Aus dem Institut für Anatomie II
der Heinrich-Heine-Universität Düsseldorf
Leiterin: Univ.-Prof. Dr. Charlotte von Gall

Effect of the Light/Dark Phase and Constant Light on Spatial Working Memory and Spine Plasticity in the Mouse Hippocampus

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von
Jane Katharina Schröder
2025

Für meine Patenkinder Toni und Fritzi.

Als Inauguraldissertation gedruckt mit der Genehmigung der
Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

gez.:
Dekan: Prof. Dr. med. Nikolaj Klöcker
Erstgutachterin: Univ.-Prof. Dr. phil. nat. Charlotte von Gall
Zweitgutacherin: Jun.-Prof. Dr. rer. nat. Nadine Erlenhardt

Parts of this paper were published:

Schröder, J.K., Abdel-Hafiz, L., Ali, A. A. H., Cousin T. C., Hallenberger, J., Rodrigues Almeida, F., Anstötz, M., Lenz, M., Vlachos, A., von Gall, C., Tundo-Lavalle, F., *Effects of the Light/Dark Phase and Constant Light on Spatial Working Memory and Spine Plasticity in the Mouse Hippocampus.* Cells, 2023. **12**(13) [1]

Zusammenfassung

Bei Säugetieren ist das zirkadiane System, das tageszeitliche Rhythmen in Physiologie und Verhalten steuert, hierarchisch aufgebaut. Ein wesentlicher Bestandteil des zirkadianen Systems ist die sog. innere Uhr, ein inneres Zeitmesssystem, das auch in Abwesenheit rhythmischer Umweltreize Rhythmen mit einer Periodenlänge von etwa 24 Stunden (h) generiert. Rhythmische Umweltstimuli, sog. Zeitgeber, wie der Hell-Dunkel-Zyklus, beeinflussen die Periode und Phase der inneren Uhr. Dauerlicht kann in einer sog. Chronodisruption resultieren, die sich negativ auf die körperliche und mentale Gesundheit auswirken kann. In der modernen Gesellschaft sind Menschen tagsüber oft einer deutlich geringeren Lichtintensität im Vergleich zum Sonnenlicht und nachts einer höheren Lichtintensität im Vergleich zum Mondlicht ausgesetzt. In dieser Studie wurde untersucht, wie sich unterschiedliche Lichtbedingungen auf das Hippocampus-abhängige Arbeitsgedächtnis und die synaptische Plastizität auswirken. Dazu wurden C57BL/6-Mäuse in drei unterschiedlichen Lichtbedingungen gehalten: In der Standardphotoperiode von 12 h Licht und 12 h Dunkelheit (LD), in konstanter Dunkelheit für 38h (DD), sowie in konstanter Beleuchtung für 38h (LL). Während in LD vorwiegend Licht-abhängige Rhythmen untersucht werden können, werden in DD die zirkadianen Rhythmen demaskiert, die auch ohne Zeitgeber ablaufen. In LL sind die zirkadianen Rhythmen hingegen abgeschwächt und das Stresshormon Corticosteron, das auch im zirkadianen System und der synaptischen Plastizität eine wichtige Rolle spielt, erhöht. Zunächst wurde das Hippocampus-abhängige Arbeitsgedächtnisleistung mittels Y-Labyrinth getestet und anschließend die Hippocampi der Mäuse hinsichtlich neuronaler Plastizität untersucht. Zum einen wurde eine Golgi-Cox-Färbung durchgeführt, um die Morphologie der Dornsynapsen (*Spines*) zu untersuchen. Zum anderen wurde Immunfluoreszenz mit Antikörpern gegen Synaptopodin und den ionotropen Glutamat-Rezeptor GluR1 durchgeführt. In LD gab es Unterschiede sowohl im Alternationsverhalten im Y-Labyrinth als auch in der Synaptopodin- und der GluR1-Immunreaktion (IR) zwischen der Licht- und der Dunkelphase. Im Gegensatz dazu gab es diese Unterschiede in DD und LL nicht, obwohl die Bewegungsaktivität rhythmisch war. Dies deutet darauf hin, dass der Tag-Nacht-Unterschied in Funktion und Struktur des Hippocampus stärker vom Licht-/Dunkelwechsel als von der Inaktivität/Aktivität der Mäuse beeinflusst wird. Darüber hinaus waren in LL sowohl Alternationsverhalten im Y-Labyrinth als auch *Spine*-Morphologie sowie Synaptopodin- und GluR1-IR deutlich gegenüber LD verändert. Dies deutet darauf hin, dass konstantes Licht für 38 h bereits zu einer Veränderung in Funktion und Struktur des Hippocampus führt. In DD waren lediglich Synaptopodin- und GluR1-IR deutlich gegenüber LD verändert. Dies deutet darauf hin, dass konstante Dunkelheit für 38 h zu subtilen Veränderungen der synaptischen Plastizität des Hippocampus führt. Es bedarf weiterer Studien um die Übertragbarkeit auf den Menschen zu untersuchen, was vor allem unter den o.g. Aspekten der modernen Lebensweise eine große Relevanz hat.

II Abstract

In mammals, the circadian system, which controls daily rhythms in physiology and behavior, is hierarchically organized. A essential component of the circadian system is the so-called internal clock, an internal time-keeping system that generates rhythms with a period length of about 24 hours (h), even in the absence of rhythmic environmental cues. Rhythmic environmental stimuli, described as zeitgeber, such as the light-dark cycle, influence the period and phase of the internal clock. Constant light exposure can lead to a phenomenon called chronodisruption, which can negatively impact physical and mental health. In modern society, people are often exposed to much lower light intensity during the day compared to the sunlight, and higher light intensity at night compared to the moonlight. In study we investigated how different light conditions affect hippocampus-dependent spatial working memory and synaptic plasticity. Therefore C57BL/6 mice were kept under three different light conditions: the standard photoperiod of 12 hours of light and 12 hours of darkness (LD), constant darkness for 38 hours (DD) and constant light for 38 hours (LL). In LD, mainly light-dependent rhythms can be studied. Circadian rhythms, which occur even without zeitgebers, are unmasked under DD conditions. In LL, circadian rhythms are weakened, and corticosterone, a stress hormone, which also plays an important role in the circadian system and synaptic plasticity, is elevated. First, hippocampus-dependent working memory performance was examined using a Y-maze, then the hippocampi of the mice were analyzed under the aspect of neural plasticity. In order to do so a Golgi-Cox staining was performed to investigate the morphology of dendritic spines. Additionally, immunohistochemistry was conducted with antibodies against synaptopodin and the ionotropic glutamate receptor GluR1. In LD, there were differences in alternations in the Y-maze as well as in synaptopodin and GluR1 immunoreactivity (IR) between the light and dark phase. In contrast, these differences were not observed in DD and LL, although the locomotor activity was still rhythmic. This suggests that the day-night difference in hippocampal function and structure is more strongly driven by the light/dark cycle than by rest/locomotor activity of the mice. Furthermore, in LL alternation behavior in the Y-maze, spine morphology, as well as synaptopodin and GluR1 IR, were significantly altered compared to LD. This suggests that constant light for 38 hours leads to changes in hippocampal function and structure. In DD, only synaptopodin and GluR1 IR were significantly altered compared to LD. This suggests that constant darkness for 38 hours leads to subtle alternations of hippocampal synaptic plasticity. Further studies are needed to investigate the applicability of these findings to humans, which is particularly relevant in light of the aspects of modern lifestyle mentioned above.

III List of abbreviations

ATP	Adenosintriphosphate
ACTH	Adrenocorticotropic Hormone
AMP	Adenosine Monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA	Analysis of Variance
BMAL 1	Brain and muscle arnt-like protein 1
Ca^{2+}	Calcium
CA	Cornu ammonis
cAMP	cyclic AMP, cyclic adenosine monophosphate
CLOCK	circadian locomotor output cycles kaput
CT	Circadian Time
CRH	Corticotropin Releasing Hormone
CRY	Cryptochrome circadian regulator
CREB1	cAMP response element-binding protein 1
D	Dark Phase
DAPI	4',6-Diamidin-2-phenylindol
DG	Dentate gyrus
DD	Dauer Dunkel/Constant Darkness
dHC	Dorsal hippocampus
EC	Enterohinal cortex
EDTA	Ethylene Diamine Tetraacetic Acid
e.g.	Exempli gratia (z.B., zum Beispiel)
ELISA	Enzyme-linked Immunosorbent Assay
ER	Endoplasmatic Reticulum
FD	Former Dark Phase
Fig.	Figure
FL	Former Light Phase
GABA	Gamma-aminobutyric acid
GluR1	Glutamate Receptor Subunit 1 (GluA1)
GRIA	Glutamate Ionotropic Receptor AMPA Type
HDAC	Histone Deacetylase
HPA	Hypothalamic-pituitary-adrenal axis
HC	Hippocampus
i.e.	Id est (d.h., das heißt)
IML	Inner molecular layer/Stratum moleculare internum

ipRGC	Intrinsically Photosensitive Ganglion Cell
IR	Immunreaktion, immunoreactivity
L	Light Phase
LA	Stratum lacunosum-moleculare
LD	Light-Dark Phase
LED	Light Emitting Diode
LL	Constant Light Phase
LSM	Laser Scanning Microscope
LTP	Long term potentiation
NGS	Normal Goat Serum
NMDA	N-Methyl-D-Aspartat
O	Stratum oriens
OML	Outer molecular layer/Stratum moleculare externum
PCR	Polymerase chain reaction
PER	Period circadian regulator
PBS	Phosphate-buffered saline
PSD	Postsynaptic density
PVN	Paraventricular nucleus
RAD	Stratum radiatum
RHT	Retinohypothalamic tract
RyR	Ryanodine Receptor
SA	Spine Apparatus
SCN	Nucleus subchiasmaticus
SEM	Standard Error of the Mean
SERCA	Sarcoplasmatic reticulum calcium ATPase
SP	Synaptopodin
Tab.	Table
vHC	Ventral hippocampus
WB	Western Blot
ZT	Zeitgeber Time

SI Units

h	Hour
kD	Kilo Dalton
lx	Lux
µm	Micrometer
mm	Milimeter

IV Contents

List of figures

List of tables

1 Introduction

1.1 Circadian rhythms

The circadian system was first described in 1729 by the French scientist Jean-Jacques d'Ortous de Mairan who kept a flower (*Mimosa pudica*) in his desk drawer and discovered that the leaves open and close even in the absence of light [2]. "Circadian" is derived from the latin term *"circa"* which means "around" and *"dies"* which means "day". Organisms have an internal clock, which keeps circadian rhythms in behavior and physiology such as rest/activity, hormone secretion, body temperature and digestion [3] even in the absence of rhythmic environmental cues with a period length of about 24h (i.e., circadian), thus summarized as the circadian system. The phase and period of circadian rhythms are synchronized/entrained by rhythmic environmental cues known as "Zeitgebers", with the light/dark cycle being the most significant. In addition to light, other factors such as climate, temperature, and social interaction can also act as Zeitgebers.

In mammals, the central circadian oscillator is the hypothalamic suprachiasmatic nucleus (SCN). The SCN controls subordinate circadian clocks in the brain and body. It generates rhythms in clock gene expression, neuronal firing, and neurotransmitter release [4]. The SCN generates circadian rhythms autonomously but also get input from e.g. the retina [5]. The connection is called the retinohypothalamic tract (RHT, Fig. 1), in which glutamate is the primary neurotransmitter [6]. Via the RHT, the information about light and darkness received by the eye is transmitted to the SCN. The SCN creates rhythmic output signals via the autonomous nervous system and the endocrine system to control subordinate circadian oscillators in other parts of the brain and peripheral organs, such as liver, heart, lungs, urinary bladder and kidneys (Fig. 1) [7]. At the cellular level, all circadian oscillators possess a molecular clock (Fig. 1), composed of transcriptional/translational feedback loop of clock genes.

The process by which circadian rhythms are synchronized to the external light/dark cycle through the detection of light is known as photoentrainment. Additionally, in nocturnal animals like mice, locomotor activity is suppressed by light, a phenomenon known as masking [8]. Both events (photoentrainment and masking) are distinct mechanisms, yet in nature, they function in a complementary manner [9].

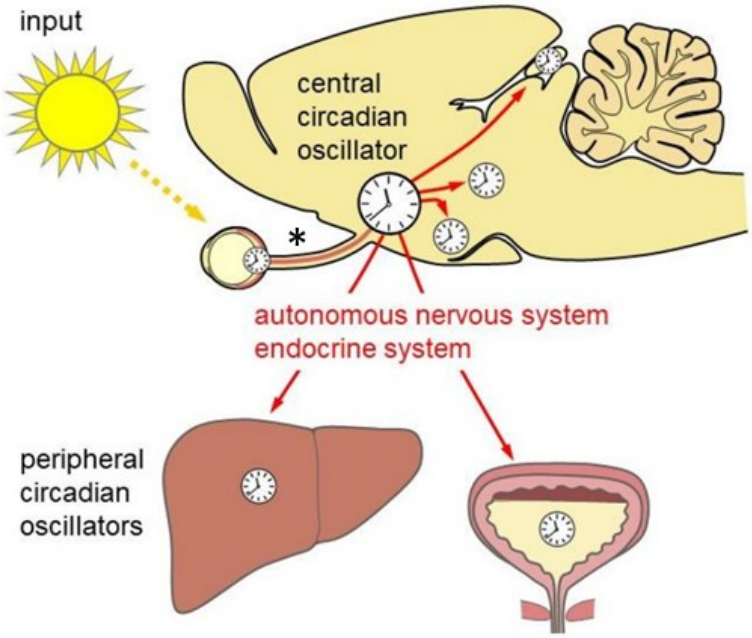


Figure 1: The mammalian circadian system. Information about light is transmitted through the retina via the retinohypothalamic tract (*RHT). It creates output signals via the autonomous nervous system and the endocrine system to central circadian oscillators like other parts of the brain and peripheral circadian oscillators like liver, heart, lungs, urinary bladder and kidneys. From Ali et al. 2020, "The Role of Purinergic Receptors in the Circadian System", International Journal of Molecular Sciences [7]. Reprinted by permission of MDPI.

In mammals, light is detected by three types of retinal cells: rods, cones, and intrinsically photosensitive retinal ganglion cells (ipRGCs) containing the photopigment melanopsin. Light information is processed not only in image formation through the visual system but also in non-image-forming responses, such as circadian photoentrainment and the pupillary light reflex [6]. The axons of the ipRGCs converge to form the RHT, which connects not only to the SCN but also to various hypothalamic regions, including the ventral preoptic area and lateral hypothalamus, involved in regulating rhythms of rest/activity, heart rate, and glucocorticoids. It also extends to other diencephalic regions, such as the habenular nuclei, and basal forebrain areas like the nucleus accumbens and medial amygdala, which play a role in adding emotional components to cognitive functions [7–9]. The elimination of the SCN in neonatal rats prevents the connection between the retina and the SCN leading to arrhythmicity [10]. Conversely, transplantation of SCN tissue into previously ablated animals results in the restoration of rhythmicity [11, 12]. Due to their adjacent location, people with tumors

in the pituitary gland that compress the SCN suffer from sleep disturbances, fluctuations in body temperature and altered hormone profiles [13, 14]. As previously mentioned, the SCN regulates subordinate circadian clocks in the brain and body through neuronal connections and hormones such as melatonin and glucocorticoids. Corticosterone, a glucocorticoid, plays a role in regulating various physiological processes, including glucose homeostasis, electrolyte balance, and stress responses, while also providing rhythmic signals to synchronize subordinate circadian oscillators [15]. In this study, we investigated how different light conditions affect hippocampus-dependent spatial working memory and synaptic plasticity. Therefore C57BL/6 mice were kept under three different light conditions: the standard photoperiod of 12 hours of light and 12 hours of darkness (LD), constant darkness for 38 hours (DD) and constant light for 38 hours (LL). In LD, mainly light-dependent rhythms can be studied. Changes that occur exclusively under LD conditions are likely influenced by additional factors such as light exposure or locomotor activity. If changes observed during LD cycle are also seen under DD conditions, they can be attributed to the circadian clock.

1.2 The molecular clockwork

Molecular processes underlying circadian rhythms are complex, and initial insights into these mechanisms were obtained through studies on the fruit fly (*Drosophila melanogaster*) [16]. In 2017, three chronobiologists were awarded the Nobel Prize in Physiology or Medicine for their discovery of the molecular foundations of circadian rhythms in this species [17]. Almost all brain regions and organs are provided with a molecular clockwork at a cellular level [9], composed of autoregulatory feedback loops of clock genes. Autonomous oscillation is mainly regulated by four clock proteins: CLOCK (circadian locomotor output cycles kaput), BMAL1 (brain and muscle arnt-like protein 1), PER (period circadian regulator) and CRY (cryptochrome circadian regulator). The transcription factors BMAL1 and CLOCK are part of the interacting positive feedback loop, while the proteins PER1, PER2, PER3, CRY1 and CRY2 exert an inhibitory effect on the transcription of clock genes indirectly by suppressing the activating function of BMAL1 and CLOCK. The transcription of period and cryptochrome genes is regulated in a rhythmic manner, driven by BMAL/CLOCK dimers. As PER and CRY proteins accumulate, they inhibit BMAL/CLOCK activity. The timely degradation of PER and CRY proteins then relieves this inhibition, enabling the start of a new 24-hour

cycle [7]. This transcriptional – translational feedback loop exists in most tissues across the body, but only the SCN can sustain persistent circadian molecular and electrophysiological oscillations ex vivo for approximately 9 days [18].

1.3 Chronodisruption

Artificial light has become an indispensable part of modern society, and the key reasons are the following: extended productivity, safety and security, technological integration and aesthetics and cultural impact. Indeed, artificial lighting enables activities to continue beyond natural daylight hours, significantly boosting productivity in workplaces, homes, and educational institutions. Furthermore, it ensures a secure environment, deterring crime and reduces accidents for people and vehicles with streetlights, traffic signals, and illuminated buildings. Artificial light is deeply embedded in modern technology, such as from LED screens to smart homes, enabling devices and systems that we rely on every day. Cities are illuminated with artificial light, creating iconic skylines and enabling night-time cultural activities like concerts, festivals, and nightlife. Despite its benefits, artificial lighting does pose challenges such as energy consumption, light pollution, and potential effects on human circadian rhythms. Exposure to even low-intensity nighttime lighting can substantially disrupt behavioral and physiological processes [19, 20]. Animals in well-lit urban areas have been observed to adjust the timing of their mating calls and delay the onset of reproduction [21, 22]. The suppressed nighttime production of melatonin appears to be the primary cause of circadian rhythm disruption [20, 23, 24]. Melatonin plays a crucial role in regulating the circadian rhythm, serving as a signal for the body's internal clock to align with the natural day-night cycle [20, 25]. Disruption of melatonin production, often caused by exposure to artificial light at night, can lead to disturbances in the circadian rhythm [20, 23, 25]. This misalignment can affect various physiological and behavioral processes, including sleep patterns, hormonal balance, and overall health [24]. A desynchronization of the circadian rhythm can lead to a chronodisruption [26]. Erren and Reiter defined chronodisruption as a "relevant disturbance of the temporal organization or order of physiology, endocrinology, metabolism and behavior" [26]. It is associated with depression, bipolar affective disorder and seasonal affective disorder [27]. Additionally, chronodisruption can worsen inflammatory responses to pathogenic stimuli, disrupt lipid metabolism [24], and even increase the risk of cancer (reviewed in

[28]). Filipski et al. demonstrated that mice with osteosarcoma xenografts, when exposed to a disrupted light/dark cycle, experienced accelerated cancer growth [29]. These mice exhibited not only altered clock gene expression but also disrupted locomotor activity [29, 30]. Chronodisruption has been also linked to several neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease [31-33]. In particular, chronic circadian disruption can lead to the accumulation of toxic proteins in the brain, which is a key feature of many neurodegenerative diseases. For example, in Alzheimer's disease, the misregulation of circadian rhythms has been shown to accelerate the buildup of amyloid plaques, which are implicated in cognitive decline. Similarly, circadian disruption can impair the clearance of neurotoxic substances, exacerbating neurodegeneration. Disruptions of circadian rhythms are frequently observed in older adults, partly due to age-related degeneration of the SCN [34, 35]. However, they are also recognized as symptoms of several neurodegenerative diseases. Hippocampus-dependent learning and cognitive functions are profoundly affected by changes in the circadian clock [36-39]. Research indicates that exposure to an experimental jet lag reduces hippocampal neurogenesis in female hamsters [40]. Disruptions in circadian rhythms can result in a noticeable reduction in hippocampal volume and significantly impair synaptic plasticity, learning, and memory formation [41-43]. Sleep deprivation after learning can influence memory consolidation and cause memory deficits through a negative impact on neuronal connectivity in the hippocampal CA1 region [44]. It also impairs long-term potentiation and the consolidation of hippocampus-dependent memories, as well as glutamate receptor expression and function and hippocampal cAMP signaling [42]. Chronic sleep deprivation even impairs neurogenesis in the hippocampus [42].

As already mentioned above the endogenous factors contributing to chronodisruption may include aging and the resulting changes in clock genes, which can lead to a decoupling of circadian oscillators. This decoupling can occur within different cell populations of the central clock and may also cause a desynchronization between the central and peripheral oscillators [45].

In conclusion, chronodisruptions can also result from exogenous factors that interfere with the body's natural circadian rhythms. These factors, including shift work, jet lag, and light exposure such as artificial light at night or light pollution, can disrupt the synchronization between the internal biological clock and the external environment.

These exogenous factors, especially when experienced over extended periods, can lead through chronodisruption to various health issues, including sleep disorders, mood disturbances, metabolic diseases, and even an increased risk of neurodegenerative conditions. Indeed, it is important to differentiate between acute and chronic chronodisruption. Acute chronodisruption is a short-term disturbance that results in temporary symptoms, whereas chronic chronodisruption is a long-term or ongoing disruption that can lead to more serious health consequences.

