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ÚČINKY PSILOCYBINU NA KONSOLIDACI PAMĚTI U POTKANA

THE EFFECT OF PSILOCYBIN ON MEMORY CONSOLIDATION IN RATS

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Prohlášení autora práce

Prohlašuji, že jsem závěrečnou práci vypracoval samostatně a uvedl veškeré použité informační zdroje. Tato práce ani její podstatná část nebyla předložena k získání jiného či stejného akademického titulu.

Bc. Kembe Chona

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Bc. Kembe Chona

Poděkování

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Abstract

Psychedelics are currently being researched due to their long-lasting antidepressant, anxiolytic and neuroplasticity inducing actions. The mechanism by which they induce these effects remains poorly understood. Here, we decided to investigate a relatively unexplored possibility. A potential interaction of psilocybin administration and sleep and their combined effects on memory consolidation. Memory formation in animals and humans is greatly influenced by sleep manipulation which led us to assess the possibility with spatial memory tasks. We hypothesized that acute psilocybin administration after learning may have a beneficial influence on memory consolidation in rats. To determine whether an interaction with sleep exists we also subjected the rats to combinations of psilocybin and control vehicle with normal sleep and sleep deprivation. Our data did not suggest such an interaction exists. Secondly, we tried to find out if psilocybin and sleep manipulation leads to changes in neuroplasticity-related events. A process that could very likely be the basis of such a proposed beneficial effect. For this purpose, we analysed the expression of the immediate-early gene Arc and the immature neuron marker doublecortin in the rat hippocampus. Doublecortin's expression was not influenced by any of the factors. However, there was a trend towards upregulation of Arc in animals that had received psilocybin and were allowed to sleep normally. Although psilocybin doesn't appear to affect memory consolidation it may increase neuroplasticity in the rat. Finally, we monitored the effect of psilocybin on sleep architecture by an EEG recording biotelemetric module. Psilocybin administration led to increased wakefulness and a reduction of paradoxical sleep compared to controls.

Keywords: psilocybin, sleep deprivation, memory consolidation, 5-HT_{2A}R, psychedelics

Abstrakt

Psychedelika jsou v současné době intenzivně zkoumána pro svůj dlouho trvající antidepresivní účinek, pozitivní vliv na úzkosti, náladu a neuroplasticitu. Mechanismus, kterým však tyto účinky způsobují je i nadále málo vysvětlen. V této práci jsme se zaměřili na aspekt jejich působení, který byl doposud téměř neprozkoumán. Je jím potenciální provázanost působení psilocybinu a vlivu spánku na konsolidaci paměti. Tvorba paměti je u zvířat i lidí velmi zásadně ovlivněna kvalitou spánku, což nás vedlo k tomu zhodnotit tyto účinky pomocí prostorových paměťových úloh. Naší hypotézou bylo, že dávka psilocybinu aplikovaná po učení by mohla mít pozitivní vliv na konsolidaci takto vzniklé paměti u potkana. Abychom určili, zda-li existuje nějaká interakce se spánkem, podrobili jsme potkany různým kombinacím aplikace psilocybinu a kontrolního vehiklu se spánkem a spánkovou deprivací. Výsledky behaviourálních úloh nepotvrdily, že by k takové interakci docházelo. Dále jsem se zabývali také vlivem psilocybinu a manipulace se spánkem na neuroplasticitu. Sledovali jsme expresi časného raného genu, Arc proteinu, a markeru nevyspělých neuronů doublecortinu v hippokampu. Exprese doublecortinu nebyla ovlivněna žádným z faktorů, nicméně exprese Arc proteinu měla v případě zvířat, která obdržela psilocybin a mohla v klidu spát, tendenci být zvýšená. Ačkoliv psilocybin se spánkem pravděpodobně nemá vliv na konsolidaci paměti, zdá se, že může vést ke zvýšené produkci markerů neuroplasticity. Závěrem jsme také pozorovali vliv psilocybinu a spánkové deprivace na architekturu spánku pomocí biotelemetrického modulu. Aplikace psilocybinu u potkanů zvyšovala bdělost a snižovala podíl REM spánku.

Klíčová slova: psilocybin, spánková deprivace, konsolidace paměti, 5-HT_{2A}R, psychedelika

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LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine, serotonin
5-HT(1-7)R	5-hydroxytryptamine receptor
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
PLC-β	beta-type phospholipase C
CA1/2/3	Cornu ammonis 1/2/3
cAMP	Cyclic AMP
CNS	Central nervous system
DCX	Doublecortin
DG	Dentate gyrus
DOI	2,5-dimethoxy-4-iodoamphetamine
DOW	Disk over water
ECoG	Electrocorticogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
EPSP	Excitatory postsynaptic potential
ERK	Extracellular-regulated kinase
GABA	γ-aminobutyric acid
GH	gentle handling
GPCR	G-protein coupled receptor
HTR	Head twitch response
IEG	Immediate early gene
IHC	Immunohistochemistry
IPSP	Inhibitory post-synaptic potential
LSD	Lysergic acid diethylamide
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
MDL 11,939	α-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol
mGlu2/3	Metabotropic glutamate receptor type 2 and 3
MWM	Morris water maze
NMDA	N-methyl-D-aspratate
NPC	Neural progenitor cells
NREMS	Non-rapid eye movement sleep
OPR	Object-place recognition task
PFC	Prefrontal cortex
PKC	Protein kinase type C
PNS	Peripheral nervous system
PPI	Prepulse inhibition
TCB-2	4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl) methylamine hydrobromide
PS	paradoxical sleep
REMS	Rapid eye movement sleep
SD	Sleep deprivation
SWS	Slow-wave sleep
TSD	Total sleep deprivation
W	wakefulness
WDS	Wet dog shake

1 INTRODUCTION

Serotonergic hallucinogens, also known as psychedelics, are a distinct group of psychoactive substances of both synthetic and natural origin which exhibit a strong influence on cognition, perception, and emotion (Nichols, 2016). Historically psychedelics have been used as valuable research tools in the quest for understanding the neurobiology of certain psychiatric diseases. This led to the introduction of the term *psychotomimetics* suggesting an ability to induce psychosis-like effects. The psychotomimetic properties of psychedelics have helped researchers gain a deeper understanding of the possible aetiology of psychotic diseases like schizophrenia and their underlying mechanisms. The psychosis models have among other things helped shape one of the most widely accepted hypotheses concerning the origins of schizophrenia — the serotonergic hypothesis.

Furthermore, an increasing body of evidence suggests that psychedelics could potentially serve as therapeutic agents for various mental disorders (major depressive disorder, drug and alcohol abuse, anxiety disorders). A point further validated by the American's FDA recent labelling of certain psychedelics (psilocybin and ketamine) as "breakthrough therapies". In addition, unlike most drugs, psychedelics appear to be free of any addictive properties or severe adverse effects. For these convincing reasons, it seems only natural that psychedelic research has experienced a renaissance in recent years.

Another interesting feature of psychedelics is their potential effect on certain mental abilities like memory, creativity or problem solving and overall wellbeing (Nichols, 2016; Schmid et al., 2015; Sweat et al., 2016). Although a large amount of the data remains anecdotal as it is based primarily on self-assessment and personal experiences there has been a surge of controlled studies looking to assess the potential benefits of psychedelic administration (Davis et al., 2020; Griffiths et al., 2016; Holze et al., 2021; Schindler et al., 2020). To date, very little effort had been made to explore the potential interaction of psychedelics and sleep and their combined effect on memory consolidation. Some authors have suggested that such an interaction may exist(Froese et al., 2018; Rambousek et al., 2014) which led us to pursue this idea in this thesis.

2 SEROTONERGIC PSYCHEDELICS AND PSILOCYBIN

The structure of classical serotonergic psychedelics is similar to the endogenous neurotransmitter serotonin (5-hydroxytryptamine or 5-HT). It is widely accepted that psychedelics mediate their effects primarily through 5-HT_{2A} receptor agonism. The physiological effects in humans are mildly sympathomimetic. The administration is typically followed by a mild increase in blood pressure and heart rate, mydriasis, and nausea. Unlike other psychoactive substances, psychedelics appear to lack any toxic or addictive properties (Nichols, 2016). Indole alkylamines psilocybin and its active metabolite psilocin (the terms may be used interchangeably for this work) are the main psychoactive alkaloids contained in hallucinogenic mushrooms (Laussmann and Meier-Giebing, 2010; Nichols, 2016). Hallucinogenic mushrooms also known as magic mushrooms are a polyphyletic group that tend to contain a mix of alkaloids psilocybin, psilocin, baeocystin and norbaeocystin. The action of baeocystin and norbaeocystin remains poorly understood but neither seems to produce any hallucinogenic effects (Sherwood et al., 2020).

2.1 EFFECTS OF PSILOCYBIN

2.1.1 EFFECTS ON HUMANS

Psilocybin's acute effects in humans are characterized by changes in cognition, perception and emotions, and even psychotic-like states, similar to early stages of schizophrenia (Nichols, 2016; Vollenweider et al., 1999). Like other psychedelics, psilocybin produces mildly sympathomimetic effects which typically manifest through an increased heart rate, blood pressure and mydriasis (Tylš et al., 2014). Other physiological symptoms may include dizziness, nausea, and drowsiness. Intoxication is usually accompanied by the attenuation of certain neurocognitive parameters (e.g., visual processing) and disruption of sensorimotor processing (Tylš et al., 2014), yet again features typically observable in psychotic patients (Vollenweider et al., 1999). The overall manner and intensity of intoxication is dose-dependent but also relies on the so-called "set and setting". Set refers to the user's mindset and psychological state before administration while setting refers to the environment in which the experience takes place. It should be noted that under uncontrolled circumstances the emotional intensity of the psychedelic experience can lead to adverse effects, especially with inexperienced

users, and may even lead to dangerous behaviour. These factors can however be controlled with a cautious approach and proper experimental environment (Johnson et al., 2008). Lower doses typically induce a heightened perception of light, colours, and contours. The user may also perceive music to be livelier and more pleasurable. Moderate doses typically lead to more pronounced visual distortions and pseudo illusions; objects begin to move and pulsate. Even after closing their eyes users may see colourful geometric patterns. Thoughts become frantic and chaotic, creativity and imagination tend to heighten while executive functioning and reasoning are greatly reduced. High doses induce changes in visual perception (pseudo illusions and illusions), sensory synaesthesia (feeling colours, hearing smells). Very high doses can also lead to profound mystical experiences characterised by a sensory disconnection from one's physical presence, a sense of unity with the universe or a seeming loss of self (ego-dissolution or ego-death) (Bayne & Carter, 2018). Psilocybin appeared to not affect spatial working memory (Carter, Burr, Pettigrew, & Vollenweider, 2005).

2.1.2 Effects on animals

The obvious lack of verbal feedback in animals leaves us to only speculate about the possible psychological effects on rodents but there are some well-recognised changes in behaviour. Another limitation lies in the fact that unlike humans, who use sight as the primary sensory domain, rodents primarily rely on tactile and olfactory stimuli (Burn, 2008). Despite these shortcomings, we can still assume that some behavioural paradigms are valid research tools for translational research. Behavioural models typically observe two classes of effects: 1) behaviours that are analogous to the effects in humans and 2) effects in models with no reliable human counterpart (Halberstadt & Geyer, 2018).

Following psilocybin administration, animals display symptoms of sympathetic arousal (Passie et al., 2002). After administering doses of around 10 mg/kg to rodents (mice and rabbits) Cerletti (1958) observed — mydriasis, piloerection, irregularities in heart rate and hyperventilation. Rodents also display a distinct behavioural effect when exposed to psilocybin – the head twitch response (HTR). HTR is a characteristic side to side head movement that occurs after administering higher doses of serotonergic psychedelics and other 5-HT_{2A} agonists (Halberstadt & Geyer, 2013). The movement is often accompanied by whole body shakes and bobs and is sometimes referred to as the "wet

dog shake" (WDS). Serotonergic psychedelics disrupt the prepulse inhibition (PPI) response in rats which indicates their capacity to impair sensorimotor gating (Halberstadt & Geyer, 2018; Ouagazzal et al., 2001; Páleníček et al., 2010, 2013; Sipes & Geyer, 1994). This notion was further supported by treatment with 5-HT_{2A} antagonists which blocked the effect on PPI (Padich et al., 1996; Sipes & Geyer, 1995). Halberstadt et al. have demonstrated that psilocybin dose-dependently reduced locomotion in mice (Halberstadt et al., 2011). Psilocin has been shown to impair memory retrieval but not memory consolidation in rats (Rambousek et al., 2014).

