasing transmitter release. Following LTP induction, PPR was higher in PD than in NS, which correlated with a significantly reduced LTP magnitude at distinct time points in OR versus RAD. Notably and distinct from basal synaptic transmission, the PD-mediated change in PPR following LTP induction persisted. Since I observed slight decrease of the slope of LFPs accompanied with increased PPF, I think likely the presynaptic mechanism including Ca^{2+} was involved in the modulation procedure by PD. Yet, I still can't exclude that postsynaptic mechanisms are involved.

4.4 D4Rs: implications in the neural network activity

4.4.1 D4 agonist pretreatment does not change the behavioral performance

The injection of a D4R agonist (PD168077, PD, 10mg/kg) 30 min ahead of the experiment had no effect in the novelty exploration task or on the contextual fear conditioning in C57BL/6N mice. In the inhibitory avoidance task, Bernaerts and Tirelli (Bernaerts & Tirelli, 2003) found that the administration of PD (0.5-10 mg/kg) right after the behavioral task dose-dependently improved memory performance in C57BL/6J mice. The possible explanations for no effect of PD in the fear conditioning task for C57BL/6N mice may include: 1) the mouse strains are different; 2) the timing of PD administration is different; 3) behavioral tasks are not exactly the same even though both paradigms evaluate contextual associative memory. Interestingly, administration of a low dose PD (0.064 mg/kg) 15 min before the locomotor experiment increases the activity, and while a high dose (0.5 mg/kg) has no effect, and both doses have no effect on appetitive or aversive conditioning (Nayak & Cassaday, 2003).

4.4.2 Manipulations of D4Rs interfere with sleeping patterns

Injections of D4R agonists or antagonists did not postpone NREM sleep, but significantly delayed REM onset. Studies in rats found that a low dose (1.5 mg/kg) of a D4 antagonist (L745870) increases the light slow wave sleep (light SWS, episodes within the NREM), while a high dose reduces (6 mg/kg) light SWS and increases REM latency by intraperitoneal administration at the beginning of light period (Cavas & Navarro, 2006). For my data analysis, I did not specify the time spent in light SWS and deep SWS, but the observation of REM delay caused by 10 mg/kg L is consistent with their observation for the dosage of 6mg/kg in rats.

In the D4R agonist treated group, the peak frequency of theta oscillation shifted to the lower band, the band power of gamma and fast gamma as well as the theta-HFO cross frequency coupling strength were significantly reduced. In contrast, the D4R antagonist did not change the band power of theta, gamma and fast gamma, but reduced the peak frequency of theta as well as the theta-HFO cross frequency coupling strength (Table 2). In vitro studies showed that activation of D4Rs augments band power of kainate induced gamma oscillation by enhancing the synchronization of fast spiking interneurons via NMDA receptor-dependent mechanism. My observations of decreased gamma band power during REM onset may employ different mechanisms.

Dopamine was shown to reversibly decrease the carbachol-induced cholinergic gamma oscillations, and the effect was mimicked by the D1 agonist SKF38393, while the D2 agonist quinpirole failed to suppress the cholinergic gamma oscillation (Weiss et al., 2003). Except for dopamine, other monoamines including norepinephrine, serotonin were shown to dose-dependently and reversibly suppress kainate and carbachol induced gamma oscillations, but to increase the power and duration of

stimulus-induced gamma oscillations after monoamines application (Wójtowicz et al., 2009).

4.4.3 D4 agonist does not alter the neural oscillatory patterns in behavioral tasks

I found fast gamma band power increased not only when the animals encounter the novel environment for the first time but also when they were re-exposed to the same context (Figure 23). This increment of fast gamma band power was even more pronounced following footshock in the fear conditioning task, not only during the training day, but also during context exposure on the second day. By injecting the D4R agonist 30 min ahead of commencement of the task on the first experimental day, the performance in both the novelty and fear conditioning tasks was not altered, and the EEG patterns were similar to the controls in general.

Conclusions

In this thesis, I described a reliable method for electrode implantation targeting the specific layers of hippocampus in mice. With this experimental paradigm, I found that stronger LTP can be induced in hippocampal OR than in RAD with the same induction protocol in mice. To replenish the earlier in vitro work, I examined the modulation of LTP and spontaneous EEG in hippocampus by manipulating D4 receptors. Activation of D4Rs decreased the basal synaptic transmission for longer time in OR than in RAD, and the PPR increased with a similar time course of synaptic transmission changing in both pathways, indicating the involvement of presynaptic mechanisms. Furthermore, administration of D4R ligands did not interfere with NREM sleep onset, but significantly postponed the onset of REM sleep, and strong modulation of EEG was also found during the onset of REM sleep. I used the novelty exploration task and classic contextual fear conditioning task to investigate the potential implication of D4Rs in learning and memory formation. The novel environment triggered an obvious band power increase in fast gamma oscillations, and the footshock in fear conditioning task enhanced the fast gamma band power even more. Correlation analysis of behavioral performance and EEG signals during para-immobility active waking revealed that memory strength is positively correlated with the fast gamma band power during the test phase of the tasks. Pretreatment of a D4R agonist 30 min before the commencement of the tasks did not change the correlation patterns, indicating D4Rs are unlikely involved in the modulating procedure in the behavioral paradigms I have examined. In conclusion, D4Rs-mediated modulation of synaptic transmission and plasticity, as well as the REM delay, unlikely contributes to learning and memory formation in the novelty exploration and contextual fear conditioning tasks.