The sleep and behavior of nocturnal rodents, such as mice, are highly sensitive to light [46]. From the literature it is well known that chronic exposure of mice to LL (for 3–4 weeks) leads to chronodisruption, resulting in elevation and disruption of circadian rhythms in corticosterone plasma levels [47], disrupted circadian rhythms in locomotor activity [14] and impaired hippocampal long-term potentiation [59]. Chronic LL also leads to depressive- and anxiety-like behavior and impaired spatial memory in mice [60]. However, the role of the circadian system and the impact of light on the structural and functional plasticity of the hippocampus remain poorly understood. To investigate the effects of acute constant light on the hippocampus, mice were exposed to acute constant light (LL) for at least 38 hours in the following study.

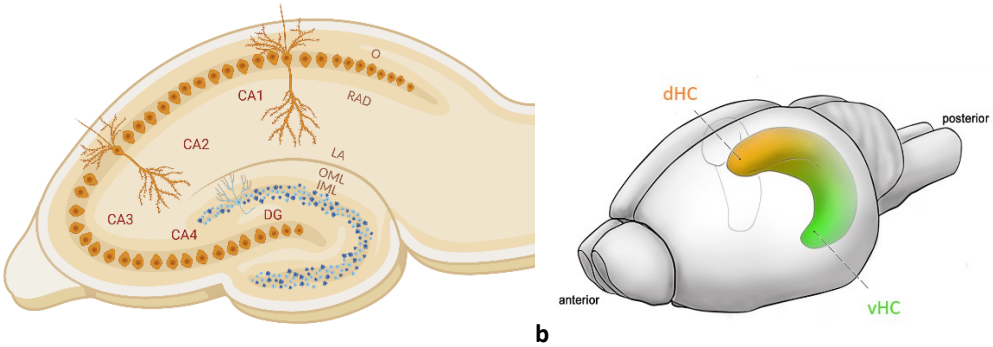
1.4 Anatomy of the hippocampus

One of the best studied brain structures is the hippocampus. It is of great scientific interest due to its important role in memory and learning. It gained significance in 1957 when there was a case study about a patient H. M. whose bilateral medial temporal lobe was removed due to therapy-refractory epilepsy and lost the ability to form new memories. The removal contained the hippocampus formation, the Entorhinal Cortex (EC) and the amygdala [48]. Scoville and Milner then concluded that these structures had to play a crucial role in the brains ability to form memories [49]. Since then, a quantity of anatomical and neurophysiological studies was performed on the hippocampus but it is still not fully understood.

The hippocampus is a paired structure and located in the brain's temporal lobe. It is the biggest part of the Archicortex. In most animals and also humans it is a curved structure which reaches from the lateral cerebral ventricle to the corpus callosum (Fig. 2).

The hippocampus contains the cornu ammonis (CA), dentate gyrus (DC) as well as the subiculum. The hippocampus formation additionally includes the entorhinal cortex (EC)

which is the adjacent cortex region. The EC is located in the parahippocampal gyrus and connected tightly to the hippocampus anatomically as well as functionally [50].



a **b**

Figure 2: The anatomy of the hippocampus

a) The hippocampus contains the dentate gyrus (DG) and the cornu ammonis (CA1-4). The neuronal cell bodies are in the pyramidal layer (stratum pyramidale) of the CA and the granular layer stratum granulosum) of the DG. N. IML = stratum moleulare interne/inner molecular layer, OML = stratum moleculare externe/outer molecular layer, LA = stratum lacunosum/moleculare, RAD = stratum radiatum, O = stratum oriens. Created in BioRender. Schröder, J. (2025) https://BioRender.com/v57e522

b) The hippocampus is a curved structure located in the temporal lobe which is divided into the dorsal hippocampus (dHC - septal) and the ventral hippocampus (vHC - temporal). Image illustrated by Christine Opfermann-Rüngeler, modified by Barr et al. "The Hippocampus as a Neural Link between Negative Affect and Vulnerability for Psychostimulant Relapse", from "The Hippocampus-Plasticity and Functions" edited by Aleš Stuchlik, 2017 [51].

The dorsal (septal) part of the hippocampus is mainly exteroceptive and important for explicit learning. It receives preprocessed sensory information from the EC. It also gets afferences from limbic structures, the hypothalamus and the raphe nuclei [52]. The ventral (temporal) hippocampus is interoceptive, it is involved in stress, emotion and affect-associated learning processes. It gets afferences from limbic structures, the hypothalamus and also the raphe nuclei [53]. Because of these significant functional differences, it made sense to investigate them separately in the following study.

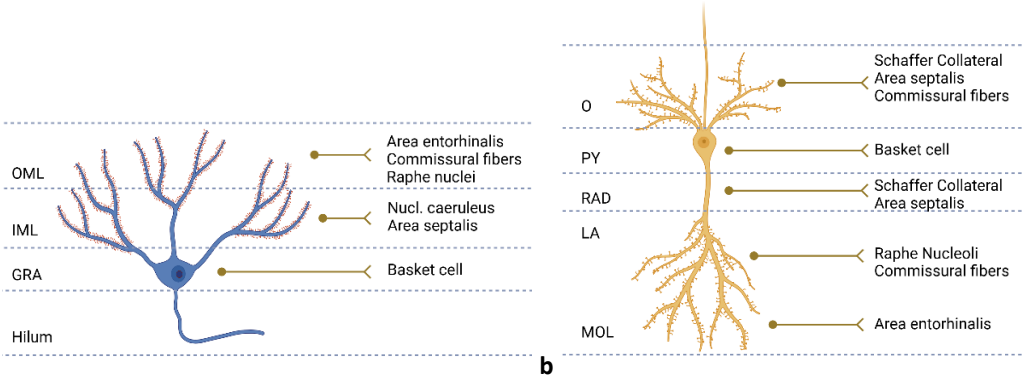


Figure 3: The microanatomy and neuronal connections of the hippocampus. a) Dentate gyrus, b) Cornu ammonis. On the left side of the DG and CA image each, the various layers of this particular area of the HC are depicted. On the right side each example of key afferent pathways and their connectivity are listed. OML = stratum moleculare externe/outer molecular layer, IML = stratum moleculare interne/inner molecular layer, GRA = stratum granulosum. O = stratum oriens, PY = stratum pyramidale, RAD = stratum radiatum, LA, MOL = stratum laconusom-moleculare. Modified from Benninghoff, Drenckhahn, Anatomie, 16. Auflage, Band 2, p. 507 Elsevier [52] a. Created in BioRender. Schröder, J. (2025) https://BioRender.com/l52y483, b. Created in BioRender. Schröder, J. (2025) https://BioRender.com/h86v526

The hippocampus is organized allocortical. Allocortical brain structures like the hippocampus and the rhinencephalon contain three to five layers: molecular layer, pyramidal layer and multiformal layer. This distinguishes it from the younger isocortex, which is built up out of six layers. Due to this microarchitecture the hippocampus is divided into the DG and CA (Fig. 3) [50]. The DG contains granula cells. Their localization forms the different layers stratum multiforme, stratum granulosum, stratum moleculare. The cornu amonis contains a pyramidal cell layer (stratum pyramidale) which is segmented in four sectors CA1 – 4. This was first described by Lorente de Nó and is due to arrangement, morphology and connectivity of the pyramidal cells [50]. The size of the neurons called granula cells are ca. 10µm. Their perikarya lay in the granular layer, their dendrites in the molecular layers. Their axons, the mossy fibers, reach in the polymorph layer to CA3 and CA4 [50]. The CA contains pyramidal cells, which are bigger than the granular cells. Their apical dendrites reach in the apical cell-poor layers stratum radiatum, lacunosum and moleculare (Fig. 3). Their basal dendrites are located in the basal cell-poor layer (stratum oriens) (Tab. 1). CA3 and CA4 pyramidal cells send information to other neurons within the hippocampus formation. Glutamatergic

granular cells and pyramidal cells are called principal cells. Besides 10% of all hippocampal neurons are not-principal cells which are inhibitory interneurons. They influence the excitatory pyramidal cells [50].

SUBREGION	CELL-POOR LAYER (APICAL)	CELL-DENSE LAYER	CELL-POOR LAYER (BASAL)
DENTATE GYRUS	Stratum moleculare	Stratum granulare	Polymorph
CA4		Stratum pyramidale	
CA3	Stratum lacunosum-moleculare Stratum radiatum Stratum lucidum	Stratum pyramidale	Stratum oriens
CA2	Stratum lacunosum-moleculare Stratum radiatum	Stratum pyramidale	Stratum oriens
CA1	Stratum lacunosum-moleculare Stratum radiatum	Stratum pyramidale	Stratum oriens
SUBICULUM	Stratum moleculare	Stratum pyramidale	Stratum oriens

Table 1: Regions and layers of the hippocampus. Modified from „Lehrbuch Histologie", Welsch, 4th edition, 2014, Urban und Fischer, p. 634 [50]. The arrangement of the cells from different (sub-)regions of the hippocampus.

Recent research showed that the connectivity of the CA3 and CA4 region are very similar so that the CA4 region is described as a elongation of the CA3 pyramidal cells rather than an independent region [50].

Many connections and loops are described within the hippocampus formation and to other regions. For simplicity I want to point out the most important ones. Afferences from other brain regions end at the dendrites of the granula cells in the DG and at the dendrites of the pyramid cells in the CA [50]. The axial connection from EC axons via DG to the CA3, CA2 and finally CA1 region is called the trisynaptic loop [54, 55]. The major cortical input is provided by the EC [54]. From the EC there are different paths into the hippocampus, one of them leads to the DG and is called perforant path. From the DG there is a mossy fiber pathway to the CA3 region. The pathway from the CA3 region to the CA1 region is called Schaffer Collateral pathway. Then CA1 is connected back to the EC which makes it a loop [54, 55].

Little is still known about the role of the circadian system and the impact of light on hippocampal synaptic plasticity. Indeed, the hippocampus [56] receives direct or indirect retinal input [9]. There are projections of the retina to the hippocampus via the SCN,

dorsal raphe nuclei (serotoninergic), the locus coeruleus (noradrenergic) and the area septalis (cholinergic) [9, 57-60].

1.5 Synaptic plasticity

For mammals, learning and memory processes are crucial for survival and development. Both are associated with formation, elimination and morphological changes of dendritic spines, summarized as synaptic plasticity [61]. Spines are small protrusions of dendrites that contain the postsynaptic elements of asymmetric synapses (Fig. 4). Asymmetric synapses are characterized by a postsynaptic density (PSD) that is thicker than the presynaptic fraction, while symmetric synapses have a PSD comparable in width to the presynaptic membrane. Consequently, asymmetric and symmetric synapses have been associated with excitatory and inhibitory signaling, respectively. Spines are structurally highly dynamic [62]. Number, shape (filopodia, stubby, thin, mushroom) and size depend on various factors such as neuronal activity, hormonal and environmental stimuli. The maturation status of a dendritic spine is linked to its stability. Stubby spines are immature dendritic spines, characterized by an indistinct neck and head compartment [63]. Thin and mushroom spines are considered mature, as they feature distinct necks and heads. Mushroom spines contain more glutamate receptors than thin spines, making them more stable, as a higher number of glutamate receptors in the spine membrane enhances stability. In fact, mushroom spines are thought to play a role in memory formation and exhibit low mobility. In contrast, thin spines are more mobile, as they can be rapidly modified by changes in neuronal activity and are associated with the process of learning new information. Spine motility depends on actin polymerization and on the levels of intracellular Ca^{2+}, which links the spine plasticity tightly to changes in synaptic activity [64]. Changes in spine morphology are closely related to functional synaptic plasticity such as long-term potentiation (LTP). LTP is a typical form of synaptic plasticity [65]. It describes the long-lasting strengthening between two glutamatergic synapses after high frequency stimulation. Memory in its different forms is distributed across many brain regions [55]. LTP as a part of synaptic plasticity was first identified in the hippocampus [55, 66]. Since then, many studies were performed to analyse electrophysiological, biochemical and molecular aspects of synaptic plasticity in the hippocampus [55, 66]. Long-lasting strengthening of glutamatergic synapses leads to LTP. Hippocampal LTP is initiated by

depolarizing postsynaptic neurons and depends on Ca^{2+} transient mediated by, e.g. NMDA receptors or voltage-gated Ca^{2+} channels. The strength of excitatory synapses is regulated in a homeostatic manner to maintain stable neuronal function in response to changes in network activity. Similarly, synaptic strength increases when there is a sustained decrease in neuronal activity or a reduction in the number of synapses [67, 68]. The compensation is driven by changes in the GluR1 subunit of postsynaptic AMPA glutamate receptors (GluR), as its insertion both enhances synaptic strength and stabilizes the spines by increasing their size [61, 69-71].

1.6 GluR1

In the mammalian central nervous system there are different neurotransmitters such as glutamate, GABA (gamma-aminobutyric acid), glycin, dopamine and serotonin. The major excitatory neurotransmitter of the mammalian cortex is glutamate [72, 73]. Glutamate receptors are located in the cell membrane of i.e. neurons and glia cells. There are ionotropic and metabotropic glutamate receptors with different subgroups each. Ionotropic glutamate receptors are NMDA, AMPA and Kainat receptors. AMPA-receptors are the primary receptors for excitatory synaptic transmission in the central nervous system. AMPAR is the abbreviation for α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid subtype of ionotropic glutamate receptors. Glutamate receptors play an important role in neuronal communication, memory, learning and are a part of synaptic plasticity [61]. AMPA receptors are involved in synaptic plasticity trough 1) synaptic insertion of Ca^{2+} and 2) trough trafficking in and out of the postsynaptic membrane [74]. AMPA-receptors are a tetrameric protein complex containing the four subunits GluR1-4 [69, 75] (also described as GluA1-4). This tetrameric complex forms a functional ion channel permeable for sodium and potassium ions [76]. AMPA-receptor subunits are dimers of dimers meaning a pair of pairs. Composition of AMPA subunits influences their function [77, 78]. Hippocampal pyramidal cells AMPA-receptors are composed of GluR1/2 and GluR2/3 receptors [79]. There are also homomeric receptors that only contain GluR1 subunits [79, 80]. They are in the intracellular reserve pool in the hippocampal CA1 neurons but can be translocated into synapses under LTP conditions [81]. GluR1 and R2 receptors are added to the synapse, and it was shown that the addition is activity-dependent [81]. Guntaupalli et al. found out that male mice with a knock-in mutation in the major GluR1 ubiquitination site exhibit enhanced as well

as deficits in short-term spatial memory and cognitive flexibility [82]. This emphasizes the importance of GluR1 in synaptic plasticity and cognition. Synaptic strength and spine stabilization are achieved through the incorporation of GluR1 into the postsynaptic density (PSD). Additionally, spine size is positively correlated with the stability and strength of synapses [83, 84]. The recruitment of AMPA receptors appears to be associated with synaptopodin [85]. Vlachos et al. showed a higher immunofluorescent intensity for glutamate in synaptopodin-positive spines compared to synaptopodin-negative spines. Furthermore, they showed that glutamate-application near the spine-head of synaptopodin-positive spines produces a larger amplitude of inward currend compared with synapptopodin negative spines. Thus, they stated that the presence of synaptopodin in spines correlates with the quantity and efficacy of glutamate receptors of the AMPA type [85]. Therefore, GluR1 is a suitable target for investigating the influence of different light conditions on synaptic plasticity.

1.7 Synaptopodin

Synaptopodin is an actin-associated protein involved in the actin-based remodeling of spine morphology [86, 87]. In the adult mouse and rat brain, synaptopodin mRNA is expressed in neurons of the olfactory bulb, cerebral cortex, striatum, and hippocampus [88]. Within the hippocampus there is a regional and laminar distribution of synatpopodin protein [88, 89], which will be discussed in the next chapter. However, it is only expressed in a subpopulation (20-30%) of dendritic spines within these regions [90]. In hippocampal principal neurons, synaptopodin expression develops during the first postnatal weeks and increases as spines mature. In the adult brain, it is localized at the spines. Synaptopodin is closely associated with the spine apparatus, which is a complex membranous structure that extends from the dendritic smooth endoplasmic reticulum into the spine neck, forming a unique organelle (Fig. 4) [91, 92]. The spine apparatus is found only in large and mushroom-shaped, mature spines [85, 90] and is involved in the intracellular release or sequestration of Ca^{2+} into the postsynapse [93, 94]. While its precise function is not yet fully understood, the spine apparatus is thought to play a role in delivering molecules into the synapse. Synaptopodin is used as a marker for the spine apparatus, as its regional distribution mirrors the expression pattern of this organelle [94]. Synaptopodin-deficient mice lack the spine apparatus organelle demonstrating that synaptopodin is required for the formation of a spine

apparatus [90]. Furthermore, synaptopodin-deficient mice demonstrate an impaired ability to express LTP *in vitro* and *in vivo* [90, 95, 96] and they show deficits in synaptic plasticity and spatial learning [90]. Mice with targeted deletion of synaptopodin also lack the compensatory increase in synaptic strength in response to denervation [67]. Therefore, synaptopodin is essential for the formation of a spine apparatus and for the regulation of synaptic plasticity. The presence of synaptopodin in spines is linked to stronger responses to glutamate and an increased prevalence of GluR1 in dendritic spines [85, 97, 98]. This is due to the mechanism in which synaptopodin is involved in regulating the AMPA receptor accumulation at excitatory postsynapses (Fig. 4) [67]. Indeed, it is associated with ryanodine receptors (RyR), intracellular Ca^{2+} channels, present throughout the ER in spines and dendrites, which in turn regulate intracellular Ca^{2+} levels and consequently the actin mediated accumulation of GluR1 clusters into dendritic spine heads [92]. In response to a decrease in neuronal network activity, homeostatic synaptic strengthening was shown to be compensated by increased synaptopodin cluster size and the number of spine apparatus stacks [67].

In conclusion, synaptopodin plays a key role in spine head expansion, the recruitment of AMPA receptors, and Ca^{2+} transients in spines. It is therefore a crucial component of the mechanisms that drive changes in synaptic strength and a major protein involved in synaptic plasticity. Furthermore, synaptopodin plays an important role in regulating homeostatic synaptic plasticity. Therefore, synaptopodin is a suitable target for investigating the influence of different light conditions on synaptic plasticity.

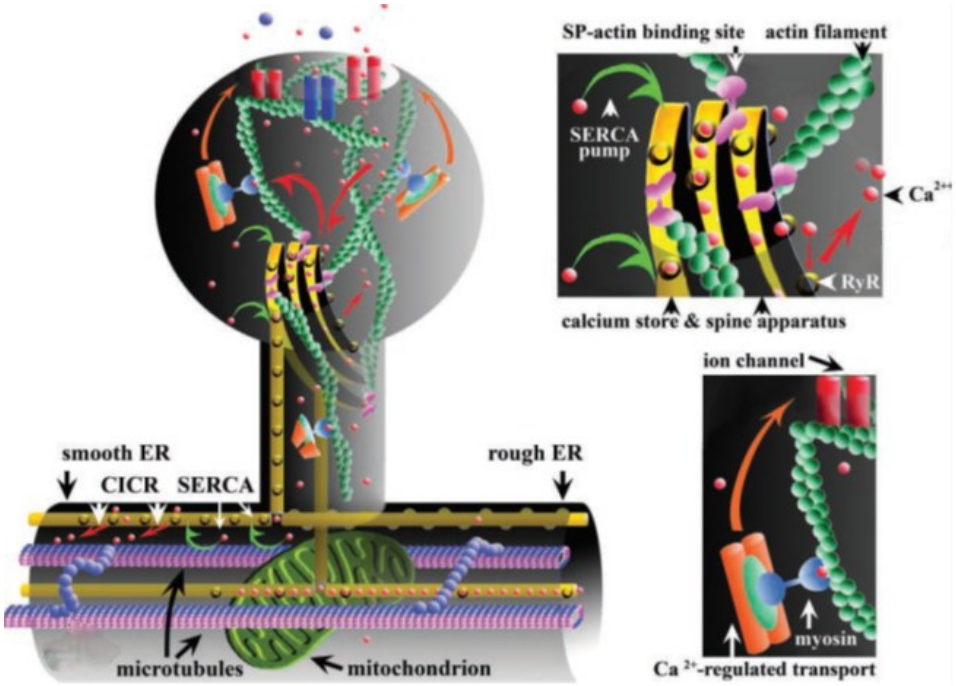


Figure 4: Schematic illustration of a spine, spine apparatus and Ca^{2+} stores. ER= Endoplasmatic recticulum, SERCA = Sarcoplasmic endoplasmic reticulum Ca^{2+} -transporting ATPase, RyR = Ryanodin-Receptor, SP= Synaptopodin. Spines are small protrusions of dendrites that contain the postsynaptic elements of asymmetric synapses. The spine apparatus is a complex membranous structure that extends from the dendritic smooth ER into the spine neck. Smooth ER does not contain ribosomes and is involved in various processes, i.e. Ca^{2+} storage. The smooth ER membrane contains SERCA which increases Ca^{2+} within the ER. Ca^{2+} release from stores is controlled by RyR. The presence of RyR is regulated by synaptopodin. Synaptopodin is an actin binding protein which regulates the Ca^{2+} dependent transport of the glutamate receptors to the PSD via myosin leading to spine head extension [92]. Segal, M., A. Vlachos, and E. Korkotian, Neuroscientist,. 16(2): p. 125-31, Copyright © 2010 by Sage Publications. Reprinted by Permission of Sage Publications.