2.2 PHARMACOLOGY

Following administration, psilocybin is rapidly dephosphorylated to form psilocin. This occurs under the acidic environment of the stomach or through phosphatases or non-specific esterases in the intestine, kidneys, and bloodstream (Horita & Weber, 1961, 1962). Psilocybin acts as a mixed agonist at serotonin receptors, mainly 5-$HT_{2A/C}$ and 5-HT_{1A} receptor subtypes (for a review, see Tylš, Páleníček, and Horáček 2014) with a predominant activity at 5-HT_{2A} receptors and, to a smaller extent, at 5-HT_{2C}R and 5-HT_{2B}R (Zhang et al., 2017; Zhang & Stackman Jr, 2015). The agonism at 5-HT_{2A}R is deemed necessary for the hallucinogenic effect in rodents (González-Maeso et al., 2007a) and humans alike (Madsen et al., 2019), but the role of other receptor subtypes remains poorly understood. In 1962, Kalberer et al. (1962) described in detail the fate of psilocin after being administered to rats. Their paper showed psilocin to be excreted in urine (65 %) and bile and faeces (15-20 %) within 8 hours following oral administration. 10-20 % of the compound persisted in the organism for a prolonged period with some metabolites being detected after several days. Around a quarter of the whole dose was shown to be excreted unaltered. The chemical structure of psilocybin, psilocin and 5-HT is shown in Fig. 1.

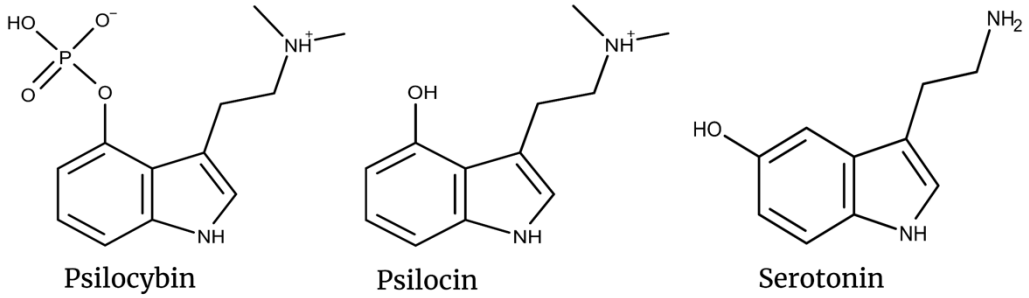

FIGURE 1: THE CHEMICAL STRUCTURES OF PSILOCYBIN, PSILOCIN, AND THE ENDOGENOUS NEUROTRANSMITTER SEROTONIN SHOWS THEIR CLOSE RESEMBLANCE (CREATED WITH WWW.BIORENDER.COM)

3 SEROTONERGIC SYSTEM

The serotonergic system is an old receptor system and plays an important role in various biological processes. Serotonin is an endogenous neurotransmitter that acts at sites in the CNS, the PNS as well as in non-neuronal tissue (e. g. gastrointestinal tract, heart). 5-HT generated in mammalian brains is produced by specialized neuronal assemblies known as the raphe nuclei (Nichols & Nichols, 2008). These key anatomical structures located in the reticular formation of the brainstem are responsible for the synthesis and distribution of 5-HT. Their efferents project to various areas of the CNS (Moore et al., 1978). This chapter will briefly overview the 5-HT receptors and their function but will mostly focus on the 5-HT_{2A}R.

5-HT receptors play a crucial role in mediating many physiological processes such as smooth muscle contraction, modulation of cognition and mood or platelet aggregation(Hoyer et al., 2002). Certain subtypes are highly expressed in the hippocampus and prefrontal cortex (PFC) and thus tightly linked to cognitive function (Hannon & Hoyer, 2008; Hoyer et al., 2002; Meneses, 1999).

Current nomenclature classifies seven groups of 5-HT receptors subtypes (5-$HT_{1A/1B/1D/1E/1F}$, 5-$HT_{2A/2B/2C}$, 5-$HT_{3A/3B}$, 5-$HT_{4A/4B/4C/4D}$, 5-$HT_{5A/5B}$, 5-HT_6 and 5-$HT_{7A/7B/7C/7D}$)(Hoyer et al., 1994; Meneses, 1999) with all except 5-HT_3 (a cation-

permeable ligand-gated ion channel) belonging to group A of the GPCR superfamily (Hannon & Hoyer, 2008; Nichols & Nichols, 2008). 5-HT_1 receptors are functionally coupled to G_i and or G_0 proteins and resulting in decreased cAMP formation (Hannon & Hoyer, 2008)., 5-HT_2 receptors to G_q, and 5-HT_4, 5-HT_6 and 5-HT_7 receptors to G_s (Meneses & Perez-Garcia, 2007). Using electrophysiology, studies have shown that activation of 5-HT_1 receptors leads to hyperpolarization(Aghajanian et al., 1990), whereas 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 cause depolarization (Hannon & Hoyer, 2008; Meneses & Perez-Garcia, 2007). The 5-HT system sends projections to virtually all parts of the forebrain (Hannon & Hoyer, 2008), which together with the ever-growing body of evidence indicates that the 5-HT system in coaction with other neurotransmitter systems (Steckler & Sahgal, 1995) plays a major role in memory and learning (Meneses & Perez-Garcia, 2007; Steckler & Sahgal, 1995). Pharmacological manipulations which induced 5-HT release or blocked 5-HT uptake indicate that elevated levels of 5-HT maintain and improve memory (du Jardin et al., 2014; Kuypers & Ramaekers, 2005; Meneses, 2003; Micheau & van Marrewijk, 1999). Conflicting results have however also been demonstrated (Carli et al., 1995). Lower levels of 5-HT or blocking of 5-HTRs on the other hand have been shown to cause memory and learning impairments (Fedotova & Ordyan, 2010; Glikmann-Johnston et al., 2015). Consolidation of learning was impaired by presynaptic activation of 5-HT_{1B}, 5-HT_3 or 5-HT_4 or blockade of postsynaptic 5-$HT_{2C/2B}$ receptors. For example, Meneses et al. (Meneses & Hong, 1997) showed consolidation was induced by presynaptic activation of 5-HT_{1A}, 5-HT_{2C}, and blockade of presynaptic 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors. blockade of postsynaptic 5-HT_{1A},5-HT_{1B},5-HT_3 or 5-HT_4 receptors. Blockade of postsynaptic 5-HT_{1A},5-HT_{1B},5-HT_3 or 5-HT_4 receptors and 5-HT depletion did not alter learning by themselves.

3.1 5-HT_{2A}R DISTRIBUTION AND FUNCTION

The 5-HT_{2A} receptor was first identified by Snyder and Peroutka in 1979 (Peroutka & Snyder, 1979). The receptor was later successfully cloned – rat variant in 1988 (Pritchett et al., 1988), the human variant in 1990 (Branchek et al., 1990). The 5-HT_2 class of receptors exhibits its effects via the $G_{\alpha q/11}$ subunit pathway. The coupling with this G-protein subunit stimulates the activity of beta-type phospholipase C (PLC-β) resulting in an increased rate of inositol phosphates hydrolysis and ultimately leading to elevated

levels of cytosolic Ca^{2+} and increasing spontaneous excitatory postsynaptic currents (Hoyer et al., 2002). Although the classical PLC-β pathway appears to be crucial it is not sufficient on its own to produce the effects of psychedelics (González-Maeso et al., 2007b; Kurrasch-Orbaugh, Watts, et al., 2003). There is substantial evidence of several non-canonical signalling pathways coupled to 5-HT_{2A} receptors (as shown in Fig. 2). That includes the actions of *Src,* β-arrestin-2, extracellular-regulated kinase (ERK), p38 mitogen-activated protein (MAP) kinase, phospholipase A_2 (downstream from ERK 1,2 and p38 MAP kinase), Akt, and phospholipase D (Barclay et al., 2011; González-Maeso et al., 2007b; Kurrasch-Orbaugh, Parrish, et al., 2003; C. L. Schmid & Bohn, 2010).

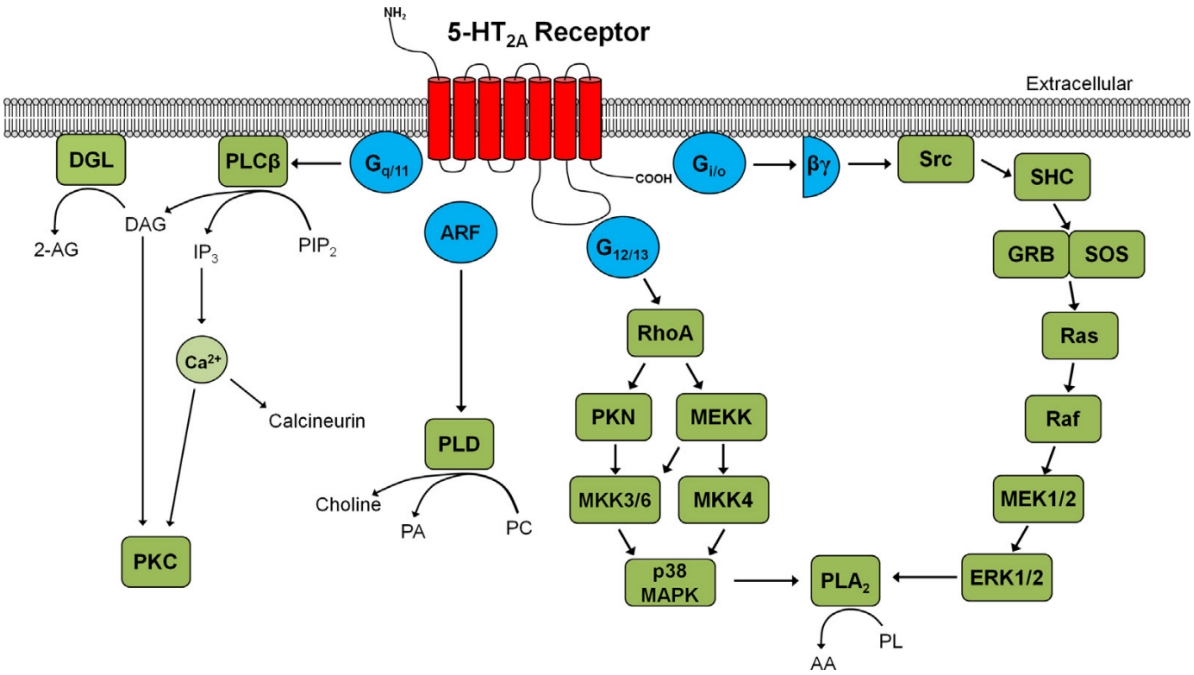

FIGURE 2: SIGNALLING PATHWAYS COUPLED TO THE 5-HT_{2A} RECEPTOR. ABBREVIATIONS: AA, ARACHIDONIC ACID; 2-AG, 2-ARACHIDONOYLGLYCEROL; ARF, ADP-RIBOSYLATION FACTOR-1; DAG, DIACYLGLYCEROL; DGL, DIACYLGLYCEROL LIPASE; ERK1/2, EXTRACELLULAR-REGULATED KINASES 1 AND 2; GRB, GROWTH FACTOR RECEPTOR-BOUND PROTEIN 2; IP_3, INOSITOL TRIPHOSPHATE; P38 MAPK, P38 MITOGEN-ACTIVATED PROTEIN KINASE; MEK1/2, MITOGEN/EXTRACELLULAR SIGNAL-REGULATED KINASES 1 AND 2; MKK3/6, MAPK KINASES 3AND 6; MKK4, MAPK KINASE 4; MEKK, MAPK KINASE KINASE; PA, PHOSPHATIDIC ACID; PC, PHOSPHATIDYLCHOLINE; PIP_2, PHOSPHATIDYLINOSITOL 4,5-BIPHOSPHATE; PKC, PROTEIN KINASE C; PKN, PROTEIN KINASE N; PL, PHOSPHOLIPIDS; PLCB, PHOSPHOLIPASE CB; PLD, PHOSPHOLIPASE D; SHC, SRC HOMOLOGY 2 DOMAIN-CONTAINING TRANSFORMING FACTOR; SOS, SON OF SEVENLESS HOMOLOG. ADOPTED FROM HALBERSTADT (2015).

The receptor's abundance throughout the CNS has been consistently verified by radioligand binding assay as well as mRNA detection. 5-HT_{2A} receptors also appear to be the primary excitatory receptors in the hippocampus due to their abundance. It should be noted that despite its apparent importance the 5-HT_{2A}R was shown to have the lowest affinity for 5-HT out of all the 5-HT receptor subtypes (Hoyer et al., 2002).

5-HT_{2A}R activation is also tightly bound with the glutamate system, namely the action of metabotropic glutamate receptors 2 and 3 (mGlu2 and mGlu3). These receptors are colocalized in the frontal cortex and have been shown to form mGlu–5-HT_{2A} complexes. Interestingly mGlu2-KO mice were shown to be unaffected by hallucinogens on both a cellular and behavioural level (Moreno et al., 2011). They also modulate the action of GABAergic interneurons (Hannon & Hoyer, 2008).
The serotonergic system mediates its effect on the CNS at three key anatomical sites;

1. Raphe nuclei are tonically active during vigilance and have been inhibited by the administration of psychedelics (Aghajanian & Hailgler, 1975; Aghajanian et al., 1970). It was subsequently shown that agonist activation at 5-HT_{1A} autoreceptors leads to presynaptic inhibition of raphe nuclei neurons (Sprouse & Aghajanian, 1988). Raphe cells can also be indirectly inhibited through stimulation of 5-HT_{2A} receptors on modulatory GABAergic neurons (Liu et al., 2000; Zhou & Hablitz, 1999). The reduced activity of rapheal neurons results in a decline of 5-HT production which in turn leads to a decrease of 5-HT distribution in the brain.