Acknowledgements

I would like to give my extreme gratitude to PD Dr. Georg Köhr for the mentorship of my PhD thesis. His enthusiastic support and guidance are appreciated so much, and his scientific curiosity, enthusiasm and hard working style will always inspire me to explore the beauty of neuroscience. I can never forget the difficulties and the frustrations during the procedure of establishing the system for in vivo electrophysiology, yet it was successfully built up with the help from my supervisor, workshop and electronic workshop in Max-Planck-Institute for Medical Research.

Many thanks also go to Prof. Dr. Andreas Draguhn, who not only serves as the second supervisor of my PhD thesis, but also supplies the opportunity for me to learn how to analysis neural oscillation signals. The help from the members in his lab including Dr. Jurij Brankack, Dr. Martin Both are appreciated in particular for teaching me the details.

I also would thank Prof. Dr. Peter H. Seeburg and Dr. Andreas T. Schaefer for serving as the other members of my thesis advisory committee, where I always gain insightful comments and advice to advance my thesis research. And I also would like to appreciate Prof. Dr. Stephan Frings, who would like to serve as the chair of the referees for my PhD defense. And also thanks go to Prof. Dr. Rainer Spanagel for supporting me to join scientific meeting.

Many thanks to Dr. Miriam A. Vogt and Prof. Dr. Peter Gass for expert assistance for behavioral experiments.

Special thanks go to all the lab members for creating a good atmosphere that reigned in the lab.

Thank you to my family for constant support and understanding.

And also thank you to the good friends in Heidlelberg for making the life outside research very colorful.

I would also like to thank the CSC and SFB 636 for supplying scholarship for me to pursue my PhD study.

References

Adamantidis A, Salvert D, Goutagny R, Lakaye B, Gervasoni D, Grisar T, Luppi PH & Fort P (2008) Sleep architecture of the melanin-concentrating hormone receptor 1-knockout mice. *Eur J Neurosci* **27**, 1793-1800.

Agnihotri N, Lopez-Garcia JC, Hawkins RD & Arancio O (1998) Morphological changes associated with long-term potentiation. *Histol Histopathol* **13**, 1155-1162.

Agster KL & Burwell RD (2013) Hippocampal and subicular efferents and afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Behav Brain Res* **254**, 50-64.

Amaral DG, Scharfman HE & Lavenex P (2007) The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res* **163**, 3-22.

Anastassiou CA, Montgomery SM, Barahona M, Buzsáki G & Koch C (2010) The effect of spatially inhomogeneous extracellular electric fields on neurons. *Journal of Neuroscience* **30**, 1925-1936.

Andersson R, Johnston A & Fisahn A (2012) Dopamine d4 receptor activation increases hippocampal gamma oscillations by enhancing synchronization of fast-spiking interneurons. *PLoS One* **7**, e40906.

Arai A, Black J & Lynch G (1994) Origins of the variations in long-term potentiation between synapses in the basal versus apical dendrites of hippocampal neurons. *Hippocampus* **4**, 1-9.

Arnsten AF, Cai JX, Steere JC & Goldman-Rakic PS (1995) Dopamine D2 receptor mechanisms contribute to age-related cognitive decline: the effects of quinpirole on memory and motor performance in monkeys. *Journal of Neuroscience* **15**, 3429-3439.

Asaka Y, Griffin AL & Berry SD (2002) Reversible septal inactivation disrupts hippocampal slow-wave and unit activity and impairs trace conditioning in rabbits (Oryctolagus cuniculus). *Behav Neurosci* **116**, 434-442.

Baarendse PJ, van Grootheest G, Jansen RF, Pieneman AW, Ogren SO, Verhage M & Stiedl O (2008) Differential involvement of the dorsal hippocampus in passive avoidance in C57bl/6J and DBA/2J mice. *Hippocampus* **18**, 11-19.

Bach ME, Barad M, Son H, Zhuo M, Lu YF, Shih R, Mansuy I, Hawkins RD & Kandel ER (1999) Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci U S A* **96**, 5280-5285.

Bannerman DM, Bus T, Taylor A, Sanderson DJ, Schwarz I, Jensen V, Hvalby O, Rawlins JNP, Seeburg PH & Sprengel R (2012) Dissecting spatial knowledge from spatial choice by hippocampal NMDA receptor deletion. *Nature Neuroscience* **15**, 1153-+.

Bartos M, Vida I & Jonas P (2007) Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci* **8**, 45-56.

Beaulieu JM & Gainetdinov RR (2011) The Physiology, Signaling, and Pharmacology of Dopamine Receptors. *Pharmacological Reviews* **63**, 182-217.

Becker N, Wierenga CJ, Fonseca R, Bonhoeffer T & Nagerl UV (2008) LTD induction causes morphological changes of presynaptic boutons and reduces their contacts with spines. *Neuron* **60**, 590-597.