1.8 Synaptopodin and GluR1 IR within the dHC and vHC (unpublished data)

Little is known about synaptopodin and GluR1 distribution within the hippocampus. Therefore, we used hippocampal slices from mice kept under LD conditions, which mimic natural light-dark cycles. Given the significant functional differences between the dHC and vHC, we compared the immunoreactivity (IR) of synaptopodin and GluR1 in both regions. Additionally, we analyzed the number and size of synaptopodin and GluR1 clusters across the different hippocampal layers. For the DG we analyzed the stratum moleculare, such as the inner molecular layer (IML) and outer molecular layer (OML) as

well as in the CA1 region of the CA stratum lacunosum/moleculare (LA), stratum radiatum (RAD) and stratum oriens (O).

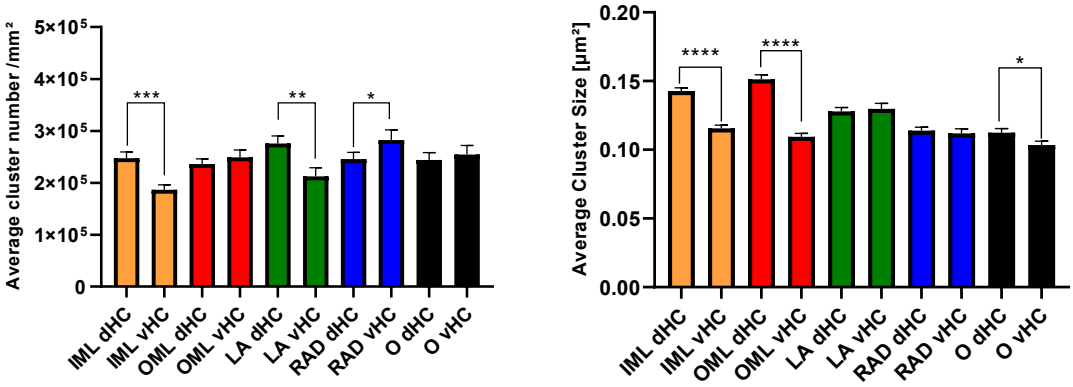


Figure 5: Synaptopodin IR in the dorsal hippocampus (dHC) and ventral hippocampus (vHC). Statistical analysis: Synaptopodin immunopuncta in dHC of one layer was compared to Synapotpodin immunopuncta in vHC in the same layer. IML = str. moleculare interne/inner molecular layer, OML = str. moleculare externe/outer molecular layer, LA = str. lacunosum/moleculare, RAD = str. radiatum, O = str. oriens. Bars represent the standard error of the mean (± SEM) of n= 9 mice per group in LD and DD and n = 15 mice per group in LL. Statistical analysis was performed using Wilcoxon Test (Cluster Number IML OML, LA) or Paired t-Test (Cluster Number RAD, O, Cluster size IML, OML, O) *p > 0.05, ** p > 0.01, *** p < 0.001, p **** < 0.0001.

Synaptopodin cluster number is significantly higher in IML and LA in the dHC compared to the vHC (Fig. 5). In RAD synaptopodin cluster number is higher in vHC than in dHC. For OML and O we did not find any significant differences. Synapopodin cluster size in the DG (IML and OML) and also O were significantly higher in the dHC compared to the vHC. In LA and RAD we did not find any significant differences either (Fig. 5).

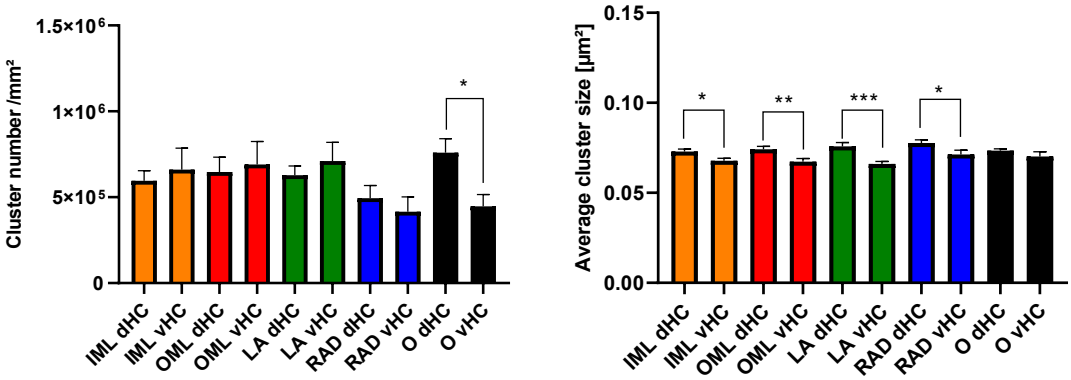


Figure 6: GluR1 IR in the dorsal hippocampus (dHC) and the ventral hippocampus (vHC). Statistical analysis: GluR1 IR in dHC of one layer was compared to GluR1 IR in vHC in the same layer. Statistical analysis was performed using Wilcoxon or Paired t-Test. IML = str. moleculare interne, OML = str. moleculare externe, LA = str. lacunosum/moleculare, RAD = str. radiatum, O = str. oriens. Bars represent the mean ± SEM of n= 9 mice per group in LD and DD and n = 15 mice per group in LL. Statistical analysis was performed using Wilcoxon Test (Cluster number O, cluster size LA) or Paired t-Test (Cluster size IML, RAD) *p > 0.05, ** p > 0.01, *** p < 0.001, p **** < 0.0001.

GluR1 cluster number is significantly higher in dHC in O compared to O in vHC. We do not see any significant differences in the other layers regarding GluR1 cluster number. In IML, OML, LA and RAD dHC cluster size is higher in dHC than in vHC (Fig. 6).

To our knowledge there are only two other studies that analyzed synaptopodin but not GluR1 distribution or IR in the different layers of the dHC and no studies that analyzed synaptopodin or GluR1 distribution in the vHC. Both studies be compared and discussed later [88, 89].

1.9 Influence of the circadian clock on synaptic plasticity

Growing evidence suggests that the circadian system modulates synaptic plasticity, for example in the hippocampi of rats and monkeys excitatory postsynaptic potentials show diurnal rhythms [99]. Chaudhury et al. showed that the magnitude of the enhancement of the population spike was greater in hippocampal LTP in rodents sacrificed in the dark phase compared to the light phase [100]. Additionally the average population spike slope was larger and the amplitude of the population spike increased more during the

night [100]. Population spike is defined as the shift in electrical potential as a consequence of the movement of ions involved in the generation of action potentials [101]. This suggests that synaptic plasticity in the hippocampus is influenced by the circadian clocks (reviewed in [102]). Several genes and proteins linked to synaptic plasticity, such as CREB1 and HDAC, exhibit time-of-day-dependent expression in the hippocampus [35]. Additionally, it was shown, that the density of dendritic spines in the hippocampus of rats fluctuates across the sleep-wake cycle [103]. However, little is known about hippocampal synaptic plasticity in animals kept under different light conditions. This is why we focused on the time-of-day-dependent changes, the effect of acute constant light (38 h) and acute constant darkness (38 h) on hippocampal spines. From the literature is known that in the somatosensory cortex the density of excitatory synapses is higher during the light phase, while the density of inhibitory synapses increases during the dark phase [84, 104]. Interestingly, under DD, the variation in excitatory synapse density was eliminated, whereas the differences in inhibitory synapse density remained. This suggests that excitatory synapses are influenced by light, whereas inhibitory synapses are regulated by the rest/locomotor activity rhythm and/or the circadian clock [104].

1.10 Corticosterone

Glucocorticoids, such as cortisol in humans and corticosterone in rodents, play a crucial role in regulating the circadian rhythm. They themselves are also regulated by the circadian system. The secretion of glucocorticoids follows a diurnal rhythm, which is tightly linked to the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the central circadian clock located in the SCN of the brain. In mice, corticosterone levels follow a diurnal rhythm, peaking during the 9th hour of the resting phase (12-hour light period) [105, 106]. This aligns with findings from other studies showing that glucocorticoid levels peak just before waking in both diurnal and nocturnal species [107]. Corticosterone levels are at their lowest during the rest phase. The synchronization of gene expression and Ca^{2+} signaling in the paraventricular nucleus (PVN) to the light cycle plays a crucial role. These processes are regulated and maintained by PVN circadian neurons, driving the pre-wake increase in corticosterone levels [108]. Glucocorticoids serve as signaling hormones that synchronize peripheral clocks in various tissues with the central clock. They regulate the expression of clock

genes, including PER, CRY, and BMAL1, in peripheral organs, ensuring circadian coherence throughout the body. Glucocorticoid receptors are widely distributed in the brain and body and their activity is regulated in a circadian manner, amplifying the systemic effects of glucocorticoid rhythms. The circadian regulation of glucocorticoids affects numerous physiological processes, including metabolism, immune function, and cognitive performance. Misalignment in glucocorticoid rhythms, as seen in shift work, jet lag, or chronic stress, is linked to metabolic disorders, immune dysregulation, and neurodegenerative conditions [109]. Corticosterone is a hormone essential to the stress response system [109]. Generally, glucocorticoids are implicated in posttraumatic stress disorder, depression, and other stress-related conditions [110, 111]. In the medical field, glucocorticoids are utilized for various purposes, including the treatment of inflammation, asthma, autoimmune diseases, and cerebral edema. Acute or chronic stress can elevate glucocorticoid levels, potentially overriding their natural rhythm and disrupting the circadian system. Persistent elevation of glucocorticoids due to stress or external factors (e.g., light exposure at night) can lead to misalignment between the central and peripheral clocks. Indeed, when mice are exposed to LL for 3–4 weeks, they exhibit elevated plasma corticosterone levels and a disrupted circadian rhythm for this hormone [112, 113]. Constant light represents a highly stressful environment for nocturnal animals, suppressing their locomotor activity rhythms and sleep-wake cycles. It weakens the SCN neural network, desynchronizes clock neurons, and disrupts rhythmic biological processes. Although this can result in arrhythmic behavior, it does not impair the SCN's capacity to generate circadian rhythms [114]. It was shown that a resynchronization of circadian corticosterone levels after a Zeitgeber shift takes more than two weeks and is faster in juvenile female mice compared with adult female mice [115].

1.11 Permission for animal study:

The animal study protocol was approved by the local government, North Rhine-Westphalia State Agency for Nature, Environment, and Consumer Protection, Germany. Approval number: 84-02.04.2013.A358; date of approval: 20 December 2013).

1.12 Aim of the study

In modern society, artificial light has become an essential component of daily life, enabling extended productivity, enhancing safety and security, and supporting technological advancements. However, the natural 24 h light/dark cycle is disrupted by reduced daytime light exposure due to indoor activities, such as office work, and increased evening light exposure from artificial sources and devices like televisions and smartphones. This pervasive use of artificial light raises concerns about its potential adverse effects on health and the environment, including circadian rhythm disruptions and light pollution. The disruption of melatonin production, often referred to as the "sleep hormone," is considered a primary cause of circadian rhythm disturbances, commonly known as chronodisruption [8]. In recent years, a growing number of products have been developed to address this issue, including daylight lamps, blue light filter applications, and "night light" settings on smartphones. The rising demand for these solutions highlights the increasing prevalence of this problem in people's daily lives. Indeed, chronic exposure to light at night can disrupt circadian rhythms, potentially leading to negative effects on overall health, including sleep disorders, metabolic dysfunctions, mental health conditions, and an increased risk of chronic diseases such as cardiovascular disease and cancer [116]. Chronodisruption has also been associated with several neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and Huntington's disease. In these conditions, chronodisruption can contribute to the accumulation of toxic proteins in the different brain areas and hinder the clearance of neurotoxic substances, thereby worsening neurodegeneration. Additionally, chronodisruption is linked to impairments in hippocampus-dependent learning and memory, as well as disruptions in synaptic plasticity [41, 43, 44]. However, there is limited understanding of how the circadian system and light exposure impact the structural and functional plasticity of the hippocampus, a key brain region involved in temporal and spatial learning and memory, which is essential for navigation. In this study we investigated how different light conditions affect hippocampus-dependent spatial working memory and synaptic plasticity. It is known from the literature that in the somatosensory cortex, the density of excitatory synapses is higher during the light phase, while the density of inhibitory synapses is higher during the dark phase [104]. Moreover, under DD, the difference in the density of excitatory synapses was abolished, whereas the differences in the inhibitory synapses persisted, indicating that excitatory synapses are influenced by light, while the inhibitory synapses are driven by the rest/locomotor activity rhythm and/or the circadian

clock [84, 104]. However, there are no studies that investigate hippocampal synaptic plasticity in animals kept under different light conditions. This is why we focused on the effect of constant light and constant darkness on excitatory synapses in the hippocampus. This study has significant social implications, as people in modern society are accustomed to much lower light intensity during the day compared to sunlight, and higher light intensity in the evening compared to moonlight, which can negatively impact the functional and structural plasticity of the hippocampus. The aim of this study is to investigate whether time-of-day-dependent morphological and molecular changes occur in excitatory synapses located on dendritic spines in the hippocampus. To achieve this, mice were kept under standard 12 h light/12 h darkness (LD) conditions. Additionally, we sought to determine whether these changes are driven by light exposure or the circadian clock. To unmask circadian rhythms and to see if DD leads to alterations, a second group of mice was kept under acute constant darkness. A third group was exposed to acute constant light. It is known that nocturnal rodents exposed to chronic constant light exhibit chronodisruption, such as disrupted rhythms in serum corticosterone levels and locomotor activity [117]. However, exposing the mice to acute constant light for at least 38 hours did not result in a disruption of circadian rhythms. Locomotor activity and corticosterone levels, which were analyzed as indicators of circadian rhythms, remained rhythmic. To test hippocampus-dependent spatial working memory, the Y-maze was performed and since the spatial working memory is related to synaptic plasticity of the dHC the structural changes of spine morphology were analyzed via Golgi–Cox-stained hippocampi. To investigate the effects of acute light on hippocampal synaptic plasticity of excitatory synapses at the molecular level, the number and the size of synaptopodin and GluR1- immunoreactive clusters were analyzed.

Our data shows that there are diurnal differences in synaptic plasticity in hippocampus that are dependent rather on light than circadian rhythms, that constant light affects hippocampal function and synaptic plasticity and that constant darkness leads to subtle alternations of hippocampal synaptic plasticity. The collected data should help to understand the origin of the negative influence of the aberrant light on the hippocampus-dependent learning and to prove our hypothesis, that the changes in structural synaptic plasticity under constant light are dependent on glucocorticoids.

2 Effects of the Light/Dark Phase and Constant Light on Spatial Working Memory and Spine Plasticity in the Mouse Hippocampus. Schröder, J.K., Abdel-Hafiz, L., Ali, A. A. H., Cousin T. C., Hallenberger, J., Rodrigues Almeida, F., Anstötz, M., Lenz, M., Vlachos, A., von Gall, C., Tundo-Lavalle, F., Cells, 2023. 12(13)

cells

MDPI

Article

Effects of the Light/Dark Phase and Constant Light on Spatial Working Memory and Spine Plasticity in the Mouse Hippocampus

Jane K. Schröder [1,2], Laila Abdel-Hafiz [1,†], Amira A. H. Ali [1,3,†], Teresa C. Cousin [1], Johanna Hallenberger [1], Filipe Rodrigues Almeida [1], Max Anstötz [1], Maximilian Lenz [4,5], Andreas Vlachos [5], Charlotte von Gall [1,*,‡] and Federica Tundo-Lavalle [1,‡]

[1] Institute of Anatomy II, Medical Faculty, Heinrich-Heine-University, Universitätsstraße 1, 40225 Düsseldorf, Germany; jane.schroeder@ukbonn.de (J.K.S.); laila.abdel-hafiz@med.uni-duesseldorf.de (L.A.-H.); amira.ali@med.uni-duesseldorf.de (A.A.H.A.); tecou100@uni-duesseldorf.de (T.C.C.); johanna.hallenberger@med.uni-duesseldorf.de (J.H.); filipe.rodriguesalmeida@med.uni-duesseldorf.de (F.R.A.); max.anstoetz@med.uni-duesseldorf.de (M.A.); federica.tundo-lavalle@med.uni-duesseldorf.de (F.T.-L.)
[2] Department of Pediatric Hematology and Oncology, Medical Faculty, University of Bonn, Venusberg-Campus 1, 53127 Bonn, Germany
[3] Department of Human Anatomy and Embryology, Faculty of Medicine, Mansoura University, El-Gomhoria St. 1, Mansoura 35516, Egypt
[4] Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover, Germany; lenz.maximilian@mh-hannover.de
[5] Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany; andreas.vlachos@anat.uni-freiburg.de
* Correspondence: charlotte.vongall@med.uni-duesseldorf.de; Tel.: +49-(0)211-8115044; Fax: +49-(0)211-8111615046
† These authors contributed equally to this work.
‡ These authors contributed equally to this work.

Citation: Schröder, J.K.; Abdel-Hafiz, L.; Ali, A.A.H.; Cousin, T.C.; Hallenberger, J.; Rodrigues Almeida, F.; Anstötz, M.; Lenz, M.; Vlachos, A.; von Gall, C.; Tundo-Lavalle, F. Effects of the Light/Dark Phase and Constant Light on Spatial Working Memory and Spine Plasticity in the Mouse Hippocampus. *Cells* **2023**, *12*, 1758. https://doi.org/10.3390/cells12131758

Academic Editor: Jean-Marie Billard

Received: 25 May 2023
Revised: 22 June 2023
Accepted: 28 June 2023
Published: 30 June 2023

Abstract: Circadian rhythms in behavior and physiology such as rest/activity and hormones are driven by an internal clock and persist in the absence of rhythmic environmental cues. However, the period and phase of the internal clock are entrained by the environmental light/dark cycle. Consequently, aberrant lighting conditions, which are increasing in modern society, have a strong impact on rhythmic body and brain functions. Mice were exposed to three different lighting conditions, 12 h light/12 h dark cycle (LD), constant darkness (DD), and constant light (LL), to study the effects of the light/dark cycle and aberrant lighting on the hippocampus, a critical structure for temporal and spatial memory formation and navigation. Locomotor activity and plasma corticosterone levels were analyzed as readouts for circadian rhythms. Spatial working memory via Y-maze, spine morphology of Golgi–Cox-stained hippocampi, and plasticity of excitatory synapses, measured by number and size of synaptopodin and GluR1-immunreactive clusters, were analyzed. Our results indicate that the light/dark cycle drives diurnal differences in synaptic plasticity in hippocampus. Moreover, spatial working memory, spine density, and size and number of synaptopodin and GluR1 clusters were reduced in LL, while corticosterone levels were increased. This indicates that acute constant light affects hippocampal function and synaptic plasticity.

Keywords: circadian; diurnal; synaptopodin; constant darkness; synapse; hippocampus; GluR1 (GluA1); corticosterone

1. Introduction

Rhythms in behavior and physiology such as rest/activity and hormone secretion, which are driven by an internal clock, persist even in the absence of rhythmic environmental cues with a period length of about 24 h (i.e., circadian) [1]. The master circadian clock resides in the hypothalamic suprachiasmatic nucleus (SCN) that controls subordinate circadian clocks in the brain and the body via neuronal connections such as the autonomic nervous system and hormones such as melatonin and glucocorticoids [2]. In particular, glucocorticoids, which play an important role in glucose homeostasis, provide a rhythmic signal for entraining subordinate circadian oscillators [3]. Glucocorticoids are also key regulators in physiological and behavioral response to stress. At the cellular level, circadian rhythms in gene expression are driven by a molecular clockwork composed of autoregulatory feedback loops of clock genes [2]. The phase and period of circadian rhythms are entrained by rhythmic environmental stimuli "Zeitgeber", of which the light/dark cycle is the most important [1]. Moreover, in nocturnal animals such as laboratory mice, bright light is highly aversive and suppresses locomotor activity (masking) [4]. Although circadian photoentrainment and masking are two separate mechanisms, they work in a complementary way in nature [5]. Rods and cones, as well as intrinsically photosensitive ganglion cells (ipRGCs) in the retina, receive the light information and provide the light input not only for image formation via the visual system but also for non-image-forming light responses such as circadian photoentrainment and the pupillary light reflex [6]. The ipRGCs directly project not only to the SCN but also to other regions in the hypothalamus such as the ventral preoptic area and lateral hypothalamus controlling rhythms in rest/activity, heart rate, and glucocorticoids, other diencephalic regions such as habenular nuclei, and basal forebrain regions such as nucleus accumbens and medial amygdala, which provide emotional components for cognitive functions [7–9]. Humans living in modern society experience much lower light intensity (400–600 lx) during the day compared to sunlight (~100,000 lx) and a higher illumination of 100–300 lx in the evening/night compared to moonlight (0.1–0.3 lx) due to the lighting conditions indoors and street illumination [9,10]. Furthermore, light-emitting devices such as TVs, computers, tablets, and smartphones, providing 30–50 lx of light, are increasingly used at night and affect circadian timing and performance [9,11]. In particular, chronic exposure to light at night can result in a disruption of circadian rhythms with potential negative consequences for health in general and mental health in particular [10]. Furthermore, sleep and behavior of mice are highly sensitive to light [12]. In the laboratory, exposure of nocturnal rodents to constant light (LL) leads to a gradually increasing disruption of circadian rhythms such as rhythms in serum corticosterone levels [13] and locomotor activity [14]. However, little is known about the role of the circadian system and the effect of light on structural and functional plasticity of the hippocampus, the key structure in temporal and spatial learning and memory that enables navigation.

Spatial working memory is correlated with hippocampal, particularly the dorsal part, structural synaptic plasticity such as formation, elimination, and morphological changes of dendritic spines [15], i.e., dendritic protrusions containing the postsynaptic part of excitatory synapses [16]. Changes in spine morphology are closely related to functional synaptic plasticity such as long-term potentiation (LTP), a long-lasting strengthening of glutamatergic synapses based on recent patterns of activity. Hippocampal LTP depends on calcium transients mediated by, e.g., NMDA receptors or voltage-gated calcium channels. Moreover, the strength of excitatory synapses is modulated in a homeostatic manner aimed at stabilizing neuronal function upon perturbations in network activity. Consistently, synaptic strength increases in response to persisting reduction in neuronal activity or reduction in the number of synapses, e.g., after denervation [17,18]. This compensatory adjustment is achieved, at least in part, through changes in the GluR1 subunit of postsynaptic AMPA glutamate receptors (GluR) [19–21], presumably via calcium-dependent negative feedback mechanisms [19,22]. Synaptic insertion of GluR1 fulfills two functions, increased synaptic strength and structural stabilization through increased spine size [23].