2. Thalamus being the key processing site for somatosensory input is essential in the action of psychedelics. 5-HT_{2A} receptors are primarily expressed in the reticular and non-specific nuclei (Cornea-Hé Bert et al., 1999). 5-HT_{2A} had been identified in the reticular nucleus, lateral geniculate nucleus, ventromedial and anterodorsal nuclei, and zona incerta (Cyr et al., 2000; Pompeiano et al., 1994). The reticular nucleus is believed to regulate the flow and exchange of information between the cortex and the thalamus. It consists of GABAergic neurons which form distinct efferent and afferent connections with other thalamic nuclei, the neocortex, the brainstem, and the basal forebrain (Nichols, 2016). Rodriguéz et. al described the presence of 2 major receptor subtypes in the reticular nucleus – 5-HT_{2A}R and 5-HT_{1A}R (Rodríguez et al., 2011). Psychedelics likely cause their specific effects by disinhibiting the regulatory function of the reticular nucleus.

3. Neocortex. 5-HT_{2A} receptor activation affects glutaminergic and GABAergic neurons and thus generates an increase in both excitatory and inhibitory postsynaptic potentials (EPSPs) (Aghajanian & Marek, 1997) and (IPSPs) (Zhou & Hablitz, 1999)(Halberstadt, 2015; Mengod et al., 2015; Nichols, 2016; Nichols & Nichols, 2008). The excitatory effect

is most pronounced in the medial PFC and lamina V (Aghajanian & Marek, 1997; Blue et al., 1988) where it increases glutamate release and leads to activation of postsynaptic AMPA receptors. This notion was further supported by microdialysis data which showed increased levels of glutamate(Muschamp et al., 2004; Scruggs et al., 2003) and GABA (Abi-Saab et al., 1999) after the administration of 5-HT_{2A} agonists. Although the effect is seemingly ambivalent it supports the basic idea of psychedelics severely disrupting the processing ability of cortical structures. Together with the impaired function of the thalamus, this leads to a substantial shift in noise to signal ratio.

3.2 5-HT_{2A}R REGULATION

Aside from triggering signal transduction cascades in the cell agonist activation of GPCRs also induces molecular mechanisms which in turn lead to decreased receptor signalling. GPCRs responsiveness to agonist stimulation is known as receptor desensitisation. Other regulatory phenomena are also recognized when describing receptors. These include the reverse process of resensitisation, a reestablishment of receptor responsiveness, and down-regulation, a reduction in the total number of functioning receptors.

Even though the exact mechanism of 5-HT_{2A} desensitisation remains incompletely understood PKC activation had been shown to play a role (Rahimian & Hrdina, 1995; Rahman et al., 1995). However, it has also been shown that down-regulating PKC isoforms had no effect on rapid (10 minutes to 2h) or delayed (6h) phases of agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) induced desensitisation, but only attenuated the intermediate phase roughly 2-4h post-treatment (Roth et al., 1995). Interestingly, 5-HT_{2A}Rs were shown to respond paradoxically to stimulation by antagonists which also resulted in decreased responsiveness (Gray & Roth, 2001).

Arrestins are likely not essential in the desensitisation of 5-HT_{2A} even though they play an important role in this cascade in other GPCRs. It's not ubiquitously expressed in the CNS and likely has a cell-specific function.

Aside from short term regulation by desensitisation GPCRs function is also modulated on a long-term scale through the process of downregulation. Several studies have linked the downregulation of 5-HT_{2A} to the development of tolerance to various psychedelics after repeated exposure. Buckholtz et. al reported that daily LSD administration resulted in selective reduction of 5-HT_{2A} (Buckholtz et al., 1985, 1988). Daily

administration of several 5-HT_{2A} agonists also lead to a similar decrease in receptor binding while an acute dose produced no change in binding (Buckholtz et al., 1988). Recently a study conducted on pigs showed that a single dose of psilocybin increased synaptic density and decreased 5-HT_{2A}R density in the hippocampus (Raval et al., 2020).

4 SLEEP

Sleep can be defined as a reversible and physiologically distinct state with reduced responsiveness to sensory stimuli and mobility. While we cannot determine a single central function of sleep, it is certainly abundant in most species of the animal kingdom and plays a key role in important neurobiological processes. It is safe to assume that sleep functions as a period of restoration for exhausted brain metabolism. However, this is only a limited portion of the various complex processes which take place in the sleeping brain.

4.1 HUMAN SLEEP

Sleep is subdivided into 2 major cyclical phases: rapid eye movement sleep (REMS, paradoxical sleep, active sleep) and non-REM sleep (NREMS) which is subdivided into 4 stages. NREMS is characterized as a deep body rest state. The so-called early sleep is typically dominated by NREMS in humans, while late sleep consists mostly of REM sleep. The sleep cycle begins with the lightest NREM stage 1 sleep. The subject can be awakened easily during this stage and EEG measurements of brain activity are characterized by transitions from alpha waves (8-12 Hz) to mixed frequencies. Followed by NREM stage 2 sleep, the sleeping subject's responsiveness to external stimuli decreases. EEG measurements begin to contain K-complexes and low amplitude sleep spindles (Diekelmann & Born, 2010). NREM sleep stages 3 and 4 form a distinct portion of sleep known as slow-wave sleep (SWS) characterized by slow-wave, high amplitude oscillations. The division into stages 3 and 4 has recently been abandoned and is now collectively referred to as stage 3 NREMS. During SWS the subject's responsiveness to stimuli further decreases. EEG measurements during SWS contain delta wave activity (0,5-3 Hz). The final stage, REM sleep, gained its name due to the occurrence of eye movements but is also characterized by muscle atonia and twitches, activation of various brain areas and changes in respiration, pulse rate and blood

pressure. REM sleep is often referred to as paradoxical sleep because it contains EEG patterns that are very similar to those found during wakefulness (Stenberg, 2007).
A sleeping subject will typically cycle through NREM and REM about 5 times per night while each cycle lasts for about 90 minutes. The proportion of REM sleep in each cycle tends to progressively increase with each new cycle (Diekelmann & Born, 2010). Fig. 3 illustrates the differences between sleep patterns typically observed in humans and rodents.

4.2 RODENT SLEEP

Unlike humans who are monophasic sleepers, rats are polyphasic sleepers. This means that they cycle through several episodes of sleep throughout both the dark (active) and light (quiescent) phases. Since rats are nocturnal species a higher percentage of sleep occurs during the light phase while having more consolidated periods of waking during the dark phase (Mong & Cusmano, 2016). Rats sleep close to 13 h daily and they have been shown to possess all the sleep stages observed in human sleep (Timo-Iaria et al., 1970).
Hoshino et al. offer an even more detailed division of distinct phases of rodent sleep based on EEG signals (Hoshino et al., 2015):

- Alert or active wake
- Relaxed or quiet wake
- Synchronized or slow-wave sleep phase I (SS_I phase)
- Synchronized or slow-wave sleep phase II (SS_{II} phase)
- Synchronized or slow-wave sleep phase III (SS_{III} phase)
- Pre-paradoxical phase
- Paradoxical phase

The actual sleep pattern of the animal does not necessarily have to follow the order of the given sequence. This may prove as somewhat of a problem when comparing rat sleep patterns with humans. For humans, a single sleep cycle is commonly understood as going from one REMS episode to another. Rats, on the other hand, will often cycle through SWS to waking without any REMS episodes (Simasko & Mukherjee, 2009). It is

still possible to say that although sleep phases will commonly fluctuate in the first sleep cycles, they become more regular as sleep progresses (Hoshino et al., 2015).

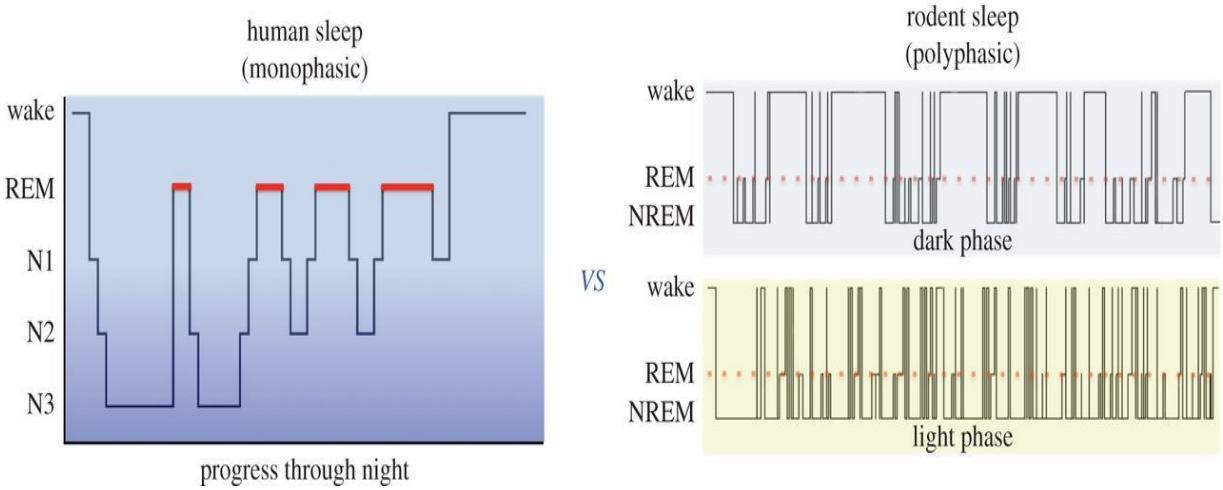

FIGURE 3: COMPARISON OF TYPICAL HYPNOGRAMS REPRESENTING THE SLEEP PATTERNS OF HUMANS AND RODENTS. ADOPTED FROM MONG & CUSMANO ET AL. (2016)

4.3 SLEEP AND MEMORY FORMATION

A major function of sleep is linked to memory. A growing majority regards sleep as an active phenomenon that utilizes regions of the brain typically active during information processing and wakefulness. Recent evidence also further suggests that sleep may serve as an ideal time window that permits the brain to consolidate new memories free from external sensory interferences (Diekelmann & Born, 2010).

Sleep itself primarily promotes memory consolidation whereas memory retrieval and encoding typically arise during wakefulness. The hippocampus is of particular interest in studies focused on memory as it appears to have an essential role in memory consolidation. Sleep is thus considered essential for the formation of declarative memory (Burgess et al., 2002; Diekelmann & Born, 2010).

Two major hypotheses are aiming to explain the mechanisms of consolidation:

1) The synaptic homeostasis hypothesis assumes that encoding of information during waking results in synapses becoming potentiated. Consolidation then arises during SWS when slow waves induce global synaptic downscaling resulting in selectively enhanced synapses and a better signal-to-noise ratio (Tononi & Cirelli, 2006).

2) The active system consolidation hypothesis assumes memories are encoded in the hippocampal and neocortical networks (Diekelmann & Born, 2010). Sleep consolidation arises from the coordinated activity of the hippocampus and the neocortex, where it promotes both quantitative and qualitative changes in memory representation. Consolidation results from selective repeated re-activation of encoded memories during SWS (M. Wilson & McNaughton, 1993). The slow oscillations generated in the neocortex drive the re-activation of traces in the hippocampus in synchrony with sharp-wave ripples and thalamo-cortical spindles. The feed-forward effect grouped with the synchronous effect leads to a transfer of the reactivated memory traces from the hippocampus into the neocortex. The arriving hippocampal output then stimulates neuroplasticity at target sites in the neocortex. It should be noted that the two models do not oppose each other they may act simultaneously to optimize memory in sleep (Diekelmann & Born, 2010).

Declarative memory in humans involves the conscious decision-related recall of events, objects, faces and spatial arrangements (Burgess et al., 2002). Tasks that utilize spatial and episodic memory are therefore often used as a valid analogy of declarative memory in animals. Memory consolidation is a process whereby new and unstable memories that were encoded during wakefulness transform into more stable representations that are integrated into the already established network of long-term memories (Diekelmann & Born, 2010). Consolidation is typically accompanied by reactivation of the neuronal ensembles which were active during the encoding phase (Kudrimoti et al., 1999; Sutherland & McNaughton, 2000; Wilson & McNaughton, 1994). The fact that this reactivation occurs during bouts of sleep or awake rest further supports the notion of sleep as being crucial in memory consolidation (Buhry et al., 2011).

A large body of evidence also favours the notion that any given amount of post-learning sleep enhances consolidation of all memory components in humans (Fischer et al., 2002; Lahl et al., 2008; Plihal & Born, 1997; Tucker et al., 2006) and animals (Borquez et al., 2014; Cai et al., 2009; Sawangjit et al., 2017) alike. Some experiments also showed a consequential increase of sleep amount (especially REM) after learning (Lucero, 1970) and improved memory function after REM sleep (Smith & Rose, 1997). Sleep also tends

to favour the consolidation of explicitly encoded memories that are relevant to the subject's behaviour (Robertson et al., 2004).

Sleep research has tried to establish how different portions of sleep contribute to the formation of different aspects of memory. SWS appears to favour the consolidation of declarative aspects of memory, while REM sleep appears to favour the formation of nondeclarative memory (Diekelmann & Born, 2010). This is in line with the hypothesis of dual-processing which assumes that SWS sleep mediates the formation of declarative, hippocampus-dependent memory (Plihal & Born, 1997) and REM sleep mediates consolidation of non-declarative, hippocampus-independent memories (Tucker et al., 2006). However other studies have shown that both types of sleep can impact either component of memory (Sara, 2017). These findings highlight the fact that no given sleep stage can be directly linked to memory consolidation, but rather the underlying neurobiological mechanisms which occur during these stages, and that some of these are present across different sleep stages.