Belluscio MA, Mizuseki K, Schmidt R, Kempter R & Buzsaki G (2012) Cross-frequency phase-phase coupling between theta and gamma oscillations in the hippocampus. *Journal of Neuroscience* **32**, 423-435.

Beninger RJ & Miller R (1998) Dopamine D1-like receptors and reward-related incentive learning. *Neurosci Biobehav Rev* **22**, 335-345.

Bernaerts P & Tirelli E (2003) Facilitatory effect of the dopamine D4 receptor agonist PD168,077 on memory consolidation of an inhibitory avoidance learned response in C57BL/6J mice. *Behav Brain Res* **142**, 41-52.

Bliss TV & Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-39.

Bliss TV & Gardner-Medwin AR (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. *J Physiol* **232**, 357-374.

Bliss TV & Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* **232**, 331-356.

Bloss EB, Puri R, Yuk F, Punsoni M, Hara Y, Janssen WG, McEwen BS & Morrison JH (2013) Morphological and molecular changes in aging rat prelimbic prefrontal cortical synapses. *Neurobiol Aging* **34**, 200-210.

Bolshakov VY, Golan H, Kandel ER & Siegelbaum SA (1997) Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. *Neuron* **19**, 635-651.

Bonnefond M & Jensen O (2012) Alpha oscillations serve to protect working memory maintenance against anticipated distracters. *Current Biology* **22**, 1969-1974.

Bosch M & Hayashi Y (2012) Structural plasticity of dendritic spines. *Curr Opin Neurobiol* **22**, 383-388.

Buzsáki G (2002) Theta oscillations in the hippocampus. *Neuron* **33**, 325-340.

Buzsáki G (2006) *Rhythms of the brain*. Oxford: Oxford University Press.

Buzsáki G, Buhl DL, Harris KD, Csicsvari J, Czeh B & Morozov A (2003) Hippocampal network patterns of activity in the mouse. *Neuroscience* **116**, 201-211.

Buzsáki G & Draguhn A (2004) Neuronal oscillations in cortical networks. *Science* **304**, 1926-1929.

Buzsáki G & Peyrache A (2013) A BOLD statement about the hippocampal-neocortical dialogue. *Trends in Cognitive Sciences* **17**, 57-59.

Buzsáki G & Silva FL (2012) High frequency oscillations in the intact brain. *Prog Neurobiol* **98**, 241-249.

Buzsáki G & Wang XJ (2012) Mechanisms of gamma oscillations. *Annu Rev Neurosci* **35**, 203-225.

Cahill L & McGaugh JL (1998) Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci* **21**, 294-299.

Caixeta FV, Cornélio AM, Scheffer-Teixeira R, Ribeiro S & Tort AB (2013) Ketamine alters oscillatory coupling in the hippocampus. *Sci Rep* **3**, 2348.

Campbell SS & Tobler I (1984) Animal sleep: a review of sleep duration across phylogeny. *Neurosci Biobehav Rev* **8**, 269-300.

Canolty RT, Edwards E, Dalal SS, Soltani M, Nagarajan SS, Kirsch HE, Berger MS, Barbaro NM & Knight RT (2006) High gamma power is phase-locked to theta oscillations in human neocortex. *Science* **313**, 1626-1628.

Canolty RT & Knight RT (2010) The functional role of cross-frequency coupling. *Trends in Cognitive Sciences* **14**, 506-515.

Cavas M & Navarro JF (2006) Effects of selective dopamine D4 receptor antagonist, L-741,741, on sleep and wakefulness in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* **30**, 668-678.

Cohen SJ, Munchow AH, Rios LM, Zhang G, Asgeirsdottir HN & Stackman RW (2013) The Rodent Hippocampus Is Essential for Nonspatial Object Memory. *Current Biology* **23**, 1685-1690.

Colgin LL (2013) Mechanisms and functions of theta rhythms. *Annu Rev Neurosci* **36**, 295-312.

Collingridge GL, Peineau S, Howland JG & Wang YT (2010) Long-term depression in the CNS. *Nat Rev Neurosci* **11**, 459-473.

Cools R & Robbins TW (2004) Chemistry of the adaptive mind. *Philos Trans A Math Phys Eng Sci* **362**, 2871-2888.

Cragg BG (1961) Olfactory and other afferent connections of the hippocampus in the rabbit, rat, and cat. *Experimental Neurology* **3**, 588-600.

Cutsuridis V & Taxidis J (2013) Deciphering the role of CA1 inhibitory circuits in sharp wave-ripple complexes. *Front Syst Neurosci* **7**, 13.

da Silva WC, Kohler CC, Radiske A & Cammarota M (2012) D1/D5 dopamine receptors modulate spatial memory formation. *Neurobiology of Learning and Memory* **97**, 271-275.

Dan Y & Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. *Physiol Rev* **86**, 1033-1048.

Davis NJ, Tomlinson SP & Morgan HM (2012) The role of beta-frequency neural oscillations in motor control. *Journal of Neuroscience* **32**, 403-404.

Defagot MC, Malchiodi EL, Villar MJ & Antonelli MC (1997) Distribution of D4 dopamine receptor in rat brain with sequence-specific antibodies. *Brain Res Mol Brain Res* **45**, 1-12.