Synaptopodin is an actin-associated protein and an essential component of the spine apparatus, which is important for the regulation of postsynaptic calcium concentration and for the actin spinoskeleton in mature spines [24–26]. Synaptopodin is associated with structural synaptic plasticity, such as motility, stability, and long-term survival of spines [27–30], and is implicated in homeostatic synaptic plasticity [17,18]. The presence of synaptopodin in spines is associated with larger responses to glutamate and enhanced prevalence of GluR1 in dendritic spines [26,31,32]. Mice with targeted deletion of synaptopodin exhibit a loss in spine apparatus, reduced hippocampal LTP, and spatial learning deficits [25], and they lack the compensatory increase in synaptic strength in response to denervation [17]. Importantly, synaptopodin cluster size corresponds to a homeostatic increase in synaptic strength, e.g., in response to reduced neuronal network activity [17].

There is increasing evidence that the circadian system modulates synaptic plasticity. The density of spines [33] and the number of synapses [34] vary throughout the day in the hippocampus and the somatosensory cortex, respectively. Moreover, multiple genes and proteins associated with synaptic plasticity, including CREB1 and HDAC, show a time-of-day-dependent expression in the hippocampus [35]. Importantly, time-of-day-dependent changes in the number of excitatory synapses located on dendritic spines in the somatosensory cortex may be light-induced as they vanish in constant dark conditions [34].

In this study, we investigated the role of the light/dark cycle and the effect of acute constant light on hippocampal function and structure in mice. The mice were kept under three distinct lighting conditions: standard condition of 12 h light/12 h darkness (LD), acute constant darkness (DD) to unmask circadian rhythms, and acute constant light (LL). Y-maze was performed in the (former) light and dark phases to test hippocampus-dependent spatial working memory. In addition, mice were sacrificed in (former) light and dark phases to analyze spine morphology, and the number and size of immunolabeled synaptopodin and GluR1 clusters in the hippocampus. We found that day/night differences in the performance in the Y-maze and in number and size of synaptopodin clusters were abolished under constant dark conditions although locomotor activity is still rhythmic. Moreover, constant light results in a significant reduction in performance in the Y-maze and in the number and size of synaptopodin clusters, as well as an increase in plasma corticosterone levels. Thus, our data suggest that the light/dark cycle, rather than rest/locomotor activity, has an enhancing effect, while constant light has a deleterious effect on functional and structural hippocampal plasticity.

2. Materials and Methods

2.1. Experimental Animals

Male C57Bl/6J mice (8–12 weeks old) were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and housed in single standard cages in light- and soundproof cabinets with automatic time switch (Beastmaster, Mannheim, Germany). The light intensity during the light phase was 400 lx. All mice had free access to food and water ad libitum. The mice were randomly assigned to three groups, which were kept under different lighting conditions. Group 1 was kept under a standard condition of 12 h light and 12 h darkness (LD) [light on at 6:00 a.m. = zeitgeber time (ZT) 00] for 16 days. Group 2 was kept under LD for 14 days, followed by at least 38 h in constant darkness (DD) [circadian time (CT) 00 = lights on in the former light phase] before being sacrificed. Group 3 was kept under LD for 14 days, followed by at least 38 h in constant light (LL) [disrupted time (DT) 00 = lights on in the former light phase] before being sacrificed. For immunofluorescence and serum analyses, mice were sacrificed every 4 h, for a total of six timepoints, starting 2 h after lights on for LD animals, 2 h after former lights on for DD, and 2 h after former lights off for LL (ZT/CT/DT 02, 06, 10, 14, 18, and 22). Mice were kept in at least 38 h of constant darkness/constant light. For Golgi staining, mice were kept as described above and sacrificed at two different timepoints: 2 h after lights/former lights on and 2 h after lights/former lights off. All animal experiments were approved by the local government, North

Rhine-Westphalia State Agency for Nature, Environment, and Consumer Protection, Germany (Approval number: 84-02.04.2013.A358) and in agreement with the international guidelines on the ethical use of animals [36]. All efforts were exerted to decrease the number and suffering of animals.

2.2. Analysis of Spontaneous Locomotor Activity

The spontaneous locomotor activity of all mice was recorded for 16 days using on-cage infrared movement detectors linked to a monitoring system (Mouse-E-Motion, infra-e-motion, Hamburg, Germany) and analyzed using Clocklab software (R2014a (8.3.0.532), Actimetrics, Wilmette, IL, USA). During the three different lighting conditions, the percentage of activity counts during the light or former light of the total activity was estimated and the relative power of the 24 h period was calculated by fast Fourier transformation.

2.3. Corticosterone Assay

For the corticosterone assay, blood was drawn from the right atrium, collected in EDTA sample tubes, and centrifuged at 4 °C for 15 min at 1400× *g*. Then, 25 µL of the blood plasma was subjected to a corticosterone ELISA Kit 2018 (assay sensitivity 2 to 22 pg/mL ab108821, Abcam, Cambridge, UK) according to the manufacturer's protocol. Optical density was analyzed using a plate reader (Multiskan FC, ThermoScientific, Waltham, MA, USA) at 450 nm. Corticosterone concentration was calculated according to the standard curve.

2.4. Exploration Activity and Hippocampus-Dependent Spatial Working Memory

To assess hippocampus-dependent spatial working memory, mice were subjected to a spontaneous spatial alternation behavior task using the Y-maze. This test utilizes the congenital tendency of mice to explore novelty. The mice frequently visit the relatively novel places that were not visited quite recently when freely allowed to choose among respective alternatives in the Y-maze. This alternation behavior is provoked by spatial novelty, thus requiring the integrity of basic spatial working memory, and is sensitive to hippocampal lesions [37,38]. The maze (length: 35 cm; width: 5.3 cm; height: 8 cm) had black plexiglass walls and an open roof. Three equally spaced arms (4.5 cm wide, 30 cm long, and 15 cm height each arm), labeled A, B, and C, were arranged radially from a triangle-shaped central platform. The Y-maze was surrounded by extra-maze visual cues, e.g., posters. In light phase testing, diffuse illumination by LED lights provided a light density of about 112 lx in the center platform and 97 lx in each of the three arms. In dark phase testing, infrared LED lights illuminated the Y-maze. A camera Gig E monochrome with infrared sensor, mounted 60 cm above the maze, linked to a computer-based tracking software system (Ethovision XT 8, Noldus, Wageningen, The Netherlands), was used for continuous tracking of the mice. Mice were habituated to the experimenter's handling before testing. At the beginning of the experiment, each animal was placed on the central platform and allowed to freely explore the maze for a total trial duration of 7 min. Successful entry into an arm was scored when the animal entered it with all four paws. The number and sequence of arm entries was manually recorded. Between sessions, the apparatus was cleaned with 70% ethanol to eliminate odor cues. The following parameters were considered: (a) total number of entries, as a measure for exploration activity; (b) correct alternations, reflected by the number of triplets/number of triads containing consecutive entries into all three arms without reentries into an arm entered during the last two entries (i.e., CAB, ABC, ACB, etc.); (c) spontaneous alternation, calculated as the number of correct alternations/(total number of entries − 2) × 100%, thus reflecting spatial working memory corrected by the exploration activity. Care was taken to ensure that the mice were stress-free during testing. Experiments were conducted in the light/former light and

dark/former dark phases by an investigator that was blinded to the experimental conditions.

2.5. Immunofluorescence and Quantitative Analysis

Mice were deeply anesthetized using ketamine/xylazine (100 mg/10 mg/kg body weight, respectively) and then transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde using a Ministar Peristaltic Pump (World Precision Instruments, Sarasota, FL, USA). Brains were removed from the skull and post-fixed for 24 h in 4% formalin. Brains were cut using a Vibratome (VT 1200S, Leica, Bensheim, Germany) into 50 µm thick free-floating sections. Coronal sections at Bregma −1.70 mm containing the dorsal hippocampus were selected for dorsal hippocampus staining [39]. Brain sections of all experimental groups were stained simultaneously to avoid variability between staining sessions. Brain sections were incubated for 1 h with 10% normal goat serum (NGS) in PBS containing 0.5% Triton X-100, to reduce unspecific binding of the secondary antibody, and subsequently incubated for 48 h at 4 °C with rabbit anti-synaptopodin (1:1000 SE-19, Sigma Aldrich, St. Louis, MO, USA) or with rabbit anti-GluR1 (1:1000, Cat. #05-855R, Millipore, Burlington, MA, USA) in PBS, 10% NGS, and 0.1% Triton X-100. After washing, sections were incubated for 3 h with Alexa 488-labeled goat anti-rabbit antibody (1:1000, Invitrogen, Waltham, MA, USA) in PBS, 10% NGS, and 0.1% Triton X-100. DAPI (4′,6-diamidino-2-phenylindol) was used to visualize cell nuclei (1:100 in PBS; 10 min). Sections were washed, transferred onto glass microscope slides, and covered with glass coverslips using Fluoromount G (Southern Biotech, Birmingham, AL, USA).

Confocal images were acquired using a Leica TCS SP8 laser-scanning microscope equipped with a Leica 63× glycerol-immersion objective lens (NA 1.3) (Leica, Bensheim, Germany) according to [40]. All high-resolution images (63× objective lens; 6.8× scan zoom) were acquired at tissue levels ~5 µm below the surface. Microscope settings such as detector gain and amplifier were kept constant during the entire image acquisition. The number and average size of synaptopodin and GluR1 clusters were analyzed in different layers of the dentate gyrus (DG) and the cornu ammonis (CA) 1 region of hippocampus. DG: inner (IML) and outer (OML) molecular layer; CA1: Stratum lacunosum (LA), stratum radiatum (RAD), and stratum oriens (O). For each layer, three visual fields (1024 × 1024 pixels, 27.54 µm × 27.54 µm = 758 µm^2) were analyzed. Quantitative analysis was performed using the ImageJ software (version 1.52) package (http://rsb.info.nih.gov/ij (accessed on 4 April 2019)). A constant threshold value in the cell free neuropil was set for all analyses. All particles above this threshold with a minimum size of 0.02 µm and a circularity of 0.01–1.00 µm were defined as clusters and counted automatically ("analyze particles" function of ImageJ software). The means of numbers and the average sizes of clusters of the three frames of each layer were calculated [40,41]. The number of clusters was normalized to mm^2. The values in the respective layers of CA1 and DG were averaged for the (former) light (ZT/CT/DT 02-10) and (former) dark phases (ZT/CT/DT 14-22), respectively. Image acquisition and analysis were performed by an investigator that was blinded to the experimental conditions.

2.6. Golgi Staining

To examine the morphology of dendritic spines, an FD-Rapid GolgiStain kit (FD NeuroTechnologies, Columbia, SC, USA) was used according to the manufacturer's instructions. Briefly, native brains were carefully dissected, washed with cold distilled water, and immersed in a 1:1 premix of solution A and B for 1 day followed by 13 days in solution A and B in darkness. Then, the brains were incubated for 72 h in solution C. Brains were frozen and sectioned on a cryostat (Leica, Bensheim, Germany) into 100 µm coronal sections throughout the rostro-caudal axis of the hippocampus. Sections were mounted onto a drop of solution C on gelatin-coated slides (Marienfeld Superior, Lauda-Königshofen, Germany) and left to dry overnight. Slides were rinsed with cold distilled water followed by impregnation in Golgi staining solution (premix of solution D and solution

E with distilled water) for 10 min. Slides were rinsed again with cold distilled water and then dehydrated in ascending alcohol concentrations (50%, 70%, 95%, and 100%), followed by xylol, and then cover-slipped using Entelan. For the analysis, six independent third-order branches of the apical dendrites of CA1 region pyramidal neurons were selected for each mouse. Z-stack images of dendritic spines were acquired using the 100× oil objective in the bright-field mode of a Keyence microscope (Keyence, Osaka, Japan). Spine morphology analysis was performed using SpineJ plugin of ImageJ software. The spine number in each dendrite segment of 50 μm was counted. The spine density was expressed as the number of spines per 50 μm dendrite length. The spine area and spine length of 20 individual spines in each dendrite segment of 50 μm were analyzed. Image acquisition and analysis were performed by an investigator that was blinded to the experimental conditions.

2.7. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software 8.0.0 (GraphPad Software, San Diego, CA, USA). Data are presented as the mean ± *SEM*. The Gaussian distribution was tested using the Kolmogorov–Smirnov normality test and visualized by QQ plots. If the data passed the normality tests, differences among groups were analyzed by one-way ANOVA followed by Sidak's post hoc test for multiple comparison. If data failed normality tests, the Kruskal–Wallis test with Dunn's multiple comparison test was used. The effects of two parameters, e.g., differences in time curves of corticosterone levels, were analyzed by two-way ANOVA on rank in Origin 2020 (OriginLab Corporation, Northampton, MA, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Spontaneous Locomotor Activity Is Rhythmic under Acute Constant Darkness and Acute Constant Light

To show the effect of the lighting conditions on rhythmic spontaneous locomotor activity, mice were kept for 14 days in a standard photoperiod of 12 h light and 12 h darkness (LD), followed by at least 38 h in LD ($n = 6$), in constant darkness (DD, $n = 6$), or in constant light (LL, $n = 18$) (Figure 1). The mice were considered as rhythmic when the percentage of locomotor activity during the light/former light phase was significantly different from the percentage of activity during the dark/former dark phase, and when the relative power of the 24 h period was not affected.

As expected for nocturnal rodents, mice in LD showed a higher proportion of spontaneous locomotor activity during the dark phase (D) as compared to the light phase (L) ($p < 0.0001$) (Figure 1A,D). Mice kept in DD showed a similar pattern of the locomotor activity with high activity during the former dark phase (FD) and low activity during the former light phase (FL) ($p = 0.0002$) (Figure 1B,D), indicating that the circadian system drives rhythmic activity in the absence of rhythmic zeitgeber. Although the pattern of locomotor activity was slightly changed under LL conditions (Figure 1C), locomotor activity was still significantly higher during the former dark phase than during the former light phase (Figure 1D) ($p = 0.0439$). There were no significant differences between the percentage activity in the L (LD) and FL (LL) or between D (LD) and FD (LL) (Figure 1D). Consistently, there was no difference in the relative power of the 24 h period, a measure for rhythmicity, among the different lighting conditions (Figure 1E). Taken together, this indicated that acute LL does not lead to arrhythmicity.

Although serum corticosterone levels of mice kept in LL are still rhythmic (effect of time, $p < 0.0001$), they are significantly higher than in mice kept in LD (effect of lighting condition, $p < 0.0001$) (Figure 1F). This indicates an activation of the endocrine stress axis in response to acute constant light.

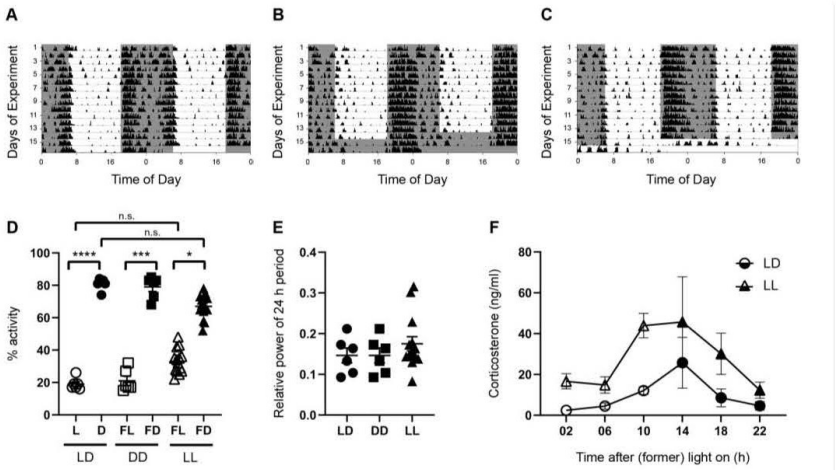


Figure 1. Rhythms in spontaneous locomotor activity and plasma corticosterone levels. Representative double-plotted actograms of spontaneous locomotor activity of mice kept for 14 days in a standard photoperiod of 12 h light (L) and 12 h darkness (D), followed by at least 38 h of (**A**) LD, (**B**) constant darkness (DD), or (**C**) constant light (LL). Black bars indicate activity. Gray boxes indicate periods of darkness. (**D**) Proportion of activity as a percentage of total activity in L or former L (FL) (open symbols), and D or former D (FD) (closed symbols) of mice kept in LD (circles), DD (squares), or LL (triangles). Bars represent the mean ± *SEM* of six mice per group (LD and DD) and 18 mice per group (LL). Kruskal–Wallis test: n.s., not significant; * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$. (**E**) Power analysis of the 24 h period in mice kept in LD, DD, and LL. Bars represent the mean ± *SEM* of six mice per group (LD and DD) and 18 mice per group (LL) (Kruskal–Wallis Test). (**F**) Serum corticosterone levels were rhythmic (effect of time) but higher in mice kept in LL than in mice kept in LD (effect of lighting condition). Two-way ANOVA on rank, $p < 0.0001$. Bars represent the mean ± *SEM* of three mice per timepoint.

3.2. Hippocampus-Dependent Spatial Working Memory Is Affected by the Light Phase and by Acute Constant Light

To show the effect of light on hippocampus-dependent working memory, mice were kept under LD, acute constant darkness (DD), or acute constant light (LL) and subjected to the Y-maze in L, FL, D, or FD (Figure 2, Videos S1–S6). As performance in the Y-maze is highly related to exploration activity, we first analyzed the number of total arm entries (Figure 2A). In mice kept in LD, the number of total arm entries was significantly higher in the dark phase than in the light phase ($p = 0.0123$) (Figure 2A). In contrast, in mice kept in DD and in mice kept in LL, the number of total arm entries was not different between FL and FD (Figure 2A). This suggests that there is no circadian rhythm in exploratory activity, in contrast to spontaneous locomotor activity (Figure 1). The number of total arm entries was significantly higher in FL of mice kept in DD than in L of mice kept in LD ($p = 0.0029$) (Figure 2A), indicating that, in mice kept in LD, the light phase suppressed exploratory activity. Similarly, the number of correct alternations, which is correlated with explorative behavior, was significantly higher in D than in L of mice kept in LD ($p = 0.0409$), not different between FL and FD of mice kept in DD or LL, and significantly lower in FD of mice kept in LL than in D of mice kept in LD ($p = 0.0461$) (Figure 2B). This suggests that light suppresses exploratory behavior-dependent spatial working memory. Moreover, spontaneous alternations, reflecting spatial working memory corrected by exploration activity, are not different between L and D in mice kept in LD but significantly higher in D of mice kept in LD than in FD of mice kept in LL ($p = 0.0217$) (Figure 2C). This indicates that acute constant light impairs spatial working memory, independent of its effect on explorative activity.

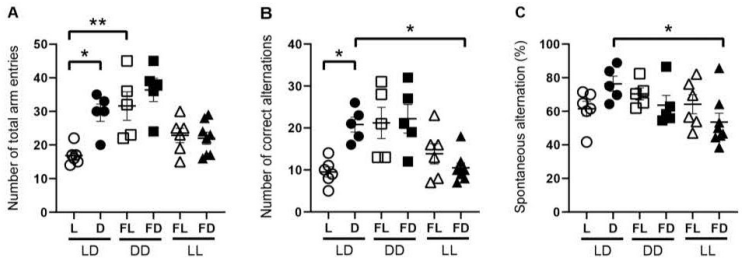


Figure 2. Hippocampus-dependent spatial working memory is affected by light. Exploration activity and correct alternations between arms in the Y-maze of mice kept in a standard photoperiod (LD) of 12 h light (L) and 12 h darkness (D), in constant darkness (DD), or in constant light (LL). The Y-maze test was performed in L or former L (open symbols) and D or former D (closed symbols) of mice kept in LD (circles), DD (squares), or LL (triangles). (**A**) Number of total arm entries reflects exploration activity, (**B**) number of correct alternations reflects the combination of exploration activity and spatial working memory, and (**C**) spontaneous alternation [calculated as the number of correct alternations/(total number of entries − 2) × 100%] reflects spatial working memory corrected for exploration activity. Bars represent the mean ± *SEM* of 6–7 mice per group. One-way ANOVA (**A**,**C**), Kruskal–Wallis test (**B**): * $p < 0.05$; ** $p < 0.01$.

3.3. *Constant Light Affects Hippocampal Spine Morphology*

Spatial working memory is related to synaptic plasticity of the dorsal hippocampus, while the ventral hippocampus is more strongly associated with emotions and affective behavior. To analyze the effects of light on hippocampal spine morphology of the apical dendrites of pyramidal cells in the CA1 region of the dorsal hippocampus, mice were kept for 14 days in LD, followed by at least 38 h LD ($n = 6$), DD ($n = 6$), or LL ($n = 6$), and sacrificed in L, FL, D, or FD (Figure 3). The spine density (Figure 3B), the spine length (Figure 3C), and the spine area (Figure 3D) were not different between L and D in mice kept in LD, and between FL and FD of mice kept in DD or LL. However, in mice kept in LL and sacrificed in FD, spine density ($p = 0.0223$) (Figure 3B), spine length ($p = 0.0272$) (Figure 3C), and spine area ($p = 0.0243$) (Figure 3D) were reduced compared to mice kept in LD and sacrificed in D. Similarly, in mice kept in LL and sacrificed in FL, the spine length ($p = 0.0018$) (Figure 3C) and spine area ($p = 0.0042$) (Figure 3D) were reduced compared to mice kept in LD and sacrificed in L. Thus, constant light leads to a reduction in the density and size of hippocampal dendritic spines.