5 SLEEP DEPRIVATION

Studying the effects of sleep deprivation (SD) on humans and animals has been one of the most important approaches in sleep research. SD produces an array of adverse effects in subjects ranging from cognitive deficits, physiological disturbances to negative mood states. Animal models of sleep SD are particularly important tools when studying the effects of SD on memory. Several SD models are available with each having its pros and cons. It must be recognized that SD models may differentiate between *partial/state-dependent* and *total sleep deprivation (TSD).* The partial or state-dependent SD models mostly targets the REM phase of sleep but can be aimed at NREM phases as well. In the case of total SD, all sleep is non-selectively restricted in the animal for a designated period. The choice of technique, duration and pattern of SD should then be based on the desired aim of the study. Even with rigorous effort and careful execution, however, prolonged intervals of truly total SD can never be achieved (Colavito et al., 2013).

No approach can fully eliminate the factor of "microsleep" (short intrusions of sleep lasting a few hundred milliseconds to several seconds) which inevitably accumulate due to SD (Friedman et al., 1979). Furthermore, selective SD on a particular section of sleep ultimately has effects on the differential distribution of sleep spent in other sleep states (Colavito et al., 2013).

Automated methods typically utilize mobile mechanical devices which force the animal to actively move to avoid an unpleasant stimulus e.g., water leading to almost complete eradication of sleep. The device can move continuously or react specifically when sleep is detected. The biggest advantage of such techniques is the fact that the strength and frequency of the stimuli can be standardized across all groups and subjects. However, the nature of the stimuli may produce results that are consequential to stress and fatigue as opposed to SD itself (Colavito et al., 2013). A popular version of this method is the "disk over water" (DOW) method (Rechtschaffen et al., 1989; Rechtschaffen & Bergmann, 1995).

Other variations include placing the animal on a treadmill or in a rotating cylinder that forces the animal to walk. The rotating cylinder method of abolishing sleep managed to reduce total sleep time to about 3,8 % of the whole 24-hour period as opposed to about 47 % of sleep time in controls (Stefurak et al., 1977). The major disadvantage of such methods is that the physical activity required for active avoidance combined with the overall stressful nature of the paradigm may falsely attribute the results to SD as opposed to general stress-related processes (Campbell et al., 2002; Guzmán-Marín et al., 2003).

Another popular set of methods of SD utilizes various platforms placed over water. These methods include the inverted flowerpot/water-tank methods and mostly target REMS. Approximately 95% of REM sleep can be eliminated through this method, but it can do the same for about 40% of NREM sleep also (Grahnstedt & Ursin, 1985; Machado et al., 2004). The technique takes advantage of the fact that REM sleep is typically associated with reduced muscle tone. This procedure also results in a considerable amount of stress for the animal, which could once again make it difficult to distinguish the effects of SD from the effects of stress (Colavito et al., 2013). The modified multiple platform paradigm allows the animal to move around the tank and to interact with cohorts resulting in reduced stress from immobility and isolation (Colavito et al., 2013; Suchecki & Tufik, 2000).

SD by gentle handling (GH) method requires direct stimulation of the animals by the researcher. This approach aims to minimize the undesirable effects of forced locomotion and the overall stress of the other paradigms. To properly execute the GH method, the experimenter must be vigilant and attentive to the animals throughout the

whole SD session. During the experiment, animals are maintained in their home cages and the experimenter keeps the animal awake by gently handling it whenever the animal begins falling asleep (Colavito et al., 2013). The wakefulness of the animal can be continuously monitored through observation or by EEG records. As for the method of stimulation, the animals may be subjected to a few stimuli ranging from gentle contact by hand or an object (e.g., a soft brush), through mild noises, to light shaking or tapping of the home cage (Colavito et al., 2013).

Even with great care, the spurious effects of SD by handling cannot be fully eliminated. SD has been correlated with increased levels of stress-related corticosterone (Hairston et al., 2001; Meerlo et al., 2008). The same can be said of repeated handling which has been associated with elevated levels of circulating corticosterone as well as alterations in locomotion compared to control untouched animals (Meerlo et al., 2008). Another potential downside of this approach lies in the fact that different animals may display different levels of wakefulness/drowsiness and thus require different levels of stimulation. Even though varying levels of stimulation may lead to variability among subjects and may pose difficulty for standardization, the technique makes up for it with reduced amounts of stress (Colavito et al., 2013).

5.1 SLEEP DEPRIVATION EFFECTS

SD can have a serious impact on a neurobiological and functional level. SD can lead to learning and memory deficits, altered behavioural patterns, the altered firing pattern of relevant neuronal assemblies, changes in expression of 5-HT receptors and 5-HT concentration at relevant sites.

The notion of SD's deleterious effects on learning and memory has firm grounds based on results from human and animal studies. SD has been shown to impair learning and spatial memory in rodents in various paradigms such as the MWM (Saygin et al., 2017; Yang et al., 2008; Zhang et al., 2017), object recognition (Palchykova et al., 2006), radial arm water maze (Zagaar et al., 2012), and novel arm recognition task (Hagewoud, Havekes, et al., 2010). Hagewoud et al. have found that animals subjected to 6 hours of SD during their normal resting phase showed a decreased consolidation of fear memory as opposed to a group in which an equal amount of SD was carried out during their normal active phase (2010). Frank et al. found that SD by gentle handling reduced REM

sleep by up to 100% and NREM by up to 92 %(1998). Saygin et al. tested the effects of SD on spatial memory using the MWM paradigm. 4 groups were tested (control, TSD, REM SD and sleep fragmentation) and after 5 days of MWM, spatial memory formation was severely impaired in all treatment groups. They also tested 5-HT_{2A}R expression in the hippocampus and found it to be significantly increased in all treatment groups (2017).

Lopez-Rodriguez et al. subjected rats to 24h SD via DOW. Using microdialysis, they measured sustained elevation of 5-HT plasma levels in the posterior hippocampus (2003).

Eydipour et al. used a total of 24h SD by multiple platforms. Two groups received different doses of a 5-HT_4 receptor agonist or antagonist. Doses of 0,1; 0,01 µg/rat but not 0,001 or 0,0001 of both agonist and antagonist reduced memory acquisition and did not affect pain perception or locomotion. TSD impaired memory acquisition while the subthreshold doses of both drugs restored TSD-induced memory acquisition impairment (2020).

6 THE HIPPOCAMPUS

The rat hippocampal formation (hippocampus) is a bilateral structure in the medial temporal lobe of the brain where it sits between the cerebral cortex and the thalamus. It has a well-organized structure that is sometimes described as two interlocked C's. The first C-like structure is formed by the so-called Cornu Ammonis (CA) also known as the hippocampus proper. It is further divided into CA1, CA2 and CA3 regions. The second C-like structure is formed by the dentate gyrus (DG). A longitudinal cross-section of the hippocampus reveals the textbook depiction of hippocampal anatomical circuitry also known as the "trisynaptic loop". The entorhinal cortex is the source of major inputs from the cortex into the hippocampus. Its strongest projections travel through a perforate path into the DG region forming the first synapse. The entorhinal cortex is further subdivided into two distinct regions in rats. The medial entorhinal cortex is typically associated with spatial processing regions of the brain. The lateral entorhinal cortex is linked to item-recognition areas like the perirhinal cortex. Both regions receive projections from the prefrontal cortex PFC and olfactory cortex, but they also send out projections between each other. The DG then projects into the CA3 through the mossy

fibre pathway forming the second synapse. CA3 projects to the CA1 through the Schaffer collateral pathway forming the third synapse. CA1 projects back into the EC, closing the loop. There is also an important additional pathway aside from the main trisynaptic loop which is formed by CA3 axons that send collaterals and form synapses with other CA3 neurons. These recurrent collaterals are thought to provide an auto-associative function of the CA3 region (Knierim, 2015).

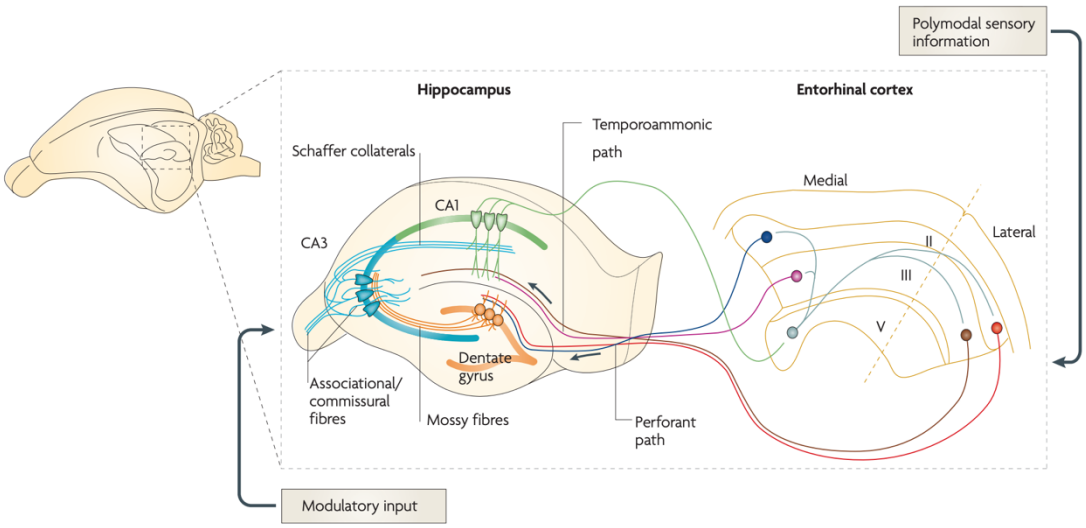

FIGURE 4 BASIC ANATOMY AND CIRCUITRY OF THE RAT HIPPOCAMPUS. ADOPTED FROM NEVES ET AL. (2008)

The trisynaptic loop was originally regarded as a unidirectional circuitry mainly contained within the lamellar portions of the hippocampus. It has since been shown, however, that the hippocampus contains various parallel processing and feedback circuits. Aside from projecting to DG, the EC also directly projects to the CA3 and CA1 regions. The CA3 region sends out a feedback projection to the DG via mossy fibres. The CA2 region which was previously regarded as a mere transition between CA3 and CA1 is now also being considered as a distinct unit with its computational significance (Knierim, 2015). The synapses are modulated at all levels by an array of inhibitory interneurons like basket or chandelier cells, which modulate and synchronize their activity.

As mentioned previously the hippocampus is an essential structure for the formation of new memories. The role in memory formation was first proposed by Scoville and Miller who observed anterograde amnesia in patients with bilateral damage of the hippocampus (Scoville & Milner, 1957). The patients lost their ability to form new long-

term memories while retaining previously acquired declarative memories. The lesions also seemed to have no impact on procedural memory and the ability to learn new motor skills. Based on these findings it was later hypothesised that the hippocampus is primarily involved in the formation of new declarative memories but has only limited importance in the formation of other non-declarative types of memory. This notion has since been supported by various neuroimaging and neurobehavioral studies. The rodent hippocampus has been intimately studied concerning spatial memory. It contains abundant populations of neurons known as "place cells" originally described in the seminal 1971 paper by O'Keefe and Dostrovsky (1971). Place cells form a neuronal representation of the external environment and individual cells' fire when the subject is at a specific physical location "linked" to that cell (Sutherland & McNaughton, 2000; M. A. Wilson & McNaughton, 1993). This applies to pyramidal cells in the CA regions, granule cells in the DG and some interneurons (O'Keefe & Dostrovsky, 1971). The hippocampus exhibits lower concentrations of the 5-HT_{2A} receptor subtype (Hannon & Hoyer, 2008; Pazos et al., 1985). Nonetheless, all the pyramidal layers of CA1, CA3, CA2 and DG have been shown to contain the receptor subtype (Cornea-Hé Bert et al., 1999; Hannon & Hoyer, 2008; Hoyer et al., 2002; López-Giménez et al., 1997; Nichols & Nichols, 2008). Similar results have been yielded when comparing primate and human brain distribution of the receptor (López-Giménez et al., 1998). Pompeiano & Pallacios et al. detected low 5-HT_{2A}R mRNA levels in pyramidal layers of CA1 and CA2, but very high levels in the posterior and ventral parts of CA3 (1994). Using in situ hybridization Wright et al. reported dense labelling in DG, CA1 and CA3 regions of the hippocampus (Wright et al., 1995). Immunohistochemical experiments also demonstrated that 5-HT_{2A}R was present in virtually all parts of the rat hippocampal formation, especially in apical dendrites of pyramidal neurons (Bombardi, 2012). 5-HT_{2A}R was also found post-synaptically on neurons of CA1 and CA3, and granule cells in the DG (Li et al., 2004).

6.1 Neuroplasticity and neurogenesis

Neuroplasticity is viewed as the potentiality of the nervous system to adjust its function and adapt to new stimuli by effectively changing its structure and functioning. Neuroplasticity typically occurs during the development of the nervous system in response to external environmental stimuli or other agents. In recent years there has

been a surge in studies oriented toward the research of adult neurogenesis and neuroplasticity.