Diba K & Buzsáki G (2007) Forward and reverse hippocampal place-cell sequences during ripples. *Nat Neurosci* **10**, 1241-1242.

Diekelmann S & Born J (2010) The memory function of sleep. *Nat Rev Neurosci* **11**, 114-126.

Dong Z, Han H, Cao J, Zhang X & Xu L (2008) Coincident activity of converging pathways enables simultaneous long-term potentiation and long-term depression in hippocampal CA1 network in vivo. *PLoS One* **3**, e2848.

Draguhn A, Traub RD, Schmitz D & Jefferys JG (1998) Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. *Nature* **394**, 189-192.

Dubrovina NI & Red'kina AV (2012) Latent inhibition and extinction of passive avoidance in mice C57BL/6J AND DBA/2J. *Ross Fiziol Zh Im I M Sechenova* **98**, 488-496.

Duffy SN & Nguyen PV (2003) Postsynaptic application of a peptide inhibitor of cAMP-dependent protein kinase blocks expression of long-lasting synaptic potentiation in hippocampal neurons. *Journal of Neuroscience* **23**, 1142-1150.

Dulawa SC, Grandy DK, Low MJ, Paulus MP & Geyer MA (1999) Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. *Journal of Neuroscience* **19**, 9550-9556.

Eichenbaum H, Dudchenko P, Wood E, Shapiro M & Tanila H (1999) The hippocampus, memory, and place cells: is it spatial memory or a memory space? *Neuron* **23**, 209-226.

El Falougy H, Kubikova E & Benuska J (2008) The microscopical structure of the hippocampus in the rat. *Bratisl Lek Listy* **109**, 106-110.

El-Ghundi M, O'Dowd BF & George SR (2007) Insights into the role of dopamine receptor systems in learning and memory. *Reviews in the Neurosciences* **18**, 37-66.

Falzone T, Gelman D, Young J & Rubinstein M (2001) Mice lacking D4 receptors display normal spatial and long-term memory but enhanced working memory. *Journal of Neurochemistry* **78**, 11-11.

Federmeier KD, Kleim JA & Greenough WT (2002) Learning-induced multiple synapse formation in rat cerebellar cortex. *Neurosci Lett* **332**, 180-184.

Floresco SB & Phillips AG (2001) Delay-dependent modulation of memory retrieval by infusion of a dopamine D1 agonist into the rat medial prefrontal cortex. *Behav Neurosci* **115**, 934-939.

Fortin DA, Srivastava T & Soderling TR (2012) Structural modulation of dendritic spines during synaptic plasticity. *Neuroscientist* **18**, 326-341.

Franklin KBJ & Paxinos G (1997) *The mouse brain in stereotaxic coordinates*. San Diego: Academic Press.

Furth KE, Mastwal S, Wang KH, Buonanno A & Vullhorst D (2013) Dopamine, cognitive function, and gamma oscillations: role of D4 receptors. *Front Cell Neurosci* **7**, 102.

Gasbarri A, Packard MG, Campana E & Pacitti C (1994a) Anterograde and retrograde tracing of projections from the ventral tegmental area to the hippocampal formation in the rat. *Brain Res Bull* **33**, 445-452.

Gasbarri A, Verney C, Innocenzi R, Campana E & Pacitti C (1994b) Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: a combined retrograde tracing and immunohistochemical study. *Brain Res* **668**, 71-79.

Geinisman Y (2000) Structural synaptic modifications associated with hippocampal LTP and behavioral learning. *Cerebral Cortex* **10**, 952-962.

Geinisman Y, deToledo-Morrell L & Morrell F (1994) Comparison of structural synaptic modifications induced by long-term potentiation in the hippocampal dentate gyrus of young adult and aged rats. *Ann N Y Acad Sci* **747**, 452-466.

Girardeau G, Benchenane K, Wiener SI, Buzsáki G & Zugaro MB (2009) Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci* **12**, 1222-1223.

Glickstein SB, Hof PR & Schmauss C (2002) Mice lacking dopamine D-2 and D-3 receptors have spatial working memory deficits. *Journal of Neuroscience* **22**, 5619-5629.

Gloveli T, Dugladze T, Rotstein HG, Traub RD, Monyer H, Heinemann U, Whittington MA & Kopell NJ (2005) Orthogonal arrangement of rhythm-generating microcircuits in the hippocampus. *Proc Natl Acad Sci U S A* **102**, 13295-13300.

Haley JE, Schaible E, Pavlidis P, Murdock A & Madison DV (1996) Basal and apical synapses of CA1 pyramidal cells employ different LTP induction mechanisms. *Learn Mem* **3**, 289-295.

Hangya B, Borhegyi Z, Szilagyi N, Freund TF & Varga V (2009) GABAergic neurons of the medial septum lead the hippocampal network during theta activity. *Journal of Neuroscience* **29**, 8094-8102.

Harris KM, Fiala JC & Ostroff L (2003) Structural changes at dendritic spine synapses during long-term potentiation. *Philos Trans R Soc Lond B Biol Sci* **358**, 745-748.