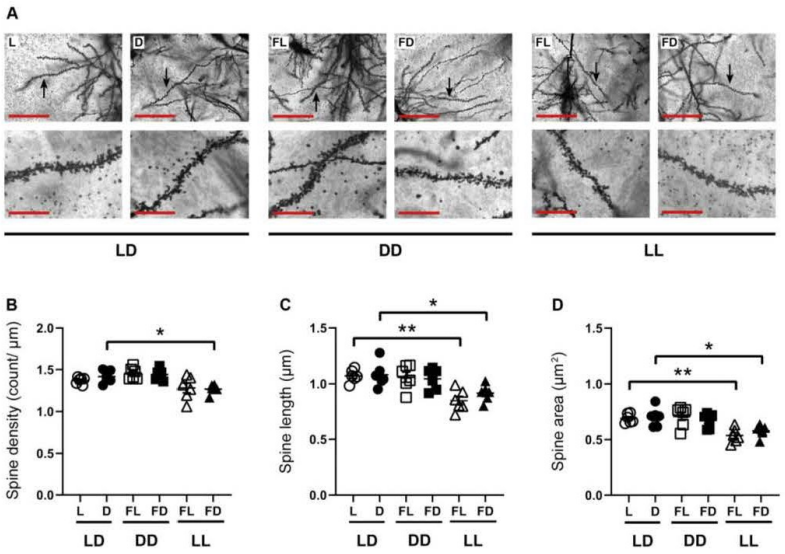


Figure 3. Hippocampal spine morphology is affected by acute constant light. (**A**) Representative microphotographs of Golgi–Cox-stained apical dendritic spines in the dorsal hippocampus of mice kept in a standard photoperiod (LD) of 12 h light (L) and 12 h darkness (D), in constant darkness (DD), or in constant light (LL), sacrificed in L, D, former L (FL), or former D (FD). Scale bar, 50 µm. Higher magnification of the dendrite indicated by the arrow. Scale bar, 12.5 µm. Quantification of (**B**) spine density, (**C**) spine length, and (**D**) spine area of mice kept in LD (circles), DD (squares), or LL (triangles) and sacrificed in L or former L (open symbols) and D or former D (closed symbols). Bars represent the mean ± *SEM* (n = 6 hippocampal slides of three mice per group). One-way ANOVA: * $p < 0.05$; ** $p < 0.01$.

3.4. Hippocampal Synaptopodin Is Affected by the Light/Dark Phase and Acute Constant Light

In order to investigate the effect of light on hippocampal synaptic plasticity at the molecular level, synaptopodin cluster numbers and sizes in both the CA1 region (Figure 4) and the DG (Figure S1) of the dorsal hippocampus were analyzed in mice kept in 12 h light/12 h darkness (LD, n = 18), constant darkness (DD, n = 18), or constant light (LL, n = 30), and sacrificed in L, FL, D, or FD. In the CA1 region of mice kept in LD, the *number* of synaptopodin clusters (Figure 4B) was higher ($p < 0.0001$), while the *size* of synaptopodin clusters (Figure 4C) was lower ($p = 0.0169$) in L than in D. This indicates that in the dorsal hippocampus of mice kept in LD, the light phase led to an increase in the *number* while the dark phase led to an increase in the *size* of synaptopodin clusters. In contrast, in mice kept in DD, the number and size of synaptopodin clusters were not different between FL and FD in both the CA1 region (Figure 4B,C) and the DG (Figure S1B,C). This indicates that there is no circadian rhythm in synaptopodin cluster dynamics in the dorsal hippocampus, in contrast to spontaneous locomotor activity (Figure 1). Moreover, the number of synaptopodin clusters was lower in FL of mice kept in DD than in L of mice kept in LD in both the CA1 ($p = 0.002$) (Figure 4B) and the DG ($p = 0.0003$) (Figure S1B). This is consistent with an induction in synaptopodin cluster number by light. However, in mice kept in LL and sacrificed in FL, the number of synaptopodin clusters was lower compared to mice kept in LD and sacrificed in L in both the CA1 region ($p < 0.0001$) (Figure 4B) and the DG ($p < 0.0001$) (Figure S1B). Similarly, the size of synaptopodin clusters was lower in mice kept in LL and sacrificed in FD compared to mice kept in LD and sacrificed in D in both the CA1 region ($p = 0.0001$) (Figure 4C) and the DG ($p < 0.0001$) (Figure S1C). This

indicates that constant light suppresses the light- and dark-induced increase in synaptopodin cluster number and size, respectively. This is consistent with the suppressive effect of constant light on hippocampus-dependent spatial working memory and spine density.

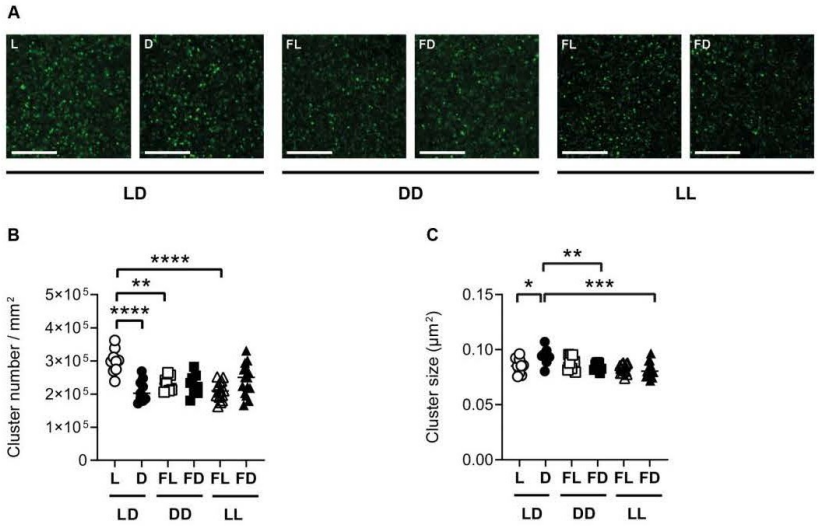


Figure 4. Synaptopodin is affected by the light/dark phase and acute constant light. (**A**) Representative confocal laser microscopic pictures of synaptopodin immunoreactive clusters in the CA1 region of the dorsal hippocampus of mice kept in a standard photoperiod (LD) of 12 h light (L) and 12 h darkness (D), in constant darkness (DD), or in constant light (LL), sacrificed in L, D, former L (FL), or former D (FD). Scale bar, 20 µm. Quantification of (**B**) cluster number and (**C**) cluster size of mice kept in LD (circles), DD (squares), or LL (triangles) and sacrificed in L or FL (open symbols) and D or FD (closed symbols). Bars represent the mean ± *SEM* of $n = 9$ mice per group in LD and DD and $n = 15$ mice per group in LL. One-way ANOVA: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

3.5. Hippocampal GluR1 Is Affected by the Light/Dark Phase and Acute Constant Light

Synaptopodin is functionally and structurally linked to AMPA-type glutamate receptors. To investigate the structural association in response to the different lighting conditions, we analyzed GluR1 in parallel sections of those used for the analysis of synaptopodin clusters. Similar to synaptopodin, in the CA1 region and in the DG of mice kept in LD, the *number* of GluR1 clusters (Figures 5B and S2B) was higher in L than in D (CA1: $p = 0.0255$; DG: $p = 0.0094$). In contrast to synaptopodin, the *size* of GluR1 clusters in the CA1 region of the hippocampus and in the DG was not different between L and D in mice kept in LD (Figures 5C and S2C). This indicates that, in the dorsal hippocampus of mice kept in LD, the light phase led to an increase in the *number* of GluR1 clusters while it had no effect on GluR1 cluster *size*. In mice kept in DD, the number and size of GluR1 clusters were not different between FL and FD in both the CA1 region (Figure 5B,C) and the DG (Figure S2B,C). This indicates that there is no circadian rhythm in GluR1 cluster number in the dorsal hippocampus, in contrast to spontaneous locomotor activity (Figure 1). Similar to synaptopodin, the number of GluR1 clusters in the CA1 region of the hippocampus, as well as in the DG, was lower in FL of mice kept in DD (CA1 and DG: $p < 0.0001$) and in FL of mice kept in LL than in L of mice kept in LD (CA1 and DG: $p < 0.0001$) (Figures 5B and S2B). This suggests that constant lighting conditions lead to a downregulation in the *number* of GluR1 in the hippocampus. In contrast to synaptopodin, the *size* of GluR1 clusters was higher in mice kept in FL (CA1: $p < 0.0001$; DG: $p = 0.0004$) and FD (CA1: $p = 0.0037$; DG: $p = 0.0008$) of mice kept in LL than in the respective phase of mice kept in LD

(Figures 5C and S2C). This increase in GluR1 cluster size may have compensated for the decrease in cluster number in LL.

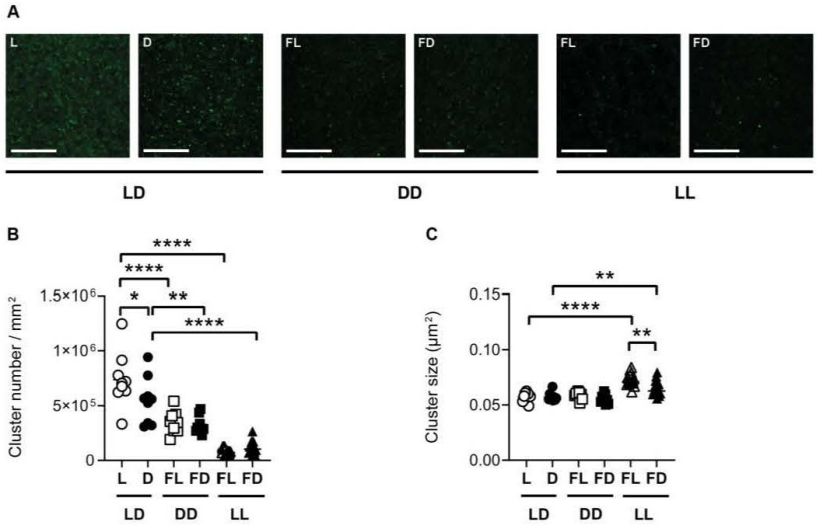


Figure 5. GluR1 is affected by the light/dark phase and acute constant light. (**A**) Representative confocal laser microscopic pictures of GluR1 immunoreactive clusters in the CA1 region of the dorsal hippocampus of mice kept in a standard photoperiod (LD) of 12 h light (L) and 12 h darkness (D), in constant darkness (DD), or in constant light (LL), sacrificed in L, D, former L (FL), or former D (FD). Scale bar, 20 µm. Quantification of (**B**) cluster number and (**C**) cluster size of mice kept for 14 days in LD (circles), DD (squares), or LL (triangles) and sacrificed in L or FL (open symbols) and D or FD (closed symbols). Bars represent the mean ± *SEM* of $n = 9$ mice per group in LD and DD and $n = 15$ mice per group in LL. One-way ANOVA: * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.

4. Discussion

Our study showed light/dark differences in hippocampus-dependent spatial working memory and in morphological equivalents of hippocampal excitatory synapses in mice kept under light/dark conditions. These differences were abolished under constant dark conditions although spontaneous locomotor activity was still rhythmic. The findings suggest that light has a greater influence on hippocampal function and the plasticity of excitatory synapses in the hippocampus than rest/locomotor activity. Furthermore, we showed that acute constant light affects hippocampus-dependent spatial working memory and structural plasticity of excitatory synapses and increases corticosterone levels.

4.1. Effect of Light/Dark Cycle

In mice kept in LD, the number of correct alternations in the Y-maze, which is dependent on hippocampal plasticity and related to exploratory activity [37], was significantly lower in the light phase than in the dark phase. This is consistent with the suppressive effect of light on activity in nocturnal animals [4], differences in effects of light and dark phase testing on behavioral readouts [42,43], and inhibition of behavioral and cognitive performance in the light phase [44] in mice. In contrast, when the mice were kept in DD to unmask circadian rhythms, there was no difference in the number of correct alternations in the Y-maze, although rhythmic spontaneous locomotor activity persisted. This indicates that the hippocampus-dependent spatial working memory is more strongly driven by light than by circadian rhythms and/or rest/locomotor activity.

It is known from the literature that, in the somatosensory cortex, the density of excitatory synapses is higher during the light phase, while the density of inhibitory synapses is higher during the dark phase [34]. Moreover, under DD, the difference in the density of excitatory synapses was abolished, whereas the differences in the inhibitory synapses persisted, indicating that excitatory synapses are influenced by light, while the inhibitory synapses are driven by the rest/locomotor activity rhythm and/or the circadian clock [34]. Therefore, we focused on the effect of the light/dark conditions on morphological equivalents of excitatory synapses. Indeed, in mice housed in LD, we found a higher *number* of synaptopodin and GluR1 clusters in the light phase than during the dark phase. In mice kept under DD conditions, the differences in the number of synaptopodin and GluR1 clusters were abolished, and the number was reduced in FL of DD compared to L of LD. This is consistent with findings in the somatosensory cortex, indicating that in both the isocortex [34] and the allocortex (this study), the density of excitatory synapses is driven by light rather than rest/locomotor activity rhythm and/or the circadian clock. Since synaptopodin is mainly expressed in mature spines of excitatory synapses [25,26], the higher number of synapotopodin clusters during the light phase might also reflect changes in spine maturation. This is consistent with an increased number of mushroom-shaped spines during the light phase in the somatosensory cortex [45], suggesting that the light phase might also promote spine maturation in the hippocampus.

Furthermore, in mice kept in LD, the *size* of synaptopodin clusters, which is related to synaptic strength [17], was higher during the dark phase than during the light phase. Thus, during the dark phase, an upscaling of synaptic strength may compensate for the reduction in the number of mature spines. Importantly in the dark phase, the larger size of synaptopodin clusters may be linked to a better performance in the Y-maze. In the primary motor and somatosensory cortices, the size of the axon–spine interface was also larger during the dark phase when the mice were awake than during the light phase when the mice were sleeping [46]. However, here, the size of the axon–spine interface increased with enforced activity in the light phase, suggesting an impact of activity on scaling of synaptic strength [46]. Unfortunately, since the study by de Vivo et al. [46] did not include constant dark conditions, it is difficult to distinguish the pure effects of light and rest/activity on scaling of synaptic strength in the primary motor and somatosensory cortices. In our study, in DD, there were no differences in the size of synaptopodin clusters and in the performance in the Y-maze between the former dark phase, when animals showed a higher locomotor activity, and the former light phase, when animals were mainly inactive/resting. This suggests that the light/dark cycle rather than the rhythm in rest/locomotor activity drives rhythmic scaling of synaptic strength (as reflected by changes in synaptopodin) in the hippocampus. In this context, it is interesting to speculate whether light/dark-induced changes in network activity account for the diurnal effects in synaptopodin cluster properties or if other factors, such as hormones, are involved in this process.

According to the literature, under LD conditions, the magnitude of hippocampal LTP in C57Bl/6 mice is generally larger during the dark phase than during the light phase [47]. This is consistent with better spatial learning associated with higher synaptic strength in the dark phase. In C3H mice, which produce melatonin in contrast to C57BL/6 mice, hippocampal LTP is still rhythmic in DD, albeit with a reduced amplitude [47]. There is evidence that the residual rhythm of LTP in C3H mice in DD [47] could be attributed to melatonin [48]. Unfortunately, LTP was not analyzed in C57BL/6 mice in DD [47], the mouse strain used in our study. Synaptopodin contributes to hippocampal LTP [25,49,50] primarily via the Ca^{2+}-dependent recruitment of GluR1 to spine synapses [26]. In contrast to the size of synaptopodin clusters, the size of GluR1 cluster was not different between L and D under LD conditions. However, this could be due to the fact that only about 20–30% of spines contain synaptopodin [27]; thus, the effect of light/darkness cannot have such a strong impact on total GluR1. Consistently, there was no difference in the density and length or area of Golgi-stained spines between light and dark phase.

In addition to melatonin, corticosterone, which can be crucially linked to stress responses, seems to be important regulator for hippocampal structural synaptic plasticity [33]. Moreover, stress-induced changes in synaptopodin expression have been reported [51]. However, like spontaneous locomotor activity and body temperature, the rhythm in corticosterone levels is controlled by the circadian clock and, therefore, persists in constant darkness [52,53]. Thus, it is the light phase rather than circadian rhythms that drives the light/dark differences observed in our study. There are several ways that light information can reach the hippocampus. The major input to the hippocampus is through the entorhinal cortex, which projects to both the dentate gyrus and the very distal apical dendrites of pyramidal neurons in the cornu ammonis subfields. The entorhinal cortex, which processes information about space [54] and time, primarily receives input from the visual and association cortices. Moreover, the entorhinal cortex receives input from the post-rhinal area, which integrates spatial and nonspatial visual information and is strongly connected to the amygdala, which provides emotional/affective components. The ipRGCs signal light information via the hypothalamus and brain stem, and via subcortical forebrain regions including the amygdala [7,8]. Neurotransmitters from the brain stem and subcortical forebrain, such as serotonin, norepinephrine, and acetylcholine, are modulators of synaptic plasticity in the hippocampus and, importantly, have a higher basal level during the dark phase compared to the light phase in nocturnal rodents [55], consistent with our hypothesis of the effect of the light/dark phase on synaptic strength. However, we found no apparent differences in the effect of the light/dark phase in the dorsal hippocampus between the CA1 region and the dentate gyrus or among the different layers that could be attributed to a modulation by specific brainstem projections.

4.2. *Effect of Acute Constant Light*

In LL, the plasma levels of corticosterone were increased and the performance in the Y-maze was reduced compared to LD. Moreover, spine density, spine length, and size of hippocampal Golgi-stained spines were reduced in LL compared to LD. This indicates that LL leads to a downscaling in the number and to a change in the morphology of excitatory synapses, thus driving a change in structural plasticity, the central cellular mechanism that underlies memory formation [56]. Spine length and spine area provide information about the maturity and strength of synapses. Mature spines, e.g., mushroom spines, have a larger spine head and contain more glutamate receptors, and they positively correlate with stability and strength of synapses [34,57]. On the other hand, the longer the spine is, the higher the degree of isolation of the spine is from its parent dendrite, which can control the effectiveness of excitatory synapse [58]. This change in structural plasticity is consistent with the suppressive effect of constant light on hippocampus-dependent spatial working memory and with the reduction in density of synaptopodin- and GluR1 clusters in LL. Interestingly, while the size of synaptopodin clusters was reduced, the size of GluR1 was increased in LL. Chronic exposure of mice to LL (for 3–4 weeks) results in elevation and disruption of circadian rhythms in corticosterone plasma levels [13], impairs hippocampal long-term potentiation [59], and disrupts circadian rhythms in locomotor activity [14]. Chronic LL also leads to depressive- and anxiety-like behavior and impaired spatial memory [60]. Similarly, mice kept for 2 weeks in aberrant light conditions of ultrashort days with 3.5 h light/3.5 h darkness (T7 cycle) showed increased corticosterone levels, increased depression-like behavior, and impaired hippocampal LTP [61]. However, it is remarkable that even acute LL used in this study had such significant effects on glucocorticoid levels, spatial memory, and hippocampal structural plasticity. Importantly, locomotor activity and corticosterone levels are still rhythmic in acute LL, indicating that the observed changes were not due to a general disruption of circadian rhythms but more likely to the increase in corticosterone levels. This is consistent with the important role of corticosterone for hippocampal structural synaptic plasticity [33] and with the stress-induced higher levels of glucocorticoids impairing spatial learn-

ing [62]. Moreover, treatment with the serotonin-reuptake inhibitor fluoxetine rescues increased corticosterone levels, as well as learning and LTP deficits induced by T7 cycle, supporting the importance of glucocorticoids in the deterioration of hippocampal function/synaptic plasticity due to aberrant light conditions [61]. However, further studies are required to prove the hypothesis that the changes in structural synaptic plasticity under LL are dependent on glucocorticoids. Interestingly, ablation of ipRGCs rescues the spatial learning deficits and the hippocampal LTP decrement induced by the T7 cycle, suggesting that the negative influence of the aberrant light cycles on hippocampus-dependent learning requires ipRGCs.

4.3. Limitations and Outlook

In this study, we used only male mice because the female estrous cycle adds a complex additional variable that is known to affect synaptic plasticity and hippocampal functions [63]. However, it is important to include female mice in future studies to investigate the interaction of sex hormones and light on structural and functional synaptic plasticity.

5. Conclusions

Our study showed that the light/dark cycle rather than rest/locomotor activity drives diurnal differences in spatial working memory and plasticity of excitatory hippocampal synapses. Moreover, acute constant light has a negative impact on spatial working memory and on the density of hippocampal excitatory synapses. Although studies on nocturnal mice have limited translational value in humans, our study may suggest that the predominant indoor activity during the day with low light intensity, as well as artificial nocturnal light, both of which are increasing in modern society, is detrimental for functional and structural plasticity of the hippocampus.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cells12131758/s1: Figure S1. Synaptopodin clusters in the dentate gyrus (DG) of the dorsal hippocampus are affected by light; Figure S2. GluR1 clusters in the dentate gyrus (DG) of the dorsal hippocampus are affected by light; Video S1. Hippocampus-dependent spatial working memory in Y-maze during light phase of LD; Video S2. Hippocampus-dependent spatial working memory in Y-maze during dark phase of LD; Video S3. Hippocampus-dependent spatial working memory in Y-maze during former light phase of DD; Video S4. Hippocampus-dependent spatial working memory in Y-maze during former dark phase of DD; Video S5. Hippocampus-dependent spatial working memory in Y-maze during former light phase of LL; Video S6. Hippocampus-dependent spatial working memory in Y-maze during former dark phase of LL.