The hippocampus has been the principal structure in the study of both neuroplasticity and adult neurogenesis. It was here that the phenomenon of long-term potentiation (LTP) was first identified (Bliss & Lømo, 1973) and it has since been described using various techniques. According to the classical view of memory and learning postulated by Donald Hebb, LTP is believed to be the cellular basis of memory formation. It proposes that synapses between co-active neurons are strengthened through mechanisms of synaptic plasticity. Subsequent activation of only a minor portion of the neuronal component will thereby lead to activation of the whole assembly (Hebb, 2005). More recent studies have shown that learning can induce LTP in the hippocampus (Whitlock et al., 2006). Aside from LTP other forms of activity-induced plasticity have also been identified in the hippocampus, including long-term depression (LTD) (Dudek & Bear, 1992), EPSP-spike potentiation(Abraham et al., 1985; Andersen et al., 1980), spike-timing-dependent plasticity (Dan & Poo, 2004) and depotentiation (Barrionuevo et al., 1980; Staubli & Lynch, 1990).

For most mammalian species adult neurogenesis, the postnatal generation and integration of new neuronal cells, occurs almost exclusively in the hippocampal DG and the olfactory bulb (Deng et al., 2010). Neurogenesis can be influenced by various factors including learning and memory tasks(Gould et al., 1999), and environmental enrichment (Kempermann, 2019) can for example stimulate neurogenesis. In contrast, physiological stress, drug abuse and other pathophysiological processes tend to down-regulate adult neurogenesis (Ohira, 2018).

A population of cells known as neuronal progenitor cells (NPCs) gives rise to newly formed neurons. NPCs possess stem cell-like capabilities which means they can differentiate into several types of cell types and have the potential of self-renewal. The subgranular zone of the DG appears to be the neurogenic region in which new cells migrate and integrate into the existing network to differentiate into granule cells (Deng et al., 2010). Doublecortin (DCX) is a microtubule-binding protein that is transiently expressed by NPCs and immature neurons generated by adult mammalian brains (Gleeson et al., 1999). DCX labelling is becoming an effective alternative to the current "gold standard" of BrdU labelling.

6.2 IEGs

It is believed that conversion of experience into a stable memory likely relies on activity-based de-novo gene transcription. Immediate early gene (IEG) expression is at the forefront of such activity-induced transcription and is caused by sensory stimuli or behavioural experience (Sun & Lin, 2016). This has led to the hypothesis of IEGs being responsible for the encoding of memories from experience-induced neuronal activity. Such events are typically followed by rapid upregulation of IEG mRNA levels leading to regulation of downstream effector genes and ultimately to alteration of synaptic activity (Guzowski et al., 2001). Consistent with this notion, suppression of IEGs have been shown to impair long-term memory consolidation while having no effect on task acquisition and short-term memory (Grimm et al., n.d.; Guzowski et al., 2000).

IEGs can be classified into two functional groups. The first group encodes regulatory transcription factors, which can affect cellular physiology by regulating the expression of specific downstream effector genes. The second group encodes functionally diverse effector proteins which directly affect cellular function.

The activity-regulated cytoskeletal-associated (*Arc)* gene, also called Arg3.1, belongs to the second group of effector IEGs. Along with Zif 268, *Arc* is believed to play the most vital role in memory consolidation and LTP. Inhibition of *Arc* expression and specific gene knock-out impaired long-term memory consolidation as well as LTP maintenance in the hippocampus (JF et al., 2000; Rodríguez et al., 2008). Upon activation in an NMDA receptor-dependent manner (Steward & Worley, 2001), *Arc* mRNA is rapidly transported from the neuronal soma to post-synaptic dendrites where it undergoes local translation (Guzowski et al., 1999; Lyford et al., 1995), especially in excitatory glutaminergic neurons in the cortex and hippocampus (Steward et al., 1998). The Arc protein interacts with components of the endocytic machinery (dynamin, endophilin) involved in receptor trafficking and selectively modulates AMPA receptor-mediated synaptic transmission (Chowdhury et al., 2006). Arc also appears to regulate spine morphology and synaptic strength through AMPA receptor internalisation which further demonstrates its role in the development of LTP and long-term memory formation (Chowdhury et al., 2006; Guzowski et al., 2000; Steward et al., 1998).

Arc is up-regulated following learning and exposure to a novel environment. Santini et al. showed that novelty exposure up-regulated Arc mRNA expression in the frontal cortex (FC) and hippocampus of mice (2011). Contrastingly, a single dose of psilocybin

induced the expression of several IEGs in rats but decreased the expression of Arc in the hippocampus (Jefsen et al., 2021a). These findings further illustrate the apparent yet poorly understood role of IEG expression in memory formation.

7 EEG RECORDING AND RODENT SLEEP

The most common form of an oscillogram, EEG, is the recording of electrical potentials generated by the brain. In humans, EEG is typically obtained by placing a set of electrodes on the scalp. In small laboratory animals like rats, recordings are usually made by perforating the skull and implanting the electrodes directly above or within the cortex. Such a setup is called an electrocorticogram (ECoG) but the term EEG is typically used interchangeably. The implanted electrodes allow for a simultaneous recording of several different areas of the brain. This is of great value for investigating the manifestations of different electrical processes within the brain during wakefulness and sleep.

Much like human EEG, rodent EEG contains fast and slow oscillations. The EEG is a rhythmic cerebral electrical signal, the observable majority of which falls in the range of 1-40 Hz. The waveforms are divided into bands known as alpha, beta, theta and delta, which denote the majority of EEGs used in clinical practice. Although the nomenclature of the divisions is arbitrary and based on clinical practice, the bands have some biological basis. Thus, some phenomena and changes are comparable to rodents. Quantitative changes across different EEG bands are determined using the so-called Fourier transform and spectral power counting. Based on EEG waveforms and power we can differentiate three categories in rodent sleep: wakefulness, REM/paradoxical sleep, and NREM sleep/SWS. These stages can also be differentiated by the variable power of an electromyogram (EMG). Wakefulness is typically accompanied by high variable EMG activity combined with medium EEG activity with power prevalent in beta (15-30 Hz) and gamma (30-100 Hz) ranges. NREM/SWS is described as having low EMG activity and high EEG activity with power concentrated in the delta (1–4 Hz) band(Hoshino et al., 2015). And finally, REM sleep is described as having very low EMG activity and medium EEG activity with power concentrated in the theta (4–8 Hz) band (Gilmour et al., 2010).

8 BIOTELEMETRY

Biotelemetry is an increasingly useful and sophisticated method for measuring various physiological parameters such as EEG, EMG, electrocardiogram (ECG), and electrooculogram (EOG), body temperature, activity etc. Telemetric recordings from unrestrained animals are thought to be superior to those from restrained or anaesthetized animals, as they allow recording the animal in a relatively normal physiological and behavioural state and can thus produce results that are more in line with what we would expect in humans (Kramer & Kinter, 2003). Classical restraining methods may induce additional stress for the animal which can, in turn, have an impact on physiological parameters, such as increased blood pressure and heart rate, temperature and lowered food intake and body weight (Kramer & Kinter, 2003). In EEG recording a standard restraining approach utilizes tethered systems in which the electrodes and cables are physically connected to a socket implanted in the animal's skull (Bertram et al., 1997). There is also the factor of increased risk of infection at the site of the implanted socket which remains exposed to the outer environment(Lundt et al., 2016). Biotelemetry allows researchers to gather large amounts of continually recorded data over longer periods. Another beneficial factor of biotelemetry is the great reduction in animal use which is in line with the current 3R approach. However, there are also some disadvantages of implantable telemetry that have been pointed out. Namely, increased difficulty of procedures required to successfully perform the implantation, and the large amount of data collected from the continuous recording (Kramer & Kinter, 2003).

9 AIM OF PROJECT

This thesis aimed to explore the effect of non-selective serotonin receptor agonist psilocybin administration combined with sleep manipulation and their effect on:

1. Memory consolidation and performance in hippocampus-dependent memory tasks in memory oriented behavioural tasks
2. Neurogenesis and expression of IEG protein Arc in the dentate gyrus of the hippocampus
3. EEG recording of distinct sleep phases during sleep manipulation (either sleep or sleep deprivation)

10 METHODS AND MATERIALS

10.1 EXPERIMENTAL ANIMALS

10.1.1. Behavioral tasks and immunohistochemistry (IHC)

The experiments were carried out on adult male Long Evans rats (Charles River, Germany). The animals were housed in pairs in standard plastic home cages on a 12:12 h light/dark cycle with access to water and standard laboratory diet ad libitum. All behavioural tests and dosing were performed during the rat's light phase. At the beginning of the experiment, the rats weighed between 280-350 g. The animals were divided into four groups (N=10) with each group receiving a combination of **treatment** and sleep **manipulation** as follow:

1. Saline + sleep
2. Saline + deprivation
3. Psilocybin + sleep
4. Psilocybin + deprivation

10.1.2. EEG recording

An additional group of 5 animals (N=5), again adult male Long Evans rats (Charles River, Germany), was used to establish a functioning paradigm for the implantation of 4ET transmitters (Data Sciences International, DSI, USA). The animals were singly housed in standard plastic home cages on a 12:12 h light/dark cycle with access to water and standard laboratory diet ad libitum. All tests and dosing were performed during the rat's light phase. At the time of the experiment, the rats weighed between 350-400 g. Our main goal was to optimize the surgical procedure and assess EEG recording to verify the efficiency of the gentle handling sleep deprivation technique we utilized for sleep manipulation.

All 5 animals received 4 consecutive combinations of treatments and sleep manipulation during the span of four weeks:

1. Saline + sleep
2. Saline + deprivation
3. Psilocybin + sleep
4. Psilocybin + deprivation

10.2 TREATMENT ADMINISTRATION REGIME

The experiments used the following compounds:

- **Psilocybin**: 5 mg/kg; dissolved in saline solution 2 ml/5 mg (0,9% NaCl)
- **Saline solution:** 0,9% NaCl was used for vehicle control groups

Both compounds were administered subcutaneously with the dosing set at 2 ml/kg of the animal's weight.

10.3 BEHAVIOURAL METHODS

The animals were first used in the OPR task. After a 2-week resting period, the animals completed the MWM task. Each of the behavioural tests combined sleep manipulation (sleep vs. sleep deprivation) with drug treatment (5 mg/kg of psilocybin vs. control vehicle). Tests and treatments were ordered so that each animal received a combination of opposing treatments before each task. Prior to testing the animals were handled for 7 days and habituated. Behaviour was recorded using Basler web-cameras (acA1300-60gc, Basler AG) and processed in Noldus Ethovision XT and Observer XT program (Noldus, NL).

Sleep deprivation

The animals were sleep-deprived by the gentle handling method (Colavito et al., 2013). After finishing the morning phase of a given task, the rats would be placed in their home cages. The experimenter would then closely observe the animals in their cages and when they showed signs of sleep or inactivity, they would gently poke the animal's body to wake it up. Animals were subjected to sleep deprivation for a period of 7 hours. The

control groups were left to sleep undisturbed in their home cages for the same amount of time.

Object place recognition

Prior to testing the animals were habituated for two consecutive days with one trial carried out on each day. During the habituation phase, the animals were placed in an empty open field arena (80x80 cm) and left to explore the box freely for 5 minutes. On the third day, the sample and test trials were conducted. We used the same open field arena, but two distinct objects (a ceramic flowerpot and a hollow plastic cylinder) were placed inside. During the sample phase, the animal was placed in the arena with the two objects placed in opposing corners at the distal side of the arena and left to explore freely for 5 minutes. The animals received treatment (saline or psilocybin) immediately after finishing the morning sample phase. After a 7-hour long period of either sleep or sleep deprivation, the test phase was carried out to assess if the animal had memorized the position of the objects. One of the objects was moved to the opposite side of the arena while the other remained in the same position as in the sample phase. The animal was then placed inside the arena and left to explore for 5 minutes. The layout and design of the experiment are shown in fig. 5. The rat's movement was tracked and recorded continuously using the Noldus Ethovision XT program (Noldus, NL). After each trial, the arena was cleaned with a 70% ethanol solution and air-dried before commencing another trial. We collected data such as time spent exploring the object moved to a novel place, the object which remained in the familiar location and total time spent exploring the objects. We considered the animal to be exploring the object when its nose moved within 1 cm of the object. Preference for the object in a novel location should then reflect the animal's place recognition memory. For the duration of the experiment, the animals were randomly assigned to one of two experimental rooms. The experimental rooms were soundproof, diffusely lit with low intensity reflected light and were virtually identical. They contained identical open field arenas with the 2 objects at identical locations. The only difference was that in each room a different object had been moved to a new location. This was done to ensure that the preference for one of the objects was based on its novel location and not just on its properties.

A preference-index (P-Index) for object exploration was calculated according to Binder et al. (2012) as the quotient of exploration time of the displaced object and total

exploration time (s): P-index = exploration of displaced object/ (exploration of displaced object + exploration of the stationary object).

P-Indexes from the sample (morning) and test (evening) trials were then used for statistical analysis of animals' place recognition memory.