Hayashi ML, Choi SY, Rao BS, Jung HY, Lee HK, Zhang D, Chattarji S, Kirkwood A & Tonegawa S (2004) Altered cortical synaptic morphology and impaired

memory consolidation in forebrain- specific dominant-negative PAK transgenic mice. *Neuron* **42**, 773-787.

He K, Lee A, Song L, Kanold PO & Lee HK (2011) AMPA receptor subunit GluR1 (GluA1) serine-845 site is involved in synaptic depression but not in spine shrinkage associated with chemical long-term depression. *Journal of Neurophysiology* **105**, 1897-1907.

Headley DB & Weinberger NM (2013) Fear conditioning enhances gamma oscillations and their entrainment of neurons representing the conditioned stimulus. *Journal of Neuroscience* **33**, 5705-5717.

Herwerth M, Jensen V, Novak M, Konopka W, Hvalby Ø & Köhr G (2012) D4 dopamine receptors modulate NR2B NMDA receptors and LTP in stratum oriens of hippocampal CA1. *Cerebral Cortex* **22**, 1786-1798.

Hodgkin AL & Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* **117**, 500-544.

Huang YY & Kandel ER (1995) D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. *Proc Natl Acad Sci U S A* **92**, 2446-2450.

Iversen SD & Iversen LL (2007) Dopamine: 50 years in perspective. *Trends in Neurosciences* **30**, 188-193.

Izquierdo I, Medina JH, Vianna MR, Izquierdo LA & Barros DM (1999) Separate mechanisms for short- and long-term memory. *Behav Brain Res* **103**, 1-11.

Jenkinson N & Brown P (2011) New insights into the relationship between dopamine, beta oscillations and motor function. *Trends in Neurosciences* **34**, 611-618.

Jentsch JD & Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)* **146**, 373-390.

Jinno S (2009) Structural organization of long-range GABAergic projection system of the hippocampus. *Front Neuroanat* **3**, 13.

Jones RS (1993) Entorhinal-hippocampal connections: a speculative view of their function. *Trends Neurosci* **16**, 58-64.

Kaibara T & Leung LS (1993) Basal versus apical dendritic long-term potentiation of commissural afferents to hippocampal CA1: a current-source density study. *Journal of Neuroscience* **13**, 2391-2404.

Kampa BM, Letzkus JJ & Stuart GJ (2006) Requirement of dendritic calcium spikes for induction of spike-timing-dependent synaptic plasticity. *J Physiol* **574**, 283-290.

Katz B & Miledi R (1968) The role of calcium in neuromuscular facilitation. *J Physiol* **195**, 481-492.

Kauer JA & Malenka RC (2007) Synaptic plasticity and addiction. *Nature Reviews Neuroscience* **8**, 844-858.

Khakpai F, Zarrindast MR, Nasehi M, Haeri-Rohani A & Eidi A (2013) The Role of Glutamatergic Pathway between Septum and Hippocampus in the Memory Formation. *Excli Journal* **12**, 41-51.

Kitamura T, Saitoh Y, Takashima N, Murayama A, Niibori Y, Ageta H, Sekiguchi M, Sugiyama H & Inokuchi K (2009) Adult neurogenesis modulates the hippocampus-dependent period of associative fear memory. *Cell* **139**, 814-827.

Klausberger T & Somogyi P (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* **321**, 53-57.

Kocsis B, Lee P & Deth R (2013) Enhancement of gamma activity after selective activation of dopamine D4 receptors in freely moving rats and in a neurodevelopmental model of schizophrenia. *Brain Struct Funct*.

Koester HJ & Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proc Natl Acad Sci U S A* **95**, 9596-9601.

Köhler C (1985) A projection from the deep layers of the entorhinal area to the hippocampal formation in the rat brain. *Neurosci Lett* **56**, 13-19.

Kotecha SA, Oak JN, Jackson MF, Perez Y, Orser BA, Van Tol HHM & MacDonald JF (2002) A D2 class dopamine receptor transactivates a receptor tyrosine kinase to inhibit NMDA receptor transmission. *Neuron* **35**, 1111-1122.

Kwon OB, Paredes D, Gonzalez CM, Neddens J, Hernandez L, Vullhorst D & Buonanno A (2008) Neuregulin-1 regulates LTP at CA1 hippocampal synapses through activation of dopamine D4 receptors. *Proc Natl Acad Sci U S A* **105**, 15587-15592.

Laurberg S (1979) Commissural and intrinsic connections of the rat hippocampus. *J Comp Neurol* **184**, 685-708.

Lee I, Hunsaker MR & Kesner RP (2005) The role of hippocampal subregions in detecting spatial novelty. *Behavioral Neuroscience* **119**, 145-153.

Lemaire N, Hernandez LF, Hu D, Kubota Y, Howe MW & Graybiel AM (2012) Effects of dopamine depletion on LFP oscillations in striatum are task- and learning-dependent and selectively reversed by L-DOPA. *Proc Natl Acad Sci U S A* **109**, 18126-18131.

Lemon N & Manahan-Vaughan D (2006) Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *Journal of Neuroscience* **26**, 7723-7729.