Author Contributions: Conceptualization, C.v.G., A.V., M.L., and F.T.-L.; methodology, J.K.S., L.A.-H., A.A.H.A., T.C.C., J.H., F.R.A. and F.T.-L.; validation, F.T.-L.; formal analysis, J.K.S., L.A.-H., T.C.C., M.A. and F.T.-L.; data curation, J.K.S., L.A.-H., T.C.C. and F.T.-L.; writing—original draft preparation, J.K.S., L.A.-H., A.A.H.A. and F.T.-L.; writing—review and editing, C.v.G. and F.T.-L.; visualization, F.T.-L.; supervision, F.T.-L.; project administration, C.v.G. All authors have read and agreed to the published version of the manuscript.

Funding: This publication is supported by Heinrich Heine University and University Hospital, Düsseldorf, Germany.

Institutional Review Board Statement: The animal study protocol was approved by the local government, North Rhine-Westphalia State Agency for Nature, Environment, and Consumer Protection, Germany (approval number: 84-02.04.2013.A358; date of approval: 20 December 2013).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data will be made available by the corresponding author on reasonable request.

Acknowledgments: The authors thank Hanna Bellert, Ralf Faßbender, Angelika Hallenberger, and Ursula Lammersen for technical support and Ilenia Tundo for graphic design.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Korf, H.-W.; von Gall, C. Circadian Physiology. In *Neuroscience in the 21st Century*, 3rd ed.; Pfaff, D.W., Volkow, N.D., Eds.; Springer Science + Business Media: Berlin/Heidelberg, Germany, 2021.
2. Reppert, S.M.; Weaver, D.R. Coordination of circadian timing in mammals. *Nature* **2002**, *418*, 935–941. https://doi.org/10.1038/nature00965.
3. Balsalobre, A.; Brown, S.A.; Marcacci, L.; Tronche, F.; Kellendonk, C.; Reichardt, H.M.; Schu¨tz, G.; Schibler, U. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* **2000**, *289*, 2344–2347. https://doi.org/10.1126/science.289.5488.2344.
4. Mrosovsky, N. Masking: History, definitions, and measurement. *Chronobiol. Int.* **1999**, *16*, 415–429. https://doi.org/10.3109/07420529908998717.
5. von Gall, C. The Effects of Light and the Circadian System on Rhythmic Brain Function. *Int. J. Mol. Sci.* **2022**, *23*, 2778. https://doi.org/10.3390/ijms23052778.
6. Hattar, S.; Liao, H.W.; Takao, M.; Berson, D.M.; Yau, K.W. Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. *Science* **2002**, *295*, 1065–1070. https://doi.org/10.1126/science.1069609.
7. Provencio, I.; Rodriguez, I.R.; Jiang, G.; Hayes, W.P.; Moreira, E.F.; Rollag, M.D. A novel human opsin in the inner retina. *J. Neurosci.* **2000**, *20*, 600–605. https://doi.org/10.1523/JNEUROSCI.20-02-00600.2000.
8. Hattar, S.; Kumar, M.; Park, A.; Tong, P.; Tung, J.; Yau, K.-W.; Berson, D.M. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J. Comp. Neurol.* **2006**, *497*, 326–349. https://doi.org/10.1002/cne.20970.
9. Rumanova, V.S.; Okuliarova, M.; Zeman, M. Differential Effects of Constant Light and Dim Light at Night on the Circadian Control of Metabolism and Behavior. *Int. J. Mol. Sci.* **2020**, *21*, 5478. https://doi.org/10.3390/ijms21155478.
10. Walker, W.H., 2nd; Walton, J.C.; DeVries, A.C.; Nelson, R.J. Circadian rhythm disruption and mental health. *Transl. Psychiatry* **2020**, *10*, 28. https://doi.org/10.1038/s41398-020-0694-0.
11. Chinoy, E.D.; Duffy, J.F.; Czeisler, C.A. Unrestricted evening use of light-emitting tablet computers delays self-selected bedtime and disrupts circadian timing and alertness. *Physiol. Rep.* **2018**, *6*, e13692. https://doi.org/10.14814/phy2.13692.
12. González, M.M.C. Dim Light at Night and Constant Darkness: Two Frequently Used Lighting Conditions That Jeopardize the Health and Well-being of Laboratory Rodents. *Front. Neurol.* **2018**, *9*, 609. https://doi.org/10.3389/fneur.2018.00609.
13. Claustrat, B.; Valatx, J.L.; Harthe, C.; Brun, J. Effect of constant light on prolactin and corticosterone rhythms evaluated using a noninvasive urine sampling protocol in the rat. *Horm. Metab. Res.* **2008**, *40*, 398–403. https://doi.org/10.1055/s-2008-1065330.
14. Mrosovsky, N. Aschoff's rule in retinally degenerate mice. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **2003**, *189*, 75–78. https://doi.org/10.1007/s00359-002-0381-z.
15. Raven, F.; Van der Zee, E.A.; Meerlo, P.; Havekes, R. The role of sleep in regulating structural plasticity and synaptic strength: Implications for memory and cognitive function. *Sleep Med. Rev.* **2018**, *39*, 3–11. https://doi.org/10.1016/j.smrv.2017.05.002.
16. Yuste, R.; Denk, W. Dendritic spines as basic functional units of neuronal integration. *Nature* **1995**, *375*, 682–684. https://doi.org/10.1038/375682a0.
17. Vlachos, A.; Ikenberg, B.; Lenz, M.; Becker, D.; Reifenberg, K.; Bas-Orth, C.; Deller, T. Synaptopodin regulates denervation-induced homeostatic synaptic plasticity. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 8242–8247. https://doi.org/10.1073/pnas.1213677110.
18. Vlachos, A.; Becker, D.; Jedlicka, P.; Winkels, R.; Roeper, J.; Deller, T. Entorhinal denervation induces homeostatic synaptic scaling of excitatory postsynapses of dentate granule cells in mouse organotypic slice cultures. *PLoS ONE* **2012**, *7*, e32883. https://doi.org/10.1371/journal.pone.0032883.
19. Turrigiano, G. Homeostatic synaptic plasticity: Local and global mechanisms for stabilizing neuronal function. *Cold Spring Harb. Perspect. Biol.* **2012**, *4*, a005736. https://doi.org/10.1101/cshperspect.a005736.
20. O'Brien, R.J.; Kamboj, S.; Ehlers, M.D.; Rosen, K.R.; Fischbach, G.D.; Huganir, R.L. Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* **1998**, *21*, 1067–1078. https://doi.org/10.1016/s0896-6273(00)80624-8.
21. Sutton, M.A.; Ito, H.T.; Cressy, P.; Kempf, C.; Woo, J.C.; Schuman, E.M. Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* **2006**, *125*, 785–799. https://doi.org/10.1016/j.cell.2006.03.040.
22. Pozo, K.; Goda, Y. Unraveling mechanisms of homeostatic synaptic plasticity. *Neuron* **2010**, *66*, 337–351. https://doi.org/10.1016/j.neuron.2010.04.028.
23. Kopec, C.D.; Real, E.; Kessels, H.W.; Malinow, R. GluR1 links structural and functional plasticity at excitatory synapses. *J. Neurosci. Off. J. Soc. Neurosci.* **2007**, *27*, 13706–13718. https://doi.org/10.1523/JNEUROSCI.3503-07.2007.
24. Mundel, P. Synaptopodin: An Actin-associated Protein in Telencephalic Dendrites and Renal Podocytes. *J. Cell Biol.* **1997**, *139*, 193–204. https://doi.org/10.1083/jcb.139.1.193.
25. Deller. Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10494–10499. https://doi.org/10.1073/pnas.1832384100.
26. Vlachos, A.; Korkotian, E.; Schonfeld, E.; Copanaki, E.; Deller, T.; Segal, M. Synaptopodin regulates plasticity of dendritic spines in hippocampal neurons. *J. Neurosci.* **2009**, *29*, 1017–1033. https://doi.org/10.1523/JNEUROSCI.5528-08.2009.

27. Bas Orth, C.; Vlachos, A.; Del Turco, D.; Burbach, G.J.; Haas, C.A.; Mundel, P.; Feng, G.; Frotscher, M.; Deller, T. Lamina-specific distribution of Synaptopodin, an actin-associated molecule essential for the spine apparatus, in identified principal cell dendrites of the mouse hippocampus. *J. Comp. Neurol.* **2005**, *487*, 227–239. https://doi.org/10.1002/cne.20539.
28. Deller, T. Actin-associated protein Synaptopodin. *J. Comp. Neurol.* **2000**, *418*, 164–181. https://doi.org/10.1002/(sici)1096-9861(20000306)418:2<164::aid-cne4>3.0.co;2-0.
29. Deller, T. Potential Role of Synaptopodin in Spine Motility by Coupling Actin to the Spine Apparatus. *Hippocampus* **2000**, *10*, 569–581. https://doi.org/10.1002/1098-1063(2000)10:5<569::AID-HIPO7>3.0.CO;2-M.
30. Yap, K.; Drakew, A.; Smilovic, D.; Rietsche, M.; Paul, M.H.; Vuksic, M.; Del Turco, D.; Deller, T. The actin-modulating protein synaptopodin mediates long-term survival of dendritic spines. *eLife* **2020**, *9*, e62944. https://doi.org/10.7554/eLife.62944.
31. Matsuzaki, M.; Ellis-Davies, G.C.; Nemoto, T.; Miyashita, Y.; Iino, M.; Kasai, H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat. Neurosci.* **2001**, *4*, 1086–1092. https://doi.org/10.1038/nn736.
32. Matsuzaki, M.; Honkura, N.; Ellis-Davies, G.C.; Kasai, H. Structural basis of long-term potentiation in single dendritic spines. *Nature* **2004**, *429*, 761–766. https://doi.org/10.1038/nature02617.
33. Ikeda, M.; Hojo, Y.; Komatsuzaki, Y.; Okamoto, M.; Kato, A.; Takeda, T.; Kawato, S. Hippocampal spine changes across the sleep-wake cycle: Corticosterone and kinases. *J. Endocrinol.* **2015**, *226*, M13–M27. https://doi.org/10.1530/joe-15-0078.
34. Jasinska, M.; Grzegorczyk, A.; Woznicka, O.; Jasek, E.; Kossut, M.; Barbacka-Surowiak, G.; Litwin, J.A.; Pyza, E. Circadian rhythmicity of synapses in mouse somatosensory cortex. *Eur. J. Neurosci.* **2015**, *42*, 2585–2594. https://doi.org/10.1111/ejn.13045.
35. Debski, K.J.; Ceglia, N.; Ghestem, A.; Ivanov, A.I.; Brancati, G.E.; Bröer, S.; Bot, A.M.; Müller, J.A.; Schoch, S.; Becker, A.; et al. The circadian hippocampus and its reprogramming in epilepsy: Impact for chronotherapeutics. *bioRxiv* **2020**, https://doi.org/10.1101/199372.
36. Kilkenny, C.; Browne, W.J.; Cuthi, I.; Emerson, M.; Altman, D.G. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *Vet. Clin. Pathol.* **2012**, *41*, 27–31. https://doi.org/10.1111/j.1939-165X.2012.00418.x.
37. Lalonde, R. The neurobiological basis of spontaneous alternation. *Neurosci. Biobehav. Rev.* **2002**, *26*, 91–104. https://doi.org/10.1016/s0149-7634(01)00041-0.
38. Naert, A.; Gantois, I.; Laeremans, A.; Vreysen, S.; Van den Bergh, G.; Arckens, L.; Callaerts-Vegh, Z.; D'Hooge, R. Behavioural alterations relevant to developmental brain disorders in mice with neonatally induced ventral hippocampal lesions. *Brain Res. Bull.* **2013**, *94*, 71–81. https://doi.org/10.1016/j.brainresbull.2013.01.008.
39. Franklin, K.B.J. *The Mouse Brain in Stereotaxic Coordinates/Keith B.J. Franklin, George Paxinos*; Elsevier: Amsterdam, The Netherlands, 2008.
40. Strehl, A.; Lenz, M.; Itsekson-Hayosh, Z.; Becker, D.; Chapman, J.; Deller, T.; Maggio, N.; Vlachos, A. Systemic inflammation is associated with a reduction in Synaptopodin expression in the mouse hippocampus. *Exp. Neurol.* **2014**, *261*, 230–235. https://doi.org/10.1016/j.expneurol.2014.04.033.
41. Lenz, M.; Ben Shimon, M.; Deller, T.; Vlachos, A.; Maggio, N. Pilocarpine-Induced Status Epilepticus Is Associated with Changes in the Actin-Modulating Protein Synaptopodin and Alterations in Long-Term Potentiation in the Mouse Hippocampus. *Neural Plast.* **2017**, *2017*, 2652560. https://doi.org/10.1155/2017/2652560.
42. Richetto, J.; Polesel, M.; Weber-Stadlbauer, U. Effects of light and dark phase testing on the investigation of behavioural paradigms in mice: Relevance for behavioural neuroscience. *Pharmacol. Biochem. Behav.* **2019**, *178*, 19–29. https://doi.org/10.1016/j.pbb.2018.05.011.
43. Tsao, C.H.; Flint, J.; Huang, G.J. Influence of diurnal phase on behavioral tests of sensorimotor performance, anxiety, learning and memory in mice. *Sci. Rep.* **2022**, *12*, 432. https://doi.org/10.1038/s41598-021-03155-5.
44. Roedel, A.; Storch, C.; Holsboer, F.; Ohl, F. Effects of light or dark phase testing on behavioural and cognitive performance in DBA mice. *Lab. Anim.* **2006**, *40*, 371–381. https://doi.org/10.1258/002367706778476343.
45. Jasinska, M.; Jasek-Gajda, E.; Woznicka, O.; Lis, G.J.; Pyza, E.; Litwin, J.A. Circadian clock regulates the shape and content of dendritic spines in mouse barrel cortex. *PLoS ONE* **2019**, *14*, e0225394. https://doi.org/10.1371/journal.pone.0225394.
46. de Vivo, L.; Bellesi, M.; Marshall, W.; Bushong, E.A.; Ellisman, M.H.; Tononi, G.; Cirelli, C. Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. *Science* **2017**, *355*, 507–510. https://doi.org/10.1126/science.aah5982.
47. Chaudhury, D.; Wang, L.M.; Colwell, C.S. Circadian regulation of hippocampal long-term potentiation. *J. Biol. Rhythm.* **2005**, *20*, 225–236. https://doi.org/10.1177/0748730405276352.
48. Wang, L.M.; Suthana, N.A.; Chaudhury, D.; Weaver, D.R.; Colwell, C.S. Melatonin inhibits hippocampal long-term potentiation. *Eur. J. Neurosci.* **2005**, *22*, 2231–2237. https://doi.org/10.1111/j.1460-9568.2005.04408.x.
49. Fukazawa, Y. Hippocampal LTP Is Accompanied by Enhanced F-Actin Content within the Dendritic Spine that Is Essential for Late LTP Maintenance In Vivo. *Neuron* **2003**, *38*, 447–460.
50. Yamazaki, M.; Matsuo, R.; Fukazawa, Y.; Ozawa, F.; Inokuchi, K. Regulated expression of an actin-associated protein, synaptopodin, during long-term potentiation. *J. Neurochem.* **2001**, *79*, 192–199. https://doi.org/10.1046/j.1471-4159.2001.00552.x.
51. Vlachos, A.; Maggio, N.; Segal, M. Lack of correlation between synaptopodin expression and the ability to induce LTP in the rat dorsal and ventral hippocampus. *Hippocampus* **2008**, *18*, 1–4. https://doi.org/10.1002/hipo.20373.
52. Filipski, E.; King, V.M.; Li, X.; Granda, T.G.; Mormont, M.C.; Liu, X.; Claustrat, B.; Hastings, M.H.; Lévi, F. Host circadian clock as a control point in tumor progression. *J. Natl. Cancer Inst.* **2002**, *94*, 690–697. https://doi.org/10.1093/jnci/94.9.690.

53. Tsinkalovsky, O.; Filipski, E.; Rosenlund, B.; Sothern, R.; Eiken, H.; Wu, M.; Claustrat, B.; Bayer, J.; Lévi, F.; Laerum, O. Circadian expression of clock genes in purified hematopoietic stem cells is developmentally regulated in mouse bone marrow. *Exp. Hematol.* **2006**, *34*, 1249–1261. https://doi.org/10.1016/j.exphem.2006.05.008.
54. Schmidt-Hieber, C.; Häusser, M. Cellular mechanisms of spatial navigation in the medial entorhinal cortex. *Nat. Neurosci.* **2013**, *16*, 325–331. https://doi.org/10.1038/nn.3340.
55. Kalén, P.; Rosegren, E.; Lindvall, O.; Björklund, A. Hippocampal Noradrenaline and Serotonin Release over 24 Hours as Measured by the Dialysis Technique in Freely Moving Rats: Correlation to Behavioural Activity State, Effect of Handling and Tail-Pinch. *Eur. J. Neurosci.* **1989**, *1*, 181–188. https://doi.org/10.1111/j.1460-9568.1989.tb00786.x.
56. Kasai, H.; Fukuda, M.; Watanabe, S.; Hayashi-Takagi, A.; Noguchi, J. Structural dynamics of dendritic spines in memory and cognition. *Trends Neurosci.* **2010**, *33*, 121–129. https://doi.org/10.1016/j.tins.2010.01.001.
57. Jasinska, M.; Woznicka, O.; Jasek-Gajda, E.; Lis, G.J.; Pyza, E.; Litwin, J.A. Circadian Changes of Dendritic Spine Geometry in Mouse Barrel Cortex. *Front. Neurosci.* **2020**, *14*, 578881. https://doi.org/10.3389/fnins.2020.578881.
58. Araya, R.; Vogels, T.P.; Yuste, R. Activity-dependent dendritic spine neck changes are correlated with synaptic strength. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E2895–E2904. https://doi.org/10.1073/pnas.1321869111.
59. Chai, A.P.; Ma, W.P.; Wang, L.P.; Cao, J.; Xu, L.; Yang, Y.X.; Mao, R.R. Chronic constant light-induced hippocampal late-phase long-term potentiation impairment in vitro is attenuated by antagonist of D1/D5 receptors. *Brain Res.* **2015**, *1622*, 72–80. https://doi.org/10.1016/j.brainres.2015.06.023.
60. Zhou, Y.; Zhang, H.K.; Liu, F.; Lei, G.; Liu, P.; Jiao, T.; Dang, Y.H. Altered Light Conditions Contribute to Abnormalities in Emotion and Cognition through HINT1 Dysfunction in C57BL/6 Mice. *Front. Behav. Neurosci.* **2018**, *12*, 110. https://doi.org/10.3389/fnbeh.2018.00110.
61. LeGates, T.A.; Altimus, C.M.; Wang, H.; Lee, H.K.; Yang, S.; Zhao, H.; Kirkwood, A.; Weber, E.T.; Hattar, S. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature* **2012**, *491*, 594–598. https://doi.org/10.1038/nature11673.
62. Krugers, H.J.; Douma, B.R.; Andringa, G.; Bohus, B.; Korf, J.; Luiten, P.G. Exposure to chronic psychosocial stress and corticosterone in the rat: Effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity. *Hippocampus* **1997**, *7*, 427–436. https://doi.org/10.1002/(sici)1098-1063(1997)7:4<427::Aid-hipo8>3.0.Co;2-f.
63. Babayan, A.H.; Kramár, E.A. Rapid effects of oestrogen on synaptic plasticity: Interactions with actin and its signalling proteins. *J. Neuroendocr.* **2013**, *25*, 1163–1172. https://doi.org/10.1111/jne.12108.

Supplementary Materials:

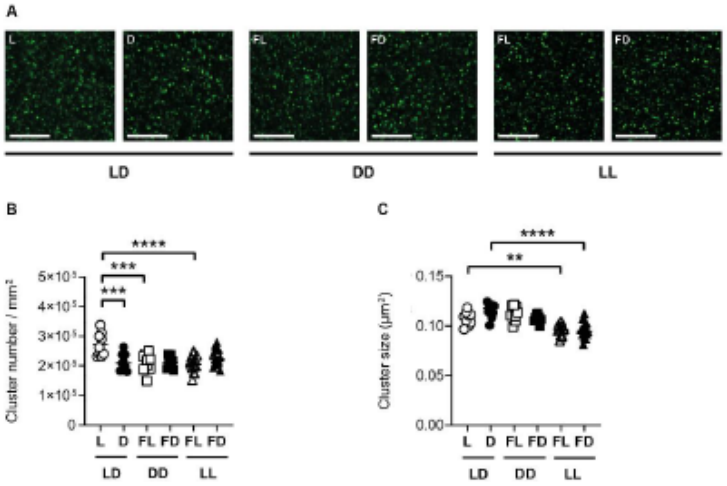


Figure S1 Synaptopodin clusters in the dentate gyrus (DG) of the dorsal hippocampus are affected by light. A) Representative confocal laser microscopic pictures of synaptopodin immunoreactive clusters in the DG of the dorsal hippocampus of mice in a standard photoperiod (LD) of 12 h light (L) and 12 h darkness (D), in constant darkness (DD) or constant light (LL) and sacrificed in L, former L (FL), D, or former D (FD). Scale bar, 20 µm. Quantification of B) cluster size, and C) cluster size of mice kept in LD (circles), DD (squares), or LL (triangles) and sacrificed in L or FL (open symbols) and D or FD (closed symbols). Bars represent mean ±SEM of n=9 mice per group in LD and DD and n=15 mice per group in LL. One-way ANOVA, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

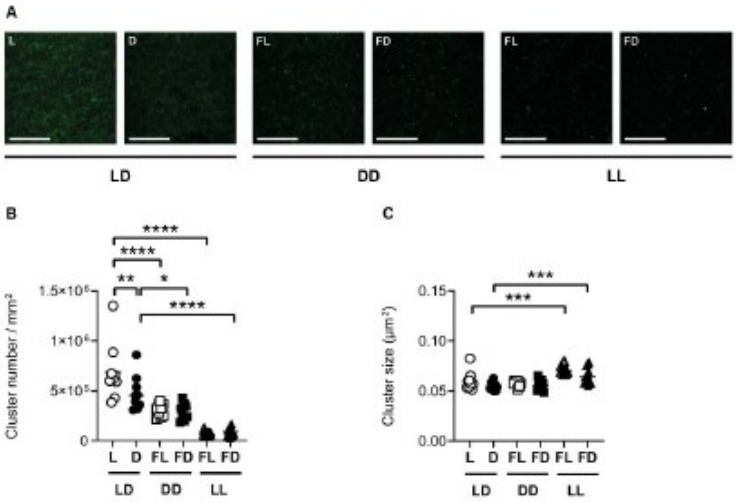


Figure S2 GluR1 clusters in the dentate gyrus (DG) of the dorsal hippocampus are affected by light. A) Representative confocal laser microscopic pictures of GluR1 immunoreactive clusters in the DG of the dorsal hippocampus of mice kept in a standard photoperiod (LD) of 12 h light (L) and 12 h darkness (D), in constant darkness (DD), or constant light (LL) and sacrificed in L, former L (FL), D, or former D (FD). Scale bar, 20 µm. Quantification of B) cluster number, and C) cluster size of mice kept in LD (circles), DD (squares), or LL (triangles) and sacrificed in L or FL (open symbols) and D or FD (closed symbols). Bars represent mean ±SEM of n=9 mice per group in LD and DD and n=15 mice per group in LL. One-way ANOVA, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

3 Discussion

The role of the circadian system, as well as the impact of light and acute exposure to constant light, on the functional and structural plasticity of the hippocampus, a crucial structure for temporal and spatial learning and memory, remains poorly understood.