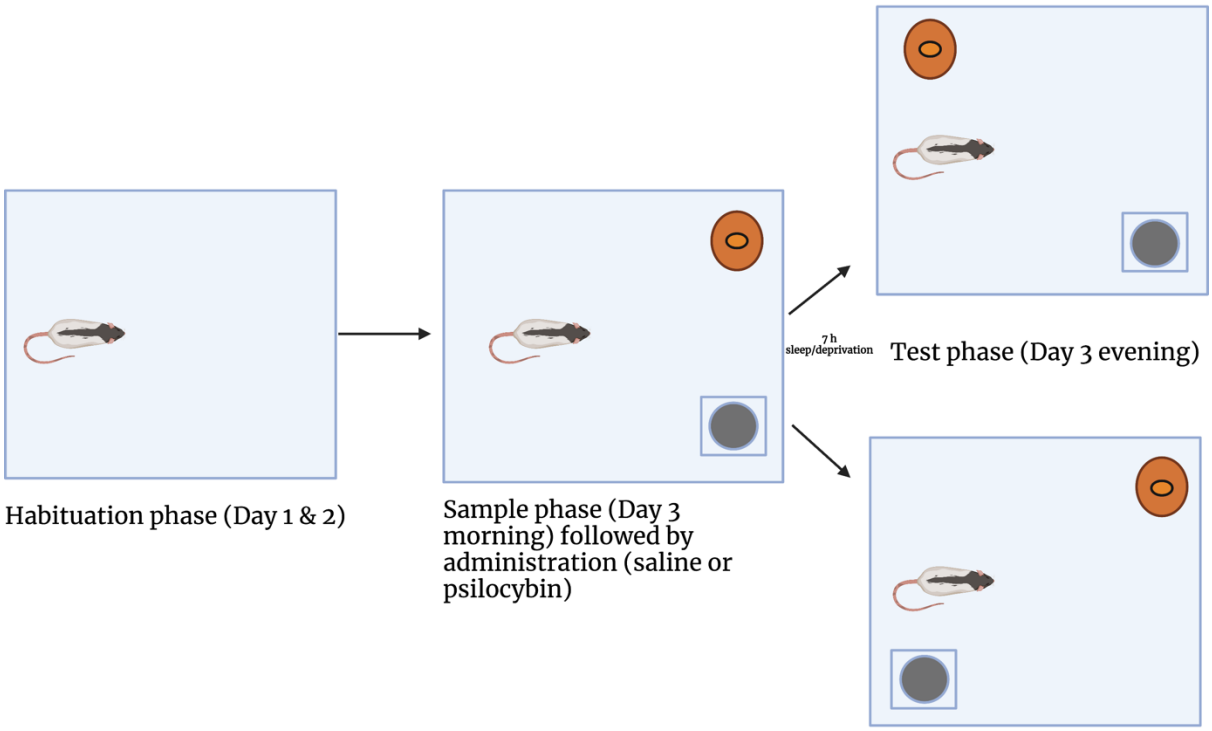

FIGURE 5. OBJECT PLACE RECOGNITION EXPERIMENT DESIGN (CREATED WITH BIORENDER.COM)

Modified Morris Water Maze test

A modified spatial version of the water maze task was used (Vorhees & Williams, 2014). The maze consisted of a round tank 180 cm in diameter and 50 cm deep filled with water. The temperature of the water was maintained at 21°C. The tank was divided into 4 imaginary quadrants (N=north, S=south, W=west, E=east). This division was maintained throughout all trials. Each of the four quadrants had a distinct visual cue in the form of a coloured shape placed at the rim of the tank. The room where the water maze was located also contained several distant visual cues. A platform made from clear plastic (10 x 26 cm) was submerged inside the water tank and placed in one of the four imaginary quadrants.

The whole experiment consisted of 3 consecutive days of multiple trials. The training phase was done within the first 2 days. On the first day, the platform was placed into the S quadrant and was visible above the water level. On the second day, the platform was placed in the N quadrant and was submerged underwater so as not to be visible. And finally, on the third day, the platform was placed in the E quadrant and submerged underwater.

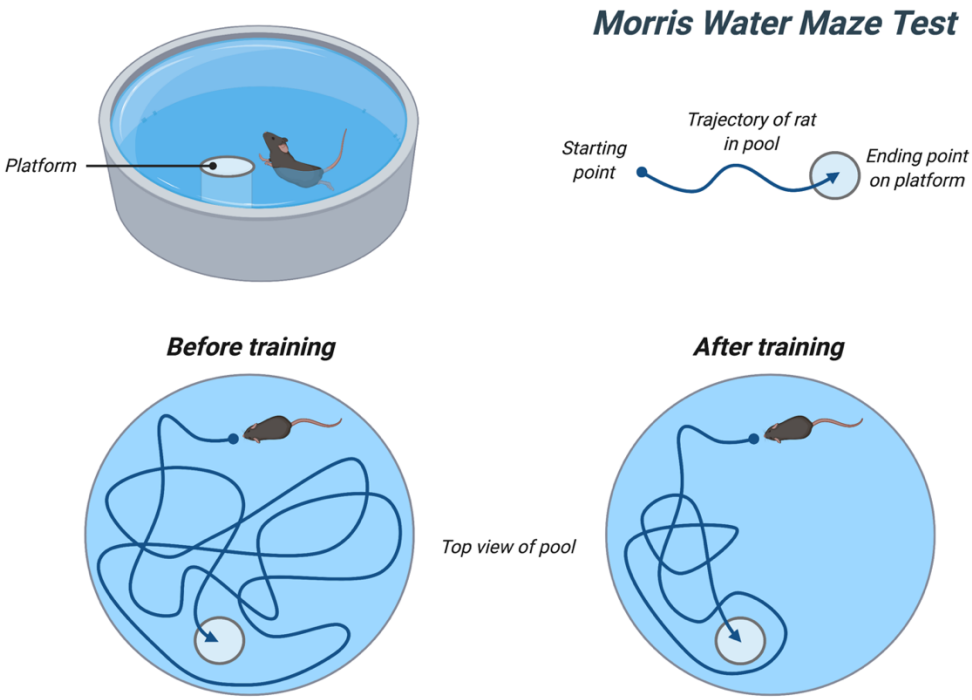

FIGURE 6 MORRIS WATER MAZE TEST (CREATED WITH BIORENDER.COM)

Each animal performed two sets of four consecutive trials every day with one set being performed in the morning (08:00) and one in the evening (16:00). Thus, each animal completed a total of 8 trials each day with a period of rest in between the 2 sets of 4 trials during the first 2 days and sleep or sleep deprivation in between the trials on the final testing day. For each of the 4 consecutive trials, the animal was placed into the tank in one of the 4 quadrants so that each starting point was covered. During the training phase, the animal was placed into the tank while facing the wall and left to swim freely until it found the platform. If it failed to find the platform within 90 seconds the experimenter gently guided the rat to the platform's position using their hand. The animal was then left to remain on the platform for 30 s to get a better overview of the

platform's position within the tank. After that, it was removed patted dry with a towel and allowed to rest for 60 s. After the short rest, the animal was once again placed inside the tank to the next starting point. The starting points alternated in a pseudo-randomized order for each of the trials. The same approach was used on days 2 and 3 but the platform was fully submerged and placed in different quadrants on each day. The design layout of the experiment is shown in fig. 7. At first, the rats wandered aimlessly around the periphery of the arena, but they quickly understood the aim of the task and their choice of trajectory became goal-oriented towards locating the hidden platform (as shown in fig. 6).

During the whole experiment, the tank was continuously video recorded using Basler web-cameras (acA1300-60gc, Basler AG). The video recordings were processed with Noldus Ethovision XT (Noldus, NL) to score for latency to escape to the platform and total trajectory length to the platform of each animal. These two variables were then used to assess if the animals were able to remember the position of the platform from the morning sample phase. Two different comparisons were used for our analysis:

1. The average escape latency and trajectory length to the platform from 2nd to 4th swim – morning vs. evening session.
2. The escape latency and trajectory length to the platform from the 1st evening swim which we consider to best represent how the animals remember the position of the platform from the morning.

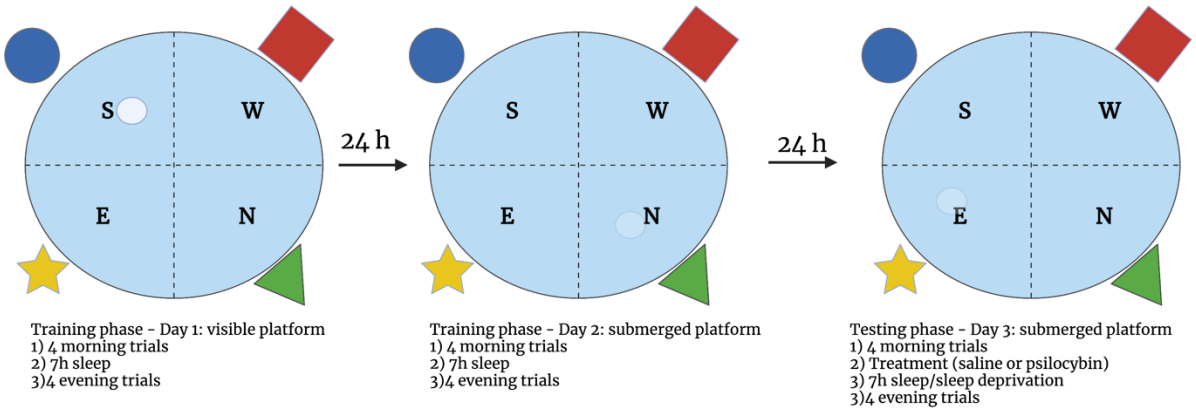

FIGURE 7: MWM EXPERIMENT DESIGN (CREATED WITH BIORENDER.COM)

Immunohistochemistry

Immediately after completing the final run of the MWM task, 3 animals were selected from each of the 4 treatment groups. The animals were anaesthetized with ether to perform transcardial perfusion via the left ventricle. The circulation was first washed using phosphate buffer saline (PBS) to remove the remaining blood and then with 4% paraformaldehyde (PFA) in 0,1M PBS. After that, the brains were immersed in PFA for 24 h and finally in a 30% sucrose in PBS solution until they sank to the bottom of the container. After being saturated with the cryoprotective sucrose solution the brains were frozen at -75 °C. The frozen brains were then cut into 50µm thick slices using cryosectioning on Leica CM1860 UV cryostat. The slices were then transferred into a well plate and stored in cryoprotective solution at -36 °C.

The staining procedure was carried out over 2 days on free-floating sections. The IEG protein Arc was selected as a possible correlate of neuroplasticity and memory formation. For phenotypic differentiation of neurons, we used immunostaining specific for immature neurons doublecortin (DCX) and mature neurons (NeuN).

On Day 1 the slices were first washed 3 times for 10 minutes with PBS. Sections were then incubated for 60 min at room temperature with 10% goat serum (NGS) in PBS and 0.3% Triton-X100 (PBST) to block non-specific protein activity. Sections were then incubated overnight at 4 °C with the primary antibodies in PBST solution containing 1% of NGS.

The following primary antibodies were used: Guinea pig anti-Doublecortin (DCX, polyclonal, 1:3000, Millipore, AB2253); Mouse anti-Neuronal nuclear antigen (NeuN, monoclonal, 1:1000, Millipore); Rabbit anti-Arc (polyclonal, 1:1000, Synaptic Systems, 156 003).

After incubating with primary antibodies, sections were washed with 0.1M PBS (3x10 min). Sections were then incubated with secondary antibodies in PBST with 1% NGS for 2 hours at room temperature and in the dark. The following secondary antibodies were used: Donkey anti-guinea pig AF488 (Jackson ImmunoResearch, 706-545-148); Goat anti-rabbit AF594 (Jackson ImmunoResearch, 111-585-003); Donkey anti-mouse AF647 (Jackson ImmunoResearch, 715-605-151), all at a concentration of 1:500. Following incubation with secondary antibodies, sections were washed in 0.1M PBS (3x10 min), mounted, and coverslipped in Prolong Gold Mounting Medium with DAPI (Vector Laboratories).

Quantification of Arc and DCX immunoreactive cells

Fluorescence was detected using a confocal microscope (Leica TCS SP8 X). DCX and Arc labelled cells were counted in the suprapyramidal and infrapyramidal blade of the dentate gyrus in both hemispheres as referred to earlier in Erwin et al. (2020)(for illustration see fig. 8). Immunoreactive cells were detected in 3 brain sections per animal, corresponding to the medial region of the hippocampus. The number of labelled cells were then counted as the average of positive cells per one section.