Lemon N & Manahan-Vaughan D (2012) Dopamine D1/D5 receptors contribute to de novo hippocampal LTD mediated by novel spatial exploration or locus coeruleus activity. *Cerebral Cortex* **22**, 2131-2138.

Leung LS & Péloquin P (2010) Cholinergic modulation differs between basal and apical dendritic excitation of hippocampal CA1 pyramidal cells. *Cerebral Cortex* **20**, 1865-1877.

Leung LS & Shen B (1995) Long-Term Potentiation at the Apical and Basal Dendritic Synapses of Ca1 after Local Stimulation in Behaving Rats. *Journal of Neurophysiology* **73**, 1938-1946.

Levy R, Ashby P, Hutchison WD, Lang AE, Lozano AM & Dostrovsky JO (2002) Dependence of subthalamic nucleus oscillations on movement and dopamine in Parkinson's disease. *Brain* **125**, 1196-1209.

Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S & Grace AA (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* **31**, 234-242.

Lörincz A, Notomi T, Tamás G, Shigemoto R & Nusser Z (2002) Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. *Nat Neurosci* **5**, 1185-1193.

MaCabe L, Prodi PD & Wörgötter F (2007) Shaping of STDP curve by interneuron and Ca2+ dynamics. *BMC Neuroscience* **July 2007, 8**, 83.

Maccaferri G & Lacaille JC (2003) Interneuron Diversity series: Hippocampal interneuron classifications--making things as simple as possible, not simpler. *Trends Neurosci* **26**, 564-571.

Madroñal N, Gruart A & Delgado-García JM (2009) Differing presynaptic contributions to LTP and associative learning in behaving mice. *Front Behav Neurosci* **3**, 7.

Maier N, Nimmrich V & Draguhn A (2003) Cellular and network mechanisms underlying spontaneous sharp wave-ripple complexes in mouse hippocampal slices. *J Physiol* **550**, 873-887.

Malenka RC (1994) Synaptic plasticity in the hippocampus: LTP and LTD. *Cell* **78**, 535-538.

Manahan-Vaughan D & Braunewell KH (1999) Novelty acquisition is associated with induction of hippocampal long-term depression. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 8739-8744.

Manahan-Vaughan D & Kulla A (2003) Regulation of depotentiation and long-term potentiation in the dentate gyrus of freely moving rats by dopamine D2-like receptors. *Cerebral Cortex* **13**, 123-135.

Matsuzaki M, Honkura N, Ellis-Davies GC & Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**, 761-766.

Missale C, Nash SR, Robinson SW, Jaber M & Caron MG (1998) Dopamine receptors: from structure to function. *Physiol Rev* **78**, 189-225.

Mockett BG, Brooks WM, Tate WP & Abraham WC (2004) Dopamine D1/D5 receptor activation fails to initiate an activity-independent late-phase LTP in rat hippocampus. *Brain Res* **1021**, 92-100.

Moncada D & Viola H (2007) Induction of long-term memory by exposure to novelty requires protein synthesis: evidence for a behavioral tagging. *Journal of Neuroscience* **27**, 7476-7481.

Money KM & Stanwood GD (2013) Developmental origins of brain disorders: roles for dopamine. *Front Cell Neurosci* **7**, 260.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE & Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learn Mem* **9**, 49-57.

Nagerl UV, Eberhorn N, Cambridge SB & Bonhoeffer T (2004) Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* **44**, 759-767.

Nakazawa K, Quirk MC, Chitwood RA, Watanabe M, Yeckel MF, Sun LD, Kato A, Carr CA, Johnston D, Wilson MA & Tonegawa S (2002) Requirement for

hippocampal CA3 NMDA receptors in associative memory recall. *Science* **297**, 211-218.

Navakkode S, Sajikumar S & Frey JU (2007) Synergistic requirements for the induction of dopaminergic D1/D5-receptor-mediated LTP in hippocampal slices of rat CA1 in vitro. *Neuropharmacology* **52**, 1547-1554.

Navakkode S, Sajikumar S, Korte M & Soong TW (2012) Dopamine induces LTP differentially in apical and basal dendrites through BDNF and voltage-dependent calcium channels (vol 19, pg 294, 2012). *Learning & Memory* **19**, 358-358.

Nayak S & Cassaday HJ (2003) The novel dopamine D4 receptor agonist (PD 168,077 maleate): doses with different effects on locomotor activity are without effect in classical conditioning. *Prog Neuropsychopharmacol Biol Psychiatry* **27**, 441-449.

Neves G, Cooke SF & Bliss TV (2008) Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. *Nat Rev Neurosci* **9**, 65-75.

Nevian T, Larkum ME, Polsky A & Schiller J (2007) Properties of basal dendrites of layer 5 pyramidal neurons: a direct patch-clamp recording study. *Nat Neurosci* **10**, 206-214.

Nevian T & Sakmann B (2004) Single spine Ca2+ signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex. *Journal of Neuroscience* **24**, 1689-1699.

Nishiyama M, Hong K, Mikoshiba K, Poo MM & Kato K (2000) Calcium stores regulate the polarity and input specificity of synaptic modification. *Nature* **408**, 584-588.