Our study showed that light is a great influencer on hippocampal function and synaptic plasticity, on the functional, structural and molecular level. The effects of the light/dark cycle and acute constant light were observed and will be detailed in the following sections.

3.1 Hippocampus-dependent spatial working memory and synaptic plasticity in the standard photoperiod (12:12)

3.1.1 Effect of the light/dark cycle

To investigate different light conditions mice were held in a standard photoperiod of 12 hours of light and 12 hours of darkness (LD). In mice kept in LD we could observe that the number of correct alternations in the Y-maze, which is dependent on hippocampal plasticity and related to exploratory activity, was significantly lower in the light phase than in the dark phase. This is consistent with the fact that light suppresses exploratory activity in nocturnal animals [118]. In contrast in mice kept in DD there was no difference in the number of correct alternations in the Y-maze, even though locomotor activity remained rhythmic. This indicates that the hippocampus-dependent spatial working memory is affected rather by light than by circadian rhythms.

In mice kept in LD we also found a higher number of synaptopodin and GluR1 IR in the light phase compared to the dark phase. In contrast, while GluR1 cluster size showed no significant differences, the average cluster size of synaptopodin was higher in the dark phase. The day/night differences observed in the number and size of synaptopodin clusters were also eliminated under constant dark conditions, indicating again a light effect. Light increased the number of synaptopodin clusters while decreasing their average size, a trait associated with synaptic strength and potentially contributing to the improved Y-maze performance during the dark phase. The average size of synaptopodin clusters and therefore spine size has been linked to stability synaptic strength [83, 84]. An increase in synaptopodin cluster size during the dark phase corresponds with enhanced spine strength at night. These changes appear to anticipate light and the associated loss of neuronal activity during the rest phase of the mice. Fewer spines are

present at night compared to the day, suggesting that enhancing synaptic strength may be a more efficient and rapid way to improve neurotransmission than increasing spine number. Importantly, this homeostatic compensatory adjustment in synaptic strength in response to fluctuations in network activity is abolished in mice with targeted deletion of synaptopodin, highlighting the critical role of this protein in maintaining synaptic plasticity [67]. The increase in synaptic strength, which is achieved by the insertion of GluR1 subunits of postsynaptic AMPA glutamate receptors, leads to the stabilization of the spines through increased spine size. According to the literature, hippocampal LTP is more pronounced during the dark phase, when synaptic strength is higher and spatial learning is improved [100]. Synaptopodin plays a role in hippocampal LTP [119] mainly through the Ca^{2+}-dependent recruitment of GluR1 to spine synapses [85]. Unlike the synaptopodin clusters, the size of GluR1 clusters did not differ between light and dark phases under LD conditions. This may be attributed to the fact that only about 20-30% of spines contain synaptopodin [120], so the light/dark cycle may not have a substantial effect on total GluR1. This is consistent with the Golgi-stained spines, where no difference between light and dark phase was observed in the density and length or area of the spines of mice kept in LD. Indeed, Golgi staining of the hippocampus labels both excitatory and inhibitory spines, enabling the analysis of structural changes in spine morphology. In contrast, synaptopodin IR represent only a subset of excitatory synapses, allowing the analysis on the molecular level. From the literature it is known, that in the somatosensory cortex, the density of excitatory synapses is, consistent with our findings in the hippocampus, higher during the light phase [104]. Moreover, the differences in the density of excitatory synapses in the somatosensory cortex were abolished in mice kept in DD, indicating that the excitatory synapses in the somatosensory cortex are also influenced by light and that in both the isocortex [83] and the allocortex [1] the density of excitatory synapses is driven by light rather than the circadian clock.

Corticosterone, the stress hormone, appears to play a crucial role in regulating hippocampal structural synaptic plasticity [103]. However, the circadian clock regulates the rhythm of corticosterone levels, as well as spontaneous locomotor activity, with these patterns persisting even in constant darkness. Thus, the diurnal differences observed in this study for mice kept in LD, appear to be influenced more by light than by circadian rhythms. But how can light influence the hippocampus that has no

connection with the visual system? Indeed, little is known about the sole effect of light on the hippocampal neuronal network. The hippocampal regions including CA1 [56] and DG receive retinal input either directly or indirectly [9]. Projections of the retina to hippocampus via the SCN, dorsal raphe nuclei (serotoninergic), the locus coeruleus (noradrenergic) and the area septalis (cholinergic) may indirectly mediate the effects of light on hippocampal learning [9, 57-60]. These neurotransmitters are key regulators of synaptic plasticity in the hippocampus [57-59] and, importantly have a higher basal level during the dark phase compared to the light phase [121] coincident with our hypothesis of the effect of the light in increased synaptic strength. However, in the dHC there were no diurnal changes between the CA1 region and the DG or among the different layers (data not shown) that could be attributed to a modulation by specific brainstem projections.

Therefore, in mice kept under light/dark conditions the morphological changes seen in hippocampal excitatory synapses correlate with the differences in hippocampus-dependent spatial working memory. These findings suggest that light has a great influence on hippocampal function, drives rhythmic scaling of synaptic strength and impacts the plasticity of excitatory synapses in the hippocampus.

3.1.2 Synaptopodin and GluR1 IR within the dHC and vHC

Within the hippocampus there is a regional and laminar specific distribution of synatpopodin protein [88, 89]. Bas Orth et al. 2005 analyzed synaptopodin immunoreactive puncta densities and measured dendritic distribution of synaptopodin-positive puncta using three-dimensional confocal reconstructions of synaptopodin-immunostained and enhanced green fluorescence protein EGFP-labeled principal neurons in the dHC [89]. In our study we found a significantly higher level of synaptopodin cluster size in the dHC, specifically in IML and LA (Fig. 5). These findings align with the results of Bas Orth et al. 2005, who also reported the highest concentration of synaptopodin immunoreactive puncta in LA within the hippocampal CA1 region in dHC [89]. They found a higher amount of synaptopodin in OML compared to IML which was not significant. They also investigated laminar specific distribution in the middle molecular layer (MML), which was even higher than in OML [89].

It was shown that synaptopodin puncta within principal neuron's dendrites were mostly (>95%) found in spines [89]. It was concluded that synaptopodin represents the spine

apparatus because of the localization. Based on these findings we may transfer our results regarding synaptopodin to spine apparatuses in general [89].

Deller et al. also found a layer specific patern of synaptopodin. They analyzed the distribution of synaptopodin mRNA in the brain of reeler mice, homozygeous control mice bred on the reeler background and C57/B16 mice [88]. In all mice they found the highest synaptopodin mRNA expression in the principal cell layer of the DG and CA. They showed that in the DG of control mice, synaptopodin immunostaining is strongest in the molecular layer. Furthermore they found that in the HC of reeler mice, the pattern of synaptopodin mRNA expressing cells reflects the location of granule and pyramidal cells that bear spines [88]. The presence of afferent fibers may influence the distribution pattern of synaptopodin [88]. Deller et al. showed in their previous work (1999) that the distribution of synaptopodin is altered after the DG was deafferentiated [122] which would support this hypothesis.

For synaptopodin we found a higher cluster number in 2 layers (IML and LA) in the dHC compared to the vHC. In RAD synaptopodin cluster number is higher in vHC than in dHC. GluR1 cluster number is significantly higher in den dHC in O. In almost all layers (IML, OML, LA and RAD) dHC cluster size is higher in dHC than in vHC. It is difficult to interpret why cluster size of GluR1 is higher in dHC compared to vHC. Little is known about synaptopodin and GluA1 distribution in the vHC and dHC because most studies focus the dHC because of its given role in learning processes. As mentioned above the dHC is mainly exteroceptive and important for explicit learning and the vHC is interoceptive, involved in stress, emotion and affect-associated learning processes. We pointed out that GluR1 plays an important role in synaptic plasticity and cognition and that synaptic strength and spine stabilization are achieved through the incorporation of GluR1 into the postsynaptic density (PSD). Spine size was shown to be positively correlated with the stability and strength of synapses [83, 84]. The recruitment of AMPA receptors appears to be associated with synaptopodin [85]. The fact synaptopodin cluster size was higher in 3 out of 5 layers and GluR1 cluster size was higher in 4 out of 5 layers can be a hint that also stability and strength of synapses is higher in the dHC where explicit learning takes place.

3.2 Effect of 38h constant darkness

To investigate different light conditions mice were held in acute constant darkness for at least 38 hours (DD). In DD, circadian rhythms, which occur even without zeitgebers, are unmasked. In this group we see a significantly higher spontaneous locomotor activity in the dark phase compared to the former light phase (FL) which means that locomotor activity is still rhythmic. In DD, synaptopodin and GluR1 IR in the dHC were significantly altered compared to LD. The number and size of synaptopodin IR were not different between the FL and former dark phase (FD) in the CA1 region and DG. Spine density, length and area showed no alterations between DD and LD or between FL and FD of mice kept in DD. The number of total arm entries in the y-maze was significantly higher in FL of mice kept in DD than in L of mice kept in LD. This indicates that the LD light phase suppresses exploratory activity. The differences between the light and dark phase seen in LD were abolished when animals were housed in DD conditions indicating that the density of excitatory synapses in the hippocampus is driven by light rather than rest/locomotor activity rhythm and/or the circadian clock. In conclusion, the results suggest that constant darkness for 38 hours leads to subtle alternations of hippocampal synaptic plasticity. Ikeda et al. showed that the number of hippocampal spines was altered under light/dark photoperiod but did not include constant darkness [103]. Jasinska et al. demonstrated that in the somatosensory cortex the density of excitatory synapses is higher during the light phase, while the density of inhibitory synapses increases during the dark phase [84, 104]. Interestingly, under DD, the variation in excitatory synapse density was eliminated, whereas the differences in inhibitory synapse density remained. This suggests that excitatory synapses are influenced by light, whereas inhibitory synapses are regulated by the rest/locomotor activity rhythm and/or the circadian clock [104]. In our study we focused on the effect of the different light conditions on excitatory synapses in the hippocampus. Our results are consistent with Jasinkas results indicating that in both the isocortex [83] and the allocortex [1] the density of excitatory synapses is driven by light rather than the circadian clock.

3.3 Effect of 38h constant light

To investigate different light conditions mice were held in acute constant light for at least 38 hours (LL). In mice kept in LL we could observe that the number of correct alternations in the Y-maze, which is dependent on hippocampal plasticity and related to exploratory activity, was significantly lower in FD of mice kept in LL than in D of mice

kept in LD. This suggests that light suppresses exploratory behavior-dependent spatial working memory. These results correlate with the results seen in LD animals. Moreover, spontaneous alternations, reflecting spatial working memory corrected by exploration activity, were significantly higher in the dark phase of mice kept in LD than in FD of mice kept in LL. This indicates that acute constant light impairs spatial working memory, independent of its effect on explorative activity. Spine density, spine length and spine area of Golgi-Cox-stained apical dendritic spines in the dHC of mice kept in LL were reduced compared to mice kept in LD thus showing that constant light also affects the hippocampal spine morphology. Indeed, changes in the morphology of spines are correlated with their maturity and strength. Mature spines, like mushroom spines, are characterized by larger spine heads and a higher concentration of glutamate receptors, which are closely linked to enhanced synaptic stability and strength [83, 84]. This change in structural plasticity suggests that constant light, which elevates stress-induced glucocorticoid levels, impairs hippocampus-dependent spatial working memory and aligns with the observed reduction in the density of synaptopodin- and GluR1-positive clusters under LL. The literature indicates that corticosterone plays a critical role in hippocampal structural synaptic plasticity [103] and that elevated levels of stress-induced glucocorticoids impair spatial learning [123]. Mice exposed to chronic LL conditions for 3–4 weeks exhibit increased and disrupted corticosterone levels [47], impaired hippocampal LTP [124], and disrupted locomotor activity rhythms [125]. The chronic exposure of mice to LL leads also to depressive- and anxiety-like behavior and impaired spatial memory [126]. Ketelauri et. al found that chronodisruption promotes caspase 1 activation in the mouse HC and lead to an activated inflammasome as well as to increased vulnerability of the brain to additional adversive stimuli [127].

In our study we exposed mice to acute constant light for at least 38 hours which did not result in a disruption of circadian rhythms. Locomotor activity and corticosterone levels, which were analyzed as indicators of circadian rhythms, remained rhythmic. However, plasma levels of corticosterone were significantly increased in animals kept in acute LL, consistent with light being a stress factor for nocturnal rodents [128]. Corticosterone levels peaked in LL and LD 14 hours after (former) light but were overall higher in LL compared to LD. Therefore, the differences seen in LL and compared to LD, which will be discussed now, are probably mainly due to the increased corticosterone levels.

However, further studies are needed to determine whether stress-induced glucocorticoids are responsible for the changes in structural synaptic plasticity under LL. It is remarkable that even acute LL used in this study had such significant effects on glucocorticoid levels, spatial memory, and hippocampal structural plasticity. Following exposure to acute stress, LTP was shown to be enhanced in the vHC and suppressed in the dHC [129]. Chronic LL also leads to depressive- and anxiety-like behavior and impaired spatial memory [130]. Discussing chronodisruption and the effects of our modern lifestyle on diurnal rhythms, behavior, and cognition highlights the need for further investigation into prolonged photoperiods. Jasinska et al. addressed this by conducting similar experiments not only in standard LD, LL, and DD conditions but also in an LD cycle with 16 hours of light and 8 hours of darkness. This extended photoperiod may mimic the human daily schedule better compared to the commonly used 12:12 light/dark cycle [104]. They investigated protein expression in the fourth layer of the somatosensory cortex in mice, as well as locomotor activity at various time points during LD (12:12, 16:8), LL, and DD conditions. Regarding locomotor activity, no significant differences were found between the 12:12 and 16:8 LD cycles. As presynaptic proteins, synaptophysin, piccolo, and neurexin were analyzed. The number of synaptophysin clusters significantly increased during the day compared to the night period in LD, which is similar to our findings for synaptopodin in the hippocampus. In mice kept under DD conditions, synaptophysin levels remained elevated during the subjective day, with an increased synaptophysin area as well. Interestingly, it was observed that fewer synaptophysin immunopuncta were present at the beginning of the subjective night in both DD and the prolonged LD (16:8) conditions.

3.4 Outlook

For future studies, it would be valuable to consider prolonged light/dark cycles with 16:8 light and darkness conditions in addition to the standard LD, DD, and LL paradigms. This extended photoperiod may better mimic the human daily schedule compared to the commonly used 12:12 light/dark cycle. Furthermore, it would be insightful to reevaluate the experiment with longer exposure in constant light, resulting in chronodisruption to see if there are any differences between acute and chronic LL.

Wosiski-Kuhn et al. stated that an increase in dendritic spine density contributes to LTP in the DG [131]. As mentioned earlier synaptopodin can act as a marker for dendritic

spines. In our study we did not measure synaptopodin or GluR1 density directly but average cluster size and number which both contributes to density. A higher number as well as higher cluster size both contribute to a higher density. Thus we may conclude that not only an increase in dendritic spine density but also an increase in synaptopodin IR cluster number and size contributes to LTP in the DG. For further studies the density should be measured besides cluster number and size.

Future studies should also include female mice, as this study focused solely on males. Research dating back to 1994 [110] has shown that variability within a gender is often greater than differences between genders in the investigated species. Despite this, male subjects remain the standard in scientific research. Including female mice in such studies would enable exploration of the interactions between sex hormones and light in shaping structural and functional synaptic plasticity.

3.5 Conclusion

Our study demonstrated that the light/dark cycle drives diurnal differences in spine morphology, as well as in synaptopodin and GluR1 clusters, thereby influencing hippocampal excitatory synapses. Moreover, the light/dark cycle drives diurnal differences in hippocampus-dependent spatial working memory. Constant darkness for 38 hours led to subtle alternations of hippocampal synaptic plasticity. Additionally, acute exposure to constant light was associated with a reduced number and size of hippocampal excitatory synapses, as well as alterations in structural plasticity, ultimately leading to impaired spatial working memory. These data were collected in nocturnal rodents which makes the transfer limited to humans. Modern society factors, such as increased indoor activity, low light intensity during the day, and artificial light during the evening and night, could be detrimental to the functional and structural plasticity of the hippocampus. These considerations should be integrated in our daily lives and addressed in the treatment of sleep and concentration disturbances.

4 References

1. Schroder, J.K., et al., *Effects of the Light/Dark Phase and Constant Light on Spatial Working Memory and Spine Plasticity in the Mouse Hippocampus.* Cells, 2023. **12**(13).
2. Mairan, J.-J.d.O.d., *Historie de l'Academie Royale des Sciences*. Observation botanique. 1729, Paris.
3. Korf, H.-W., & von Gall, C. , *Circadian Physiology*, in *Neuroscience in the 21st Century*. 2021. p. 1813-1845.
4. Welsh, D.K., J.S. Takahashi, and S.A. Kay, *Suprachiasmatic nucleus: cell autonomy and network properties.* Annu Rev Physiol, 2010. **72**: p. 551-77.
5. Morin, L.P. and C.N. Allen, *The circadian visual system, 2005.* Brain Res Rev, 2006. **51**(1): p. 1-60.
6. Ebling, F.J., et al., *FosB in the suprachiasmatic nucleus of the Syrian and Siberian hamster.* Brain Res Bull, 1996. **41**(5): p. 257-68.
7. Ali, A.A.H., G.A. Avakian, and C.V. Gall, *The Role of Purinergic Receptors in the Circadian System.* Int J Mol Sci, 2020. **21**(10).
8. Mrosovsky, N., *Masking: history, definitions, and measurement.* Chronobiol Int, 1999. **16**(4): p. 415-29.
9. von Gall, C., *The Effects of Light and the Circadian System on Rhythmic Brain Function.* Int J Mol Sci, 2022. **23**(5).
10. Mosko, S. and R.Y. Moore, *Neonatal suprachiasmatic nucleus ablation: absence of functional and morphological plasticity.* Proc Natl Acad Sci U S A, 1978. **75**(12): p. 6243-6.
11. Sollars, P.J. and G.E. Pickard, *Restoration of circadian behavior by anterior hypothalamic grafts containing the suprachiasmatic nucleus: graft/host interconnections.* Chronobiol Int, 1998. **15**(5): p. 513-33.
12. Ralph, M.R. and M.N. Lehman, *Transplantation: a new tool in the analysis of the mammalian hypothalamic circadian pacemaker.* Trends Neurosci, 1991. **14**(8): p. 362-6.
13. Sagan, K.P., et al., *Quality of Life and Sleep in Patients with Pituitary Adenoma in Relation to Tumor Type and Compression of the Optic Chiasm.* J Clin Med, 2021. **10**(9).
14. Moore, R.Y. and S.J. C., *GABA is the principal neurotransmitter of the circadian system.* Neuroscience letters, 1993. **150**: p. 112-116.
15. Balsalobre, A., et al., *Resetting of circadian time in peripheral tissues by glucocorticoid signaling.* Science, 2000. **289**(5488): p. 2344-7.
16. Konopka, R.J. and S. Benzer, *Clock mutants of Drosophila melanogaster.* Proc Natl Acad Sci U S A, 1971. **68**(9): p. 2112-6.
17. Huang, R.C., *The discoveries of molecular mechanisms for the circadian rhythm: The 2017 Nobel Prize in Physiology or Medicine.* Biomed J, 2018. **41**(1): p. 5-8.
18. Liu, C. and S.M. Reppert, *GABA synchronizes clock cells within the suprachiasmatic circadian clock.* Neuron, 2000. **25**(1): p. 123-8.
19. Longcore, T. and C. Rich, *Ecological light pollution.* Frontiers in Ecology and the Environment, 2004. **2**(4): p. 191-198.
20. Jones, T.M., et al., *Melatonin: a possible link between the presence of artificial light at night and reductions in biological fitness.* Philos Trans R Soc Lond B Biol Sci, 2015. **370**(1667).
21. Baker, B.J. and J.M.L. Richardson, *The effect of artificial light on male breeding-season behaviour in green frogs, Rana clamitans melanota.* Canadian Journal of Zoology, 2006. **84**(10): p. 1528-1532.