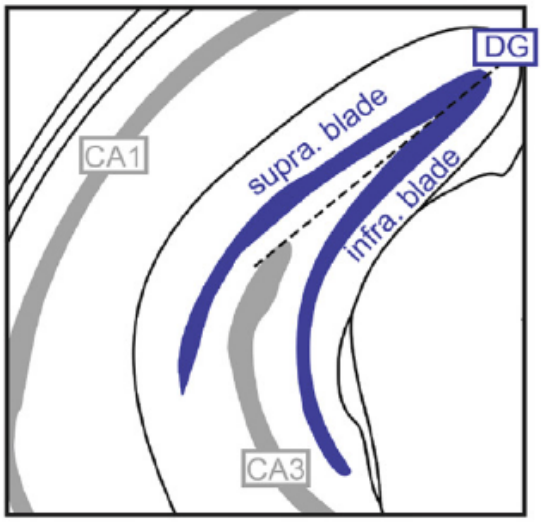

FIGURE 8: THE STRUCTURAL ORGANIZATION OF HIPPOCAMPAL REGIONS IN A CROSS-SECTION. ADOPTED FROM ERWIN ET AL.(2020)

10.4 EEG RECORDING

10.4.1 SURGICAL PROCEDURE

After 1 week of handling, the animals were anaesthetized by inhalation of Isoflurane (2.5–3%) and underwent a surgical procedure in which a telemetric device DSI 4ET (Data Sciences International, DSI, USA) had been implanted. A longitudinal incision was made on the animals back and the implant was placed subcutaneously on both sides of the animal's lower abdomen. A second incision was made on the cranial region of the head. The wires from the implant were then pushed through the subcutaneous space on the back of the neck so that they could be placed in the designated regions of the brain. The placement of the telemetric device is shown in fig. 9.

After exposing the external connective tissue, a total of 10 holes were drilled in the skull. Three were the exact locations of our areas of interest - frontal association, primary somatosensory, and primary visual cortices (Bregma [A=0/L=0], F1 - [A= +4,8 mm/L= -1,3 mm], P1 - [A= -3,0 mm/ L= +3,9 mm], V1 - [A= -5,6 mm/L= +3,9 mm]). Another four were drilled above the cerebellum and three more in other, undisturbed areas for the placement of stainless-steel screws.

Gold-plated pins were soldered to the ends of wires (except the one for EMG) before starting the surgery. Three of the sensing electrodes were inserted into the

stereotactically determined drilled holes. Four reference electrodes for each of the channels had been placed above the cerebellum. Three stainless steel screws were attached to increase the durability of the implant and the entire wound was sealed with a thin layer of dental cement. Bare wire of EMG electrode had been sewn into the back neck muscles to monitor the animal's physical activity. After the electrodes were in place the skin was positioned back into place and sutured. The recovery phase following surgery lasted for 3 weeks. The first week after surgery the animals were supplied with appropriate doses of pain killers (meloxicam 1mg/kg once a day) and antibiotics (enrofloxacin 10 mg/kg once a day).

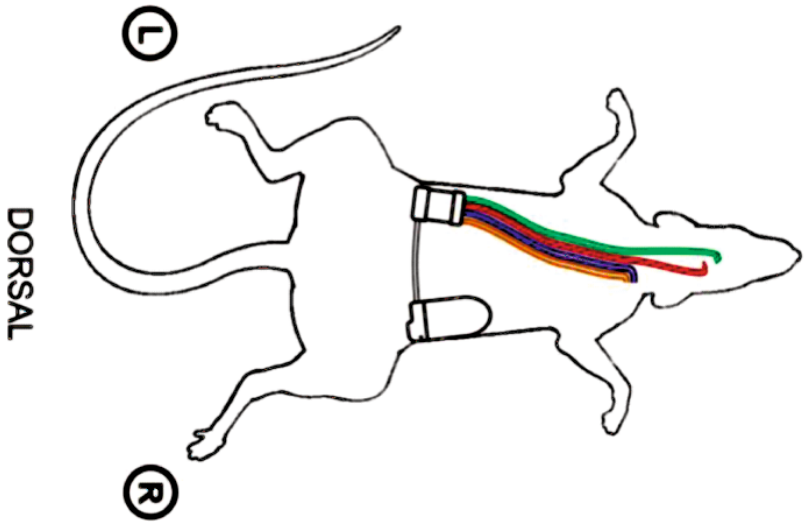

FIGURE 9: PLACEMENT OF TELEMETRY MODULE

10.4.2 SIGNAL RECORDING

EEG was recorded continuously from the animals using a telemetric module. The 4ET transmitter can measure four biopotential channels, temperature and general locomotor activity in rats and other small laboratory animals. Each of the channels can be used to record any combination of physiological signals such as EEG, EMG, ECG, EOG. The 4ET transmitter is a dual-module device consisting of two modules that are electrically and physically interconnected. The sensing module receives power from the telemetry module. The thermal sensor and bipotential leads are included in the sensing module. The battery and data transmission circuitry are contained in the telemetric

module. The data is received via a receiver which is a flat board placed underneath the home cages. The telemetry module receives the physiologic data from the sensing module and transmits them telemetrically to the receiver. This allowed us to measure the change in electric potentials continuously while keeping the animals in a familiar environment. It receives the signal via radiofrequency emitted from the 4ET transmitter. The telemetry module contains a magnetic switch to turn it on and off. When the device is on, an audible tone can be heard with the 4ET radio. The signals were recorded using the Dataquest A.R.T. software (Data Sciences International, DSI, USA) at a sampling frequency of 500Hz.

Vigilance state analysis

The identification of vigilance states was performed by analysing EEG and EMG signals using the NeuroScore software version X (DSI, St-Paul, MN, USA). Vigilance state identification was performed on 10-s epochs, and three different states were identified (Fig. 10):
Wakefulness (W), slow-wave sleep (SWS) and paradoxical sleep (PS).

Criteria for vigilance state attribution were:

- Wakefulness was attributed when the animal actively moved in the cage and/or low-amplitude, rapid-frequency EEG was accompanied by high-amplitude EMG activity.
- SWS was scored when EEG showed slow waves (low-frequency waves (~1–4 Hz) and low EMG activity.
- Paradoxical sleep was scored when EEG showed predominant theta activity (~4–8 Hz)

Even though the time of recording was 8 hours for every animal, a different number of artefacts in each recording forced us to calculate the vigilance state duration as a percentage of time spent by wakefulness, slow-wave sleep, and paradoxical sleep. A representative sample of each vigilance state as displayed in the NeuroScore analyzer can be seen in fig. 10.

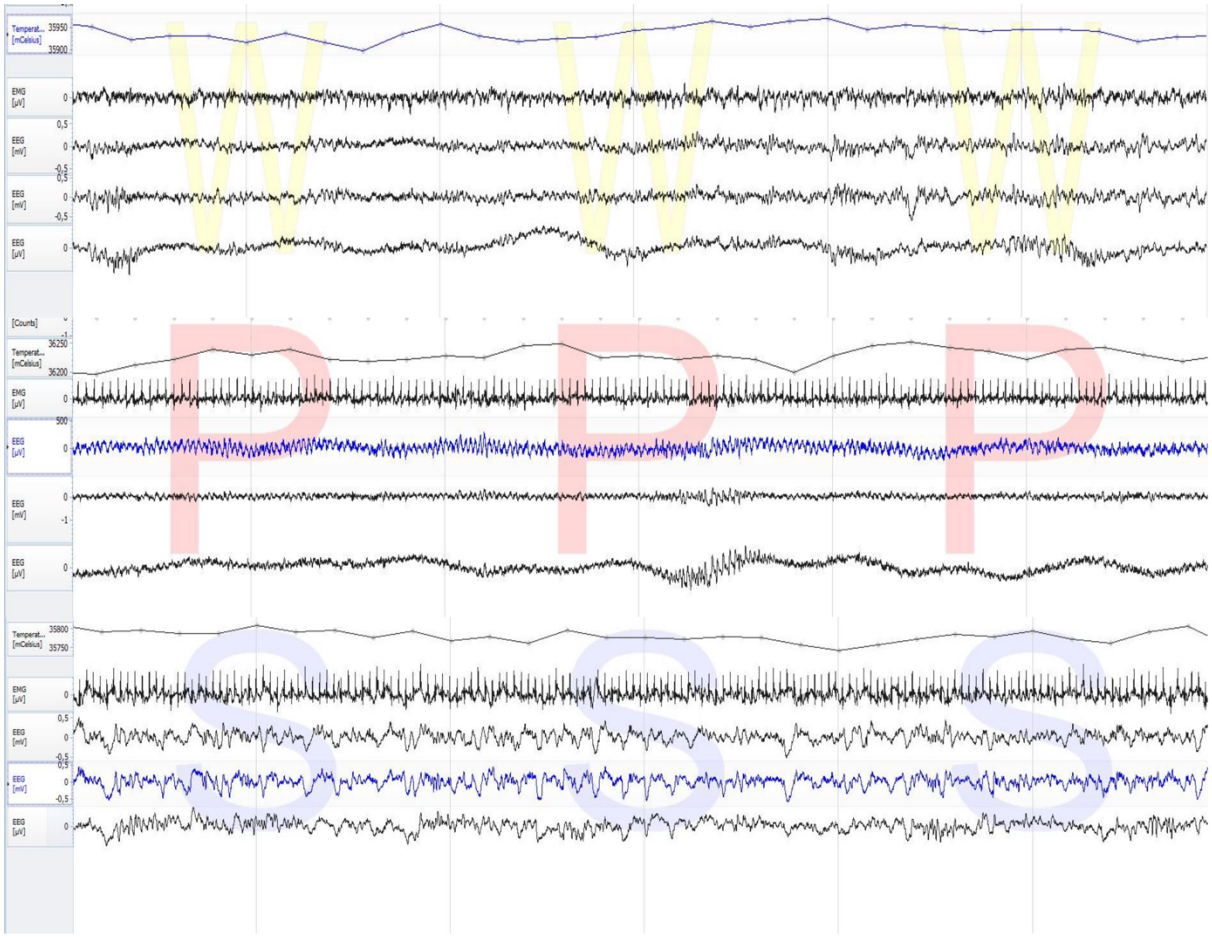

FIGURE 10 REPRESENTATIONS OF THE INDIVIDUAL VIGILANCE STATES (W: WAKEFULNESS, P: PARADOXICAL SLEEP, S: SLOW-WAVE SLEEP) DATA RECORDED IN THE ACQUISITION AND SHOWN IN THE NEUROSCORE PROGRAM

10.5 STATISTICAL ANALYSES

Experimental data are presented as mean ± standard deviation (SD) and 'n' refers to the number of rats per group. Statistically significant differences were accepted when the p-value was < 0.05. All the statistical analyses were conducted using IBM SPSS version 25 and GraphPad Prism 8.0.

Normality of data distribution and homogeneity of variances were verified using the Shapiro-Wilk Normality test and Levene's test, respectively. Where Mauchly's test of sphericity was significant, Greenhouse-Geisser (Greenhouse-Geisser estimate of sphericity (ε) < 0.75) corrections are reported. Data that showed normal distribution and homogeneity of variances were analysed separately using parametric tests including Two-Way/Three-Way Analysis of Variance and One-Way/Two-Way/Three-Way Repeated Measures Analyses of Variance (Two-Way/Three-Way ANOVA and One-Way/Two-Way/Three-Way RM ANOVA, respectively). When these assumptions are not matched, data were evaluated by a non-parametric Kruskal-Wallis test. The statistical methods are summarized in Tab. 1. Significant main effects and/or factors´ interactions were followed by Tukey (for a parametric test) and Dunn´s post hoc test (for the non-parametric test) where it was relevant.

PROCEDURE	INDEPENDENT VARIABLE	METHOD	BETWEEN-SUBJECT FACTOR	WITHIN-SUBJECT FACTOR
OPR task	P-index	RM ANOVA	treatment (saline/psilocybin) manipulation (sleep/deprivation)	time (morning/evening)
MWM task	Average trajectory length (cm)	RM ANOVA	treatment (saline/psilocybin) manipulation (sleep/deprivation)	time (morning/evening)
	Average escape latency (s)	RM ANOVA	treatment (saline/psilocybin) manipulation (sleep/deprivation)	time (morning/evening)
	Trajectory length of 1st evening swim (cm)	Kruskal-Wallis test	group (saline + sleep/saline + deprivation/psilocybin + sleep/psilocybin + deprivation)	-
	Latency of escape of 1st evening swim (s)	Kruskal-Wallis test	group (saline + sleep/saline + deprivation/psilocybin + sleep/psilocybin + deprivation)	-
EEG recording	Time spent with wakefulness (%)	RM ANOVA	-	Repeated treatment+manipulation (saline + sleep/saline + deprivation/psilocybin + sleep/psilocybin + deprivation)
	Time spent with SWS (%)	RM ANOVA	-	Repeated treatment+manipulation (saline + sleep/saline + deprivation/psilocybin + sleep/psilocybin + deprivation)
	Time spent with PS (%)	RM ANOVA	-	Repeated treatment+manipulation (saline + sleep/saline + deprivation/psilocybin + sleep/psilocybin + deprivation)
IHC analysis	Number of positively stained cells	Three-way ANOVA	treatment (saline/psilocybin) manipulation (sleep/deprivation) position (supra/infra blade)	- - -

11 RESULTS

11.1 OBJECT PLACE RECOGNITION TASK

Across the treatments, manipulations, and measurements, *P-indexes* displayed normal distribution and equal variances. According to results of Three-Way RM ANOVA, values of *P-index* significantly varied across the different time of measurements ($F_{(1, 36)} = 4.598$, $p = 0.039$), whereas the rest of the tested factors or factors´ interactions did not have any effect.

Further investigation of the significant main effect using the Tukey post hoc test revealed an increased investigation of the displaced object in the evening test by all animals ($p = 0.039$) (Fig. 11).