Nyhus E & Curran T (2010) Functional role of gamma and theta oscillations in episodic memory. *Neurosci Biobehav Rev* **34**, 1023-1035.

O'Keefe J & Conway DH (1978) Hippocampal place units in the freely moving rat: why they fire where they fire. *Exp Brain Res* **31**, 573-590.

Okada K & Okaichi H (2010) Functional cooperation between the hippocampal subregions and the medial septum in unreinforced and reinforced spatial memory tasks. *Behav Brain Res* **209**, 295-304.

Otmakhova NA & Lisman JE (1996) D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. *Journal of Neuroscience* **16**, 7478-7486.

Ozaki I & Hashimoto I (2005) Neural mechanisms of the ultrafast activities. *Clin EEG Neurosci* **36**, 271-277.

Pitkänen A, Pikkarainen M, Nurminen N & Ylinen A (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat - A review. *Parahippocampal Region* **911**, 369-391.

Ponnusamy R, Nissim HA & Barad M (2005) Systemic blockade of D2-like dopamine receptors facilitates extinction of conditioned fear in mice. *Learn Mem* **12**, 399-406.

Ramirez S, Liu X, Lin PA, Suh J, Pignatelli M, Redondo RL, Ryan TJ & Tonegawa S (2013) Creating a False Memory in the Hippocampus. *Science* **341**, 387-391.

Rasch B & Born J (2013) About Sleep's Role in Memory. *Physiological Reviews* **93**, 681-766.

Rossato JI, Bevilaqua LR, Izquierdo I, Medina JH & Cammarota M (2009) Dopamine controls persistence of long-term memory storage. *Science* **325**, 1017-1020.

Sajikumar S & Frey JU (2004) Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. *Neurobiology of Learning and Memory* **82**, 12-25.

Sawaguchi T & Goldmanrakic PS (1991) D1 Dopamine-Receptors in Prefrontal Cortex - Involvement in Working Memory. *Science* **251**, 947-950.

Scheffzük C, Kukushka VI, Vyssotski AL, Draguhn A, Tort ABL & Brankack J (2011) Selective Coupling between Theta Phase and Neocortical Fast Gamma Oscillations during REM-Sleep in Mice. *PLoS One* **6**.

Scoville WB & Milner B (1957) Loss of Recent Memory after Bilateral Hippocampal Lesions. *Journal of Neurology Neurosurgery and Psychiatry* **20**, 11-21.

Seidenbecher T, Laxmi TR, Stork O & Pape HC (2003) Amygdalar and hippocampal theta rhythm synchronization during fear memory retrieval. *Science* **301**, 846-850.

Shires KL, Da Silva BM, Hawthorne JP, Morris RG & Martin SJ (2012) Synaptic tagging and capture in the living rat. *Nat Commun* **3**, 1246.

Smith CC & Greene RW (2012) CNS dopamine transmission mediated by noradrenergic innervation. *Journal of Neuroscience* **32**, 6072-6080.

Sood P, Idris NF, Cole S, Grayson B, Neill JC & Young AM (2011) PD168077, a D(4) receptor agonist, reverses object recognition deficits in rats: potential role

for D(4) receptor mechanisms in improving cognitive dysfunction in schizophrenia. *J Psychopharmacol* **25**, 792-800.

Stramiello M & Wagner JJ (2008) D1/5 receptor-mediated enhancement of LTP requires PKA, Src family kinases, and NR2B-containing NMDARs. *Neuropharmacology* **55**, 871-877.

Strange PG & Neve K (2013) Dopamine receptors. *Tocris Bioscience Scientific Review Series*.

Suh J, Foster DJ, Davoudi H, Wilson MA & Tonegawa S (2013) Impaired hippocampal ripple-associated replay in a mouse model of schizophrenia. *Neuron* **80**, 484-493.

Suh J, Rivest AJ, Nakashiba T, Tominaga T & Tonegawa S (2011) Entorhinal cortex layer III input to the hippocampus is crucial for temporal association memory. *Science* **334**, 1415-1420.

Surmeier DJ (2007) Dopamine and working memory mechanisms in prefrontal cortex. *J Physiol* **581**, 885.

Swant J & Wagner JJ (2006) Dopamine transporter blockade increases LTP in the CA1 region of the rat hippocampus via activation of the D3 dopamine receptor. *Learning & Memory* **13**, 161-167.

Tort AB, Kramer MA, Thorn C, Gibson DJ, Kubota Y, Graybiel AM & Kopell NJ (2008) Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. *Proc Natl Acad Sci U S A* **105**, 20517-20522.

Toth K, Borhegyi Z & Freund TF (1993) Postsynaptic Targets of Gabaergic Hippocampal-Neurons in the Medial Septum Diagonal Band of Broca Complex. *Journal of Neuroscience* **13**, 3712-3724.

Toth K, Freund TF & Miles R (1997) Disinhibition of rat hippocampal pyramidal cells by GABAergic afferents from the septum. *Journal of Physiology-London* **500**, 463-474.

Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L & Deisseroth K (2009) Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* **324**, 1080-1084.

Twyver HBV (1967) Analysis of sleep cycles in the rodent. *The University of Florida*.