22. Kempenaers, B., et al., *Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds.* Curr Biol, 2010. **20**(19): p. 1735-9.
23. Chinoy, E.D., J.F. Duffy, and C.A. Czeisler, *Unrestricted evening use of light-emitting tablet computers delays self-selected bedtime and disrupts circadian timing and alertness.* Physiol Rep, 2018. **6**(10): p. e13692.
24. Baez-Ruiz, A., et al., *Food in synchrony with melatonin and corticosterone relieves constant light disturbed metabolism.* J Endocrinol, 2017. **235**(3): p. 167-178.
25. Lewy, A.J., *Melatonin and human chronobiology.* Cold Spring Harb Symp Quant Biol, 2007. **72**: p. 623-36.
26. Erren, T.C. and R.J. Reiter, *Light Hygiene: Time to make preventive use of insights--old and new--into the nexus of the drug light, melatonin, clocks, chronodisruption and public health.* Med Hypotheses, 2009. **73**(4): p. 537-41.
27. Srinivasan, V., et al., *Melatonin in mood disorders.* World J Biol Psychiatry, 2006. **7**(3): p. 138-51.
28. Reiter, R.J., et al., *Light at night, chronodisruption, melatonin suppression, and cancer risk: a review.* Crit Rev Oncog, 2007. **13**(4): p. 303-28.
29. Filipski, E., et al., *Host circadian clock as a control point in tumor progression.* J Natl Cancer Inst, 2002. **94**(9): p. 690-7.
30. Filipski, E., et al., *Effects of chronic jet lag on tumor progression in mice.* Cancer Res, 2004. **64**(21): p. 7879-85.
31. Homolak, J., et al., *Circadian Rhythm and Alzheimer's Disease.* Med Sci (Basel), 2018. **6**(3).
32. Lauretti, E., et al., *Circadian rhythm dysfunction: a novel environmental risk factor for Parkinson's disease.* Mol Psychiatry, 2017. **22**(2): p. 280-286.
33. Pfeffer, M., et al., *Disturbed sleep/wake rhythms and neuronal cell loss in lateral hypothalamus and retina of mice with a spontaneous deletion in the ubiquitin carboxyl-terminal hydrolase L1 gene.* Neurobiol Aging, 2012. **33**(2): p. 393-403.
34. Leng, Y., et al., *Association between circadian rhythms and neurodegenerative diseases.* The Lancet Neurology, 2019. **18**(3): p. 307-318.
35. Nakamura, T.J., et al., *Age-related decline in circadian output.* J Neurosci, 2011. **31**(28): p. 10201-5.
36. Dufort-Gervais, J., V. Mongrain, and J. Brouillette, *Bidirectional relationships between sleep and amyloid-beta in the hippocampus.* Neurobiol Learn Mem, 2019. **160**: p. 108-117.
37. Krause, A.J., et al., *The sleep-deprived human brain.* Nat Rev Neurosci, 2017. **18**(7): p. 404-418.
38. Shokri-Kojori, E., et al., *beta-Amyloid accumulation in the human brain after one night of sleep deprivation.* Proc Natl Acad Sci U S A, 2018. **115**(17): p. 4483-4488.
39. Zhu, L. and P.C. Zee, *Circadian rhythm sleep disorders.* Neurol Clin, 2012. **30**(4): p. 1167-91.
40. Gibson, E.M., et al., *Experimental 'jet lag' inhibits adult neurogenesis and produces long-term cognitive deficits in female hamsters.* PLoS One, 2010. **5**(12): p. e15267.
41. Havekes, R. and T. Abel, *The tired hippocampus: the molecular impact of sleep deprivation on hippocampal function.* Curr Opin Neurobiol, 2017. **44**: p. 13-19.
42. Kreutzmann, J.C., et al., *Sleep deprivation and hippocampal vulnerability: changes in neuronal plasticity, neurogenesis and cognitive function.* Neuroscience, 2015. **309**: p. 173-90.
43. Musiek, E.S. and D.M. Holtzman, *Mechanisms linking circadian clocks, sleep, and neurodegeneration.* Science, 2016. **354**(6315): p. 1004-1008.

44. Havekes, R., et al., *Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1.* Elife, 2016. **5**.
45. Garaulet, M. and P. Gomez-Abellan, *Chronobiology and obesity.* Nutr Hosp, 2013. **28 Suppl 5**: p. 114-20.
46. Gonzalez, M.M.C., *Dim Light at Night and Constant Darkness: Two Frequently Used Lighting Conditions That Jeopardize the Health and Well-being of Laboratory Rodents.* Front Neurol, 2018. **9**: p. 609.
47. Claustrat, B., et al., *Effect of constant light on prolactin and corticosterone rhythms evaluated using a noninvasive urine sampling protocol in the rat.* Horm Metab Res, 2008. **40**(6): p. 398-403.
48. Scoville, W.B., *The limbic lobe in man.* J Neurosurg, 1954. **11**(1): p. 64-6.
49. Scoville, W.B. and B. Milner, *Loss of recent memory after bilateral hippocampal lesions.* J Neurol Neurosurg Psychiatry, 1957. **20**(1): p. 11-21.
50. Ulrich Welsch, W.K., Thomas Deller, *Lehrbuch Histologie*. Vol. 4. 2014, München: Elsevier Urban & Fischer.
51. Jeffrey L. Barr, B.B., Gina L. Forster, *The Hippocampus as a Neural Link between Negative Affect and Vulnerability for Psychostimulant Relapse*, in *The Hippocampus - Plasticity and Functions*. 2018.
52. A. Benninghoff, D.D., *Anatomie, Makroskopische Anatomie, Embryologie und Histologie des Menschen*. Vol. 16. 2004, Würzburg: Prof. Dr. Detlev Drenckhahn.
53. Fanselow, M.S. and H.W. Dong, *Are the dorsal and ventral hippocampus functionally distinct structures?* Neuron, 2010. **65**(1): p. 7-19.
54. Knierim, J.J., *The hippocampus.* Curr Biol, 2015. **25**(23): p. R1116-21.
55. Neves, G., S.F. Cooke, and T.V. Bliss, *Synaptic plasticity, memory and the hippocampus: a neural network approach to causality.* Nat Rev Neurosci, 2008. **9**(1): p. 65-75.
56. Lo, L. and D.J. Anderson, *A Cre-dependent, anterograde transsynaptic viral tracer for mapping output pathways of genetically marked neurons.* Neuron, 2011. **72**(6): p. 938-50.
57. Maity, S., et al., *Norepinephrine triggers metaplasticity of LTP by increasing translation of specific mRNAs.* Learn Mem, 2015. **22**(10): p. 499-508.
58. Ovsepian, S.V., *Differential cholinergic modulation of synaptic encoding and gain control mechanisms in rat hippocampus.* Neurosci Res, 2008. **61**(1): p. 92-8.
59. Lopes, J.P., A. Pliássova, and R.A. Cunha, *The physiological effects of caffeine on synaptic transmission and plasticity in the mouse hippocampus selectively depend on adenosine A(1) and A(2A) receptors.* Biochem Pharmacol, 2019. **166**: p. 313-321.
60. Richetto, J., M. Polesel, and U. Weber-Stadlbauer, *Effects of light and dark phase testing on the investigation of behavioural paradigms in mice: Relevance for behavioural neuroscience.* Pharmacol Biochem Behav, 2019. **178**: p. 19-29.
61. Turrigiano, G., *Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function.* Cold Spring Harb Perspect Biol, 2012. **4**(1): p. a005736.
62. Gray, E.G., *Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex.* Nature, 1959. **183**(4675): p. 1592-3.
63. Jasinska, M., et al., *Circadian clock regulates the shape and content of dendritic spines in mouse barrel cortex.* PLoS One, 2019. **14**(11): p. e0225394.
64. Bosch, M. and Y. Hayashi, *Structural plasticity of dendritic spines.* Curr Opin Neurobiol, 2012. **22**(3): p. 383-8.
65. Fukazawa', Y., et al., *Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo.* Neuron, 2003. **38**(3): p. 447-460.

66. Bliss, T.V., G.L. Collingridge, and R.G.M. Morris, *The Hippocampus Book*. 2007: Oxford Univ. Press, New York.
67. Vlachos, A., et al., *Synaptopodin regulates denervation-induced homeostatic synaptic plasticity.* Proc Natl Acad Sci U S A, 2013. **110**(20): p. 8242-7.
68. Vlachos, A., et al., *Entorhinal denervation induces homeostatic synaptic scaling of excitatory postsynapses of dentate granule cells in mouse organotypic slice cultures.* PLoS One, 2012. **7**(3): p. e32883.
69. Kopec, C.D., et al., *GluR1 links structural and functional plasticity at excitatory synapses.* J Neurosci, 2007. **27**(50): p. 13706-18.
70. O'Brien, R.J., et al., *Activity-dependent modulation of synaptic AMPA receptor accumulation.* Neuron, 1998. **21**(5): p. 1067-78.
71. Sutton, M.A., et al., *Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis.* Cell, 2006. **125**(4): p. 785-99.
72. von Kitzing, E., P. Jonas, and B. Sakmann, *Quantal analysis of excitatory postsynaptic currents at the hippocampal mossy fiber-CA3 pyramidal cell synapse.* Adv Second Messenger Phosphoprotein Res, 1994. **29**: p. 235-60.
73. Petroff, O.A., *GABA and glutamate in the human brain.* Neuroscientist, 2002. **8**(6): p. 562-73.
74. Ge, Y. and Y.T. Wang, *GluA1-homomeric AMPA receptor in synaptic plasticity and neurological diseases.* Neuropharmacology, 2021. **197**: p. 108708.
75. Hollmann, M. and S. Heinemann, *Cloned glutamate receptors.* Annu Rev Neurosci, 1994. **17**: p. 31-108.
76. Mano, I. and V.I. Teichberg, *A tetrameric subunit stoichiometry for a glutamate receptor-channel complex.* Neuroreport, 1998. **9**(2): p. 327-31.
77. Ayalon, G., et al., *Two regions in the N-terminal domain of ionotropic glutamate receptor 3 form the subunit oligomerization interfaces that control subtype-specific receptor assembly.* J Biol Chem, 2005. **280**(15): p. 15053-60.
78. Tanaka, H., et al., *The AMPAR subunit GluR2: still front and center-stage.* Brain Res, 2000. **886**(1-2): p. 190-207.
79. Sans, N., et al., *Aberrant formation of glutamate receptor complexes in hippocampal neurons of mice lacking the GluR2 AMPA receptor subunit.* J Neurosci, 2003. **23**(28): p. 9367-73.
80. Wenthold, R.J., et al., *Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons.* J Neurosci, 1996. **16**(6): p. 1982-9.
81. Ju, W., et al., *Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors.* Nat Neurosci, 2004. **7**(3): p. 244-53.
82. Guntupalli, S., et al., *Ubiquitination of the GluA1 Subunit of AMPA Receptors Is Required for Synaptic Plasticity, Memory, and Cognitive Flexibility.* J Neurosci, 2023. **43**(30): p. 5448-5457.
83. Jasinska, M., et al., *Circadian rhythmicity of synapses in mouse somatosensory cortex.* Eur J Neurosci, 2015. **42**(8): p. 2585-94.
84. Jasinska, M., et al., *Circadian Changes of Dendritic Spine Geometry in Mouse Barrel Cortex.* Front Neurosci, 2020. **14**: p. 578881.
85. Vlachos, A., et al., *Synaptopodin regulates plasticity of dendritic spines in hippocampal neurons.* J Neurosci, 2009. **29**(4): p. 1017-33.
86. Deller, T., et al., *Plasticity of synaptopodin and the spine apparatus organelle in the rat fascia dentata following entorhinal cortex lesion.* J Comp Neurol, 2006. **499**(3): p. 471-84.

87. Deller, T., et al., *Actin-associated protein synaptopodin in the rat hippocampal formation: localization in the spine neck and close association with the spine apparatus of principal neurons.* J Comp Neurol, 2000. **418**(2): p. 164-81.
88. Deller, T., et al., *Laminar distribution of synaptopodin in normal and reeler mouse brain depends on the position of spine-bearing neurons.* J Comp Neurol, 2002. **453**(1): p. 33-44.
89. Bas Orth, C., et al., *Lamina-specific distribution of Synaptopodin, an actin-associated molecule essential for the spine apparatus, in identified principal cell dendrites of the mouse hippocampus.* J Comp Neurol, 2005. **487**(3): p. 227-39.
90. Deller, T., et al., *Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity.* Proc Natl Acad Sci U S A, 2003. **100**(18): p. 10494-9.
91. Segal, M., *Dendritic spines, synaptic plasticity and neuronal survival: activity shapes dendritic spines to enhance neuronal viability.* Eur J Neurosci, 2010. **31**(12): p. 2178-84.
92. Segal, M., A. Vlachos, and E. Korkotian, *The spine apparatus, synaptopodin, and dendritic spine plasticity.* Neuroscientist, 2010. **16**(2): p. 125-31.
93. Korkotian, E., M. Frotscher, and M. Segal, *Synaptopodin regulates spine plasticity: mediation by calcium stores.* J Neurosci, 2014. **34**(35): p. 11641-51.
94. Korkotian, E. and M. Segal, *Synaptopodin regulates release of calcium from stores in dendritic spines of cultured hippocampal neurons.* J Physiol, 2011. **589**(Pt 24): p. 5987-95.
95. Deller, T., et al., *Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity.* Proceedings of the National Academy of Sciences of the United States of America, 2003. **100**(18): p. 10494-10499.
96. Jedlicka, P., et al., *A role for the spine apparatus in LTP and spatial learning.* Behav Brain Res, 2008. **192**(1): p. 12-9.
97. Matsuzaki, M., et al., *Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons.* Nat Neurosci, 2001. **4**(11): p. 1086-92.
98. Matsuzaki, M., et al., *Structural basis of long-term potentiation in single dendritic spines.* Nature, 2004. **429**(6993): p. 761-6.
99. Barnes, C.A., et al., *Circadian rhythm of synaptic excitability in rat and monkey central nervous system.* Science, 1977. **197**(4298): p. 91-2.
100. Chaudhury, D., L.M. Wang, and C.S. Colwell, *Circadian regulation of hippocampal long-term potentiation.* J Biol Rhythms, 2005. **20**(3): p. 225-36.
101. McNaughton, B.L., R.M. Douglas, and G.V. Goddard, *Synaptic enhancement in fascia dentata: cooperativity among coactive afferents.* Brain Res, 1978. **157**(2): p. 277-93.
102. Frank, M.G., *Circadian Regulation of Synaptic Plasticity.* Biology (Basel), 2016. **5**(3).
103. Ikeda, M., et al., *Hippocampal spine changes across the sleep-wake cycle: corticosterone and kinases.* J Endocrinol, 2015. **226**(2): p. M13-27.
104. Jasinska, M., et al., *Light-Modulated Circadian Synaptic Plasticity in the Somatosensory Cortex: Link to Locomotor Activity.* International Journal of Molecular Sciences, 2024. **25**.
105. Liston, C., et al., *Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance.* Nat Neurosci, 2013. **16**(6): p. 698-705.
106. Jaszczyk, A., et al., *Overnight Corticosterone and Gene Expression in Mouse Hippocampus: Time Course during Resting Period.* Int J Mol Sci, 2023. **24**(3).
107. Barriga, C., et al., *Circadian rhythm of melatonin, corticosterone and phagocytosis: effect of stress.* J Pineal Res, 2001. **30**(3): p. 180-7.
108. Jones, J.R., et al., *Circadian neurons in the paraventricular nucleus entrain and sustain daily rhythms in glucocorticoids.* Nat Commun, 2021. **12**(1): p. 5763.

109. Charmandari, E., C. Tsigos, and G. Chrousos, *Endocrinology of the stress response.* Annu Rev Physiol, 2005. **67**: p. 259-84.
110. Gaffey, A.E., et al., *Alterations in Systemic and Cognitive Glucocorticoid Sensitivity in Depression.* Biol Psychiatry Cogn Neurosci Neuroimaging, 2019. **4**(3): p. 310-320.
111. Szeszko, P.R., A. Lehrner, and R. Yehuda, *Glucocorticoids and Hippocampal Structure and Function in PTSD.* Harv Rev Psychiatry, 2018. **26**(3): p. 142-157.
112. Okuliarova, M., et al., *Dim Light at Night Disturbs Molecular Pathways of Lipid Metabolism.* Int J Mol Sci, 2020. **21**(18).
113. Mishra, I., et al., *Light at night disrupts diel patterns of cytokine gene expression and endocrine profiles in zebra finch (Taeniopygia guttata).* Sci Rep, 2019. **9**(1): p. 15833.
114. Ohta, H., S. Yamazaki, and D.G. McMahon, *Constant light desynchronizes mammalian clock neurons.* Nat Neurosci, 2005. **8**(3): p. 267-9.
115. Weinert, D., et al., *Resynchronization of the circadian corticosterone rhythm after a light/dark shift in juvenile and adult mice.* Chronobiol Int, 1994. **11**(4): p. 222-31.
116. Walker, W.H., 2nd, et al., *Circadian rhythm disruption and mental health.* Transl Psychiatry, 2020. **10**(1): p. 28.
117. Alaasam, V.J., et al., *Light at night disrupts nocturnal rest and elevates glucocorticoids at cool color temperatures.* J Exp Zool A Ecol Integr Physiol, 2018. **329**(8-9): p. 465-472.
118. Hoffmann, J., R. Palme, and J.A. Eccard, *Long-term dim light during nighttime changes activity patterns and space use in experimental small mammal populations.* Environ Pollut, 2018. **238**: p. 844-851.
119. Yamazaki, M., et al., *Regulated expression of an actin-associated protein, synaptopodin, during long-term potentiation.* J Neurochem, 2001. **79**(1): p. 192-9.
120. Verbich, D., et al., *Rewiring neuronal microcircuits of the brain via spine head protrusions--a role for synaptopodin and intracellular calcium stores.* Acta Neuropathol Commun, 2016. **4**: p. 38.
121. Kalén, P., et al., *Hippocampal Noradrenaline and Serotonin Release over 24 Hours as Measured by the Dialysis Technique in Freely Moving Rats: Correlation to Behavioural Activity State, Effect of Handling and Tail-Pinch.* Eur J Neurosci, 1989. **1**(3): p. 181-188.
122. Deller, T., et al., *Changes in the actin-associated protein synaptopodin in the rat fascia dentata after entorhinal cortex lesion: morphological evidence for a role in the dendritic plasticity of CNS neurons.* Ann Anat 1999. **181**.
123. Krugers, H.J., et al., *Exposure to chronic psychosocial stress and corticosterone in the rat: effects on spatial discrimination learning and hippocampal protein kinase Cgamma immunoreactivity.* Hippocampus, 1997. **7**(4): p. 427-36.
124. Chai, A.P., et al., *Chronic constant light-induced hippocampal late-phase long-term potentiation impairment in vitro is attenuated by antagonist of D1/D5 receptors.* Brain Res, 2015. **1622**: p. 72-80.
125. Mrosovksky, N., *Aschoff's rule in retinally degenerate mice.* J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol., 2003(189): p. 75-78.
126. Zhou, Y., et al., *Altered Light Conditions Contribute to Abnormalities in Emotion and Cognition Through HINT1 Dysfunction in C57BL/6 Mice.* Front Behav Neurosci, 2018. **12**: p. 110.
127. Ketelauri, P., et al., *Acute Circadian Disruption Due to Constant Light Promotes Caspase 1 Activation in the Mouse Hippocampus.* Cells, 2023. **12**(14).
128. Kumari, R., et al., *Differential response of diurnal and nocturnal mammals to prolonged altered light-dark cycle: a possible role of mood associated endocrine, inflammatory and antioxidant system.* Chronobiol Int, 2021. **38**(11): p. 1618-1630.

129. Bartsch, J.C., et al., *Stress-Induced Enhanced Long-Term Potentiation and Reduced Threshold for N-Methyl-D-Aspartate Receptor- and beta-Adrenergic Receptor-Mediated Synaptic Plasticity in Rodent Ventral Subiculum.* Front Mol Neurosci, 2021. **14**: p. 658465.
130. Soler, J.E., et al., *Light modulates hippocampal function and spatial learning in a diurnal rodent species: A study using male nile grass rat (Arvicanthis niloticus).* Hippocampus, 2018. **28**(3): p. 189-200.
131. Wosiski-Kuhn, M. and A.M. Stranahan, *Transient increases in dendritic spine density contribute to dentate gyrus long-term potentiation.* Synapse, 2012. **66**(7): p. 661-4.

ACKNOWLEDGEMENT

It is a genuine pleasure to express my deep gratitude to the following people who have contributed significantly to the realization of this work:

Prof. Dr. Charlotte von Gall, for being an outstanding mentor. Your constant support, encouragement, and welcoming attitude made me feel like a valued member of the team, and your guidance was instrumental in making this work possible.

Jun.-Prof. Dr. Nadine Erlenhardt, for introducing new and intriguing perspectives from your area of research, enriching this study.

Dr. Federica Tundo-Lavalle, for your exceptional supervision and unwavering support. I greatly valued our teamwork and collaboration over the past few years. From you, I learned invaluable lessons about structured work and overcoming challenges in scientific research. Your dedication to this project and your open-minded approach have been truly inspiring.

Dr. Amira Ali, for your encouragement, insightful discussions, and for always thinking of us when encountering relevant literature. Your thoughtful contributions have significantly enriched this work.

Dr. Laila Abdel-Hafiz, for your valuable contributions to this study.

Angelika and Johanna Hallenberger, for your professional and emotional support. Your camaraderie made the long hours in the lab much more enjoyable and added a spark of joy to the hard work.

Hanna Bellert, Ursula Lammersen, and Ralf Faßbender, for your encouragement and for introducing me to the methodologies essential for this research.

Thank you, Ilenia Tundo, for your excellent graphic design contributions.

Finally, I would like to acknowledge the support provided by Heinrich Heine University Düsseldorf, which played a vital role in enabling this study.