Uhlhaas PJ & Singer W (2010) Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* **11**, 100-113.

Varga V, Hangya B, Kranitz K, Ludanyi A, Zemankovics R, Katona I, Shigemoto R, Freund TF & Borhegyi Z (2008) The presence of pacemaker HCN channels identifies theta rhythmic GABAergic neurons in the medial septum. *J Physiol* **586**, 3893-3915.

Vertes RP, Hoover WB & Viana Di Prisco G (2004) Theta rhythm of the hippocampus: subcortical control and functional significance. *Behav Cogn Neurosci Rev* **3**, 173-200.

Wang SH, Redondo RL & Morris RG (2010) Relevance of synaptic tagging and capture to the persistence of long-term potentiation and everyday spatial memory. *Proc Natl Acad Sci U S A* **107**, 19537-19542.

Wang X, Zhong P, Gu ZL & Yan Z (2003) Regulation of NMDA receptors by dopamine D-4 signaling in prefrontal cortex. *Journal of Neuroscience* **23**, 9852-9861.

Wang XB, Yang Y & Zhou Q (2007) Independent expression of synaptic and morphological plasticity associated with long-term depression. *Journal of Neuroscience* **27**, 12419-12429.

Warthen DM, Wiltgen BJ & Provencio I (2011) Light enhances learned fear. *Proc Natl Acad Sci U S A* **108**, 13788-13793.

Weinberger M & Dostrovsky JO (2011) A basis for the pathological oscillations in basal ganglia: the crucial role of dopamine. *Neuroreport* **22**, 151-156.

Weinberger M, Mahant N, Hutchison WD, Lozano AM, Moro E, Hodaie M, Lang AE & Dostrovsky JO (2006) Beta oscillatory activity in the subthalamic nucleus and its relation to dopaminergic response in Parkinson's disease. *Journal of Neurophysiology* **96**, 3248-3256.

Weiss T, Veh RW & Heinemann U (2003) Dopamine depresses cholinergic oscillatory network activity in rat hippocampus. *Eur J Neurosci* **18**, 2573-2580.

Whitlock JR, Heynen AJ, Shuler MG & Bear MF (2006) Learning induces long-term potentiation in the hippocampus. *Science* **313**, 1093-1097.

Wilkerson A & Levin ED (1999) Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats. *Neuroscience* **89**, 743-749.

Winson J (1978) Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science* **201**, 160-163.

Witter MP, Groenewegen HJ, Lopes da Silva FH & Lohman AH (1989) Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. *Prog Neurobiol* **33**, 161-253.

Wójtowicz AM, van den Boom L, Chakrabarty A, Maggio N, Haq RU, Behrens CJ & Heinemann U (2009) Monoamines block kainate- and carbachol-induced gamma-oscillations but augment stimulus-induced gamma-oscillations in rat hippocampus in vitro. *Hippocampus* **19**, 273-288.

Xing B, Kong H, Meng X, Wei SG, Xu M & Li SB (2010) Dopamine D1 but not D3 receptor is critical for spatial learning and related signaling in the hippocampus. *Neuroscience* **169**, 1511-1519.

Yasumoto F, Negishi T, Ishii Y, Kyuwa S, Kuroda Y & Yoshikawa Y (2004) Endogenous dopamine maintains synchronous oscillation of intracellular calcium in primary cultured-mouse midbrain neurons. *Cell Mol Neurobiol* **24**, 51-61.

Yeragani VK, Tancer M, Chokka P & Baker GB (2010) Arvid Carlsson, and the story of dopamine. *Indian J Psychiatry* **52**, 87-88.

Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A & Buzsáki G (1995) Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *Journal of Neuroscience* **15**, 30-46.

Yuen EY & Yan Z (2009) Dopamine D4 receptors regulate AMPA receptor trafficking and glutamatergic transmission in GABAergic interneurons of prefrontal cortex. *Journal of Neuroscience* **29**, 550-562.

Yuen EY & Yan Z (2011) Cellular mechanisms for dopamine D4 receptor-induced homeostatic regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. *J Biol Chem* **286**, 24957-24965.

Yuen EY, Zhong P & Yan Z (2010) Homeostatic regulation of glutamatergic transmission by dopamine D4 receptors. *Proc Natl Acad Sci U S A* **107**, 22308-22313.

Zhang K, Grady CJ, Tsapakis EM, Andersen SL, Tarazi FI & Baldessarini RJ (2004) Regulation of working memory by dopamine D4 receptor in rats. *Neuropsychopharmacology* **29**, 1648-1655.

Zhou Q, Homma KJ & Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* **44**, 749-757.

Zucker RS (1989) Short-term synaptic plasticity. *Annu Rev Neurosci* **12**, 13-31.

Zucker RS & Regehr WG (2002) Short-term synaptic plasticity. *Annu Rev Physiol* **64**, 355-405.

Zweifel LS, Fadok JP, Argilli E, Garelick MG, Jones GL, Dickerson TM, Allen JM, Mizumori SJ, Bonci A & Palmiter RD (2011) Activation of dopamine neurons is critical for aversive conditioning and prevention of generalized anxiety. *Nat Neurosci* **14**, 620-626.