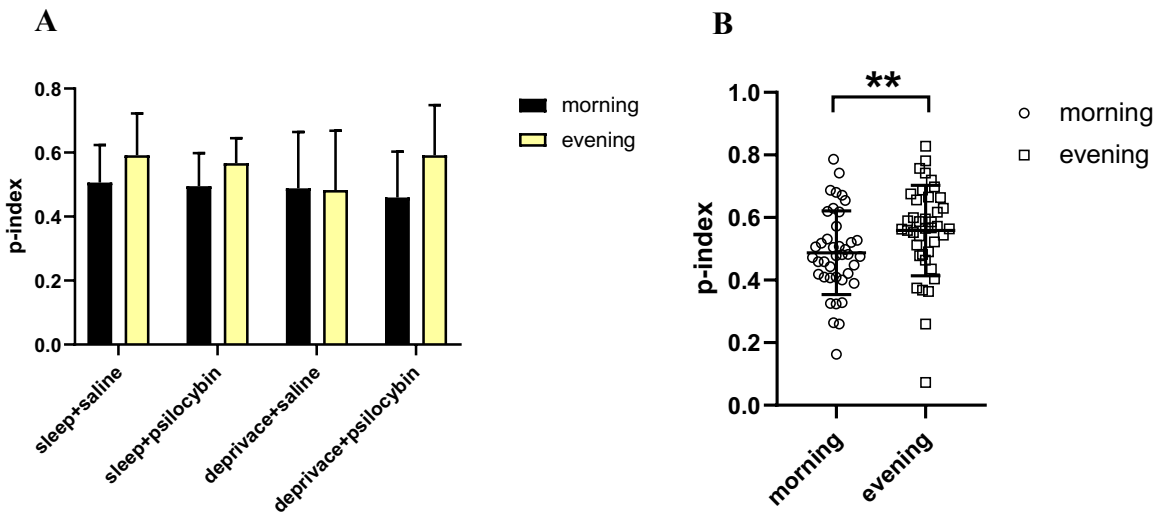

FIGURE 11: DATA REPRESENTING THE P-INDEXES IN THE MORNING AND EVENING SESSION OF OBJECT PLACE RECOGNITION TASK. (A) RESULTS WITHIN TREATMENT AND MANIPULATION IN GROUPS. (B) OVERALL RESULTS FOR MORNING/EVENING. P-INDEX REPRESENTS A PREFERENCE FOR TIME SPENT EXPLORING THE DISPLACED OBJECT (0-1). DATA SHOWN ARE COLUMNS/INDIVIDUAL VALUES WITH MEANS± SD; N = 10 FOR ALL GROUPS. **P < 0.01, SIGNIFICANTLY DIFFERENT AS INDICATED; RM ANOVA FOLLOWED BY TUKEY POST HOC TESTS.

11.2 MORRIS WATER MAZE

Average escape latency and trajectory length

Both tested parameters (i.e. escape latency and trajectory length) displayed normal distribution and homogenous variances across the different treatments, manipulation, and measurements. Analysis of escape latencies revealed a significant effect of manipulation ($F_{(1, 36)} = 8.461$, $p = 0.006$) and a tendency for its interaction with time of the measurement ($F_{(1, 36)} = 2.874$, $p = 0.099$). The significant main effect of manipulation was followed by the Tukey post hoc test which revealed increased escape latency in the sleep-deprived group ($p = 0.006$).

Similar pattern was pronounced also for trajectory length, nevertheless, there is only non-significant trend for both manipulation ($F_{(1, 36)} = 4.077$, $p = 0.051$) and interaction between manipulation and time of measurement ($F_{(1, 36)} = 3.225$, $p = 0.081$). None of the further factors or their interactions had an effect.

Based on the significant main effect and presented trends, deprivation tended to disturb the performance of rats (i.e. decreased escape latency and trajectory length) in the evening trials (Fig. 12 & 13).

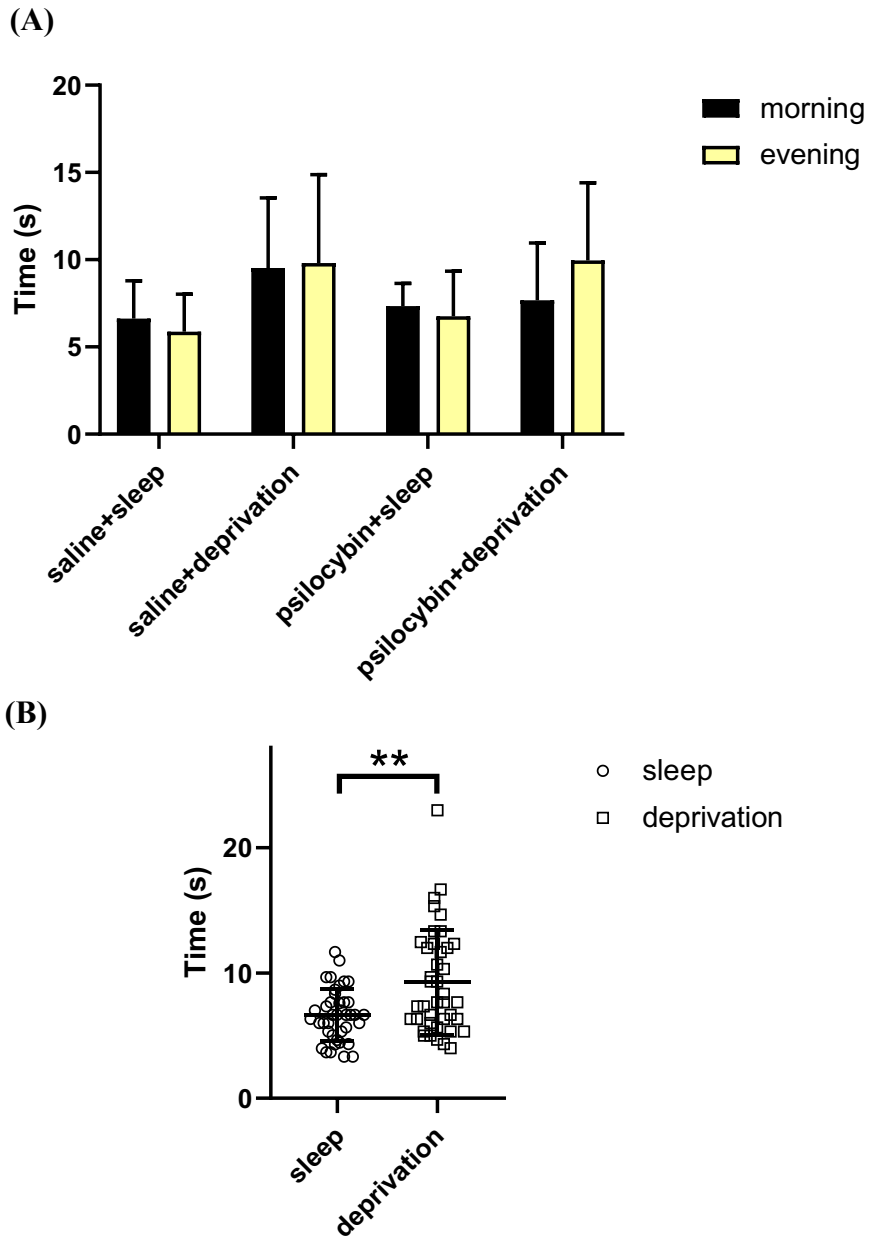

FIGURE 12: DATA REPRESENT AVERAGE ESCAPE LATENCY TO THE PLATFORM IN THE MORNING AND EVENING SESSION OF MORRIS WATER MAZE – (A) RESULTS WITHIN TREATMENT AND MANIPULATION IN GROUPS. (B) SHOWS OVERALL RESULTS FOR SLEEP/DEPRIVATION. DATA SHOWN ARE COLUMNS/INDIVIDUAL VALUES WITH MEANS ± SD; N = 10 FOR ALL GROUPS. **P < 0.01, SIGNIFICANTLY DIFFERENT AS INDICATED; RM ANOVA FOLLOWED BY TUKEY POST HOC TESTS.

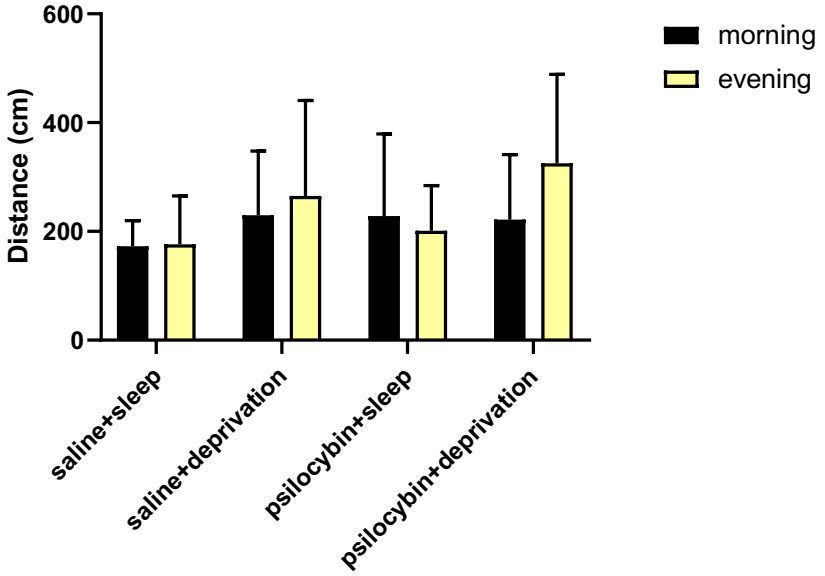

FIGURE 13: AVERAGE TRAJECTORY LENGTH TO THE PLATFORM IN THE MORNING AND EVENING SESSION OF MORRIS WATER MAZE – RESULTS WITHIN TREATMENT AND MANIPULATION IN GROUPS. DATA SHOWN ARE COLUMNS WITH MEANS ± SD; N = 10 FOR ALL GROUPS. RM ANOVA.

First evening swim

Measurements of escape latency and trajectory length in the first evening swim did not display normal distribution and equal variances.

Escape latency

Kruskal-Wallis test proved a significant difference in median escape latency between groups ($H(4) = 8.927$, $p = 0.030$). Dunn's multiple comparison test revealed a significant increase of escape latency in animals that received saline and were sleep-deprived (saline + deprivation) compared to control animals (saline + sleep) ($p = 0.045$). For illustration see Fig. 14.

Trajectory length

According to the Kruskal-Wallis test, there was no significant difference in median trajectory length between groups. For illustration see Fig. 14.

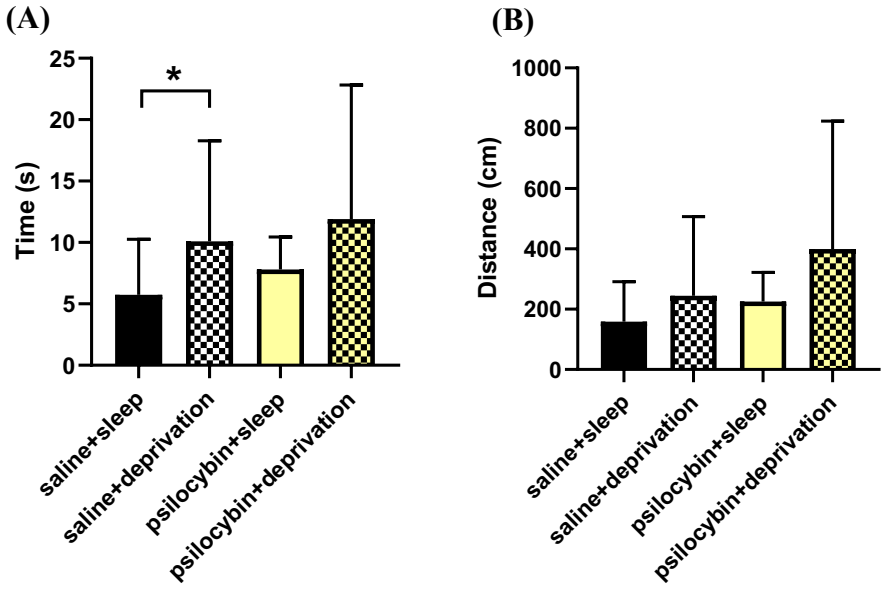

FIGURE 14: ESCAPE LATENCY (A) AND TRAJECTORY LENGTH (B) TO THE PLATFORM IN THE FIRST EVENING SWIM IN MORRIS WATER MAZE – RESULTS WITHIN TREATMENT AND MANIPULATION IN GROUPS. DATA SHOWN ARE COLUMNS WITH MEANS± SD; N = 10 FOR ALL GROUPS. *P < 0.05, SIGNIFICANTLY DIFFERENT AS INDICATED KRUSKAL-WALLIS TEST FOLLOWED BY DUNN'S MULTIPLE COMPARISON TEST.

11.3 IHC

DCX

Three-way ANOVA revealed a significant effect of position on the number of labelled cells ($F_{(1, 16)} = 8.882$, $p = 0.009$). Manipulation and treatment itself did not have a significant effect on the number of labelled cells, but we found a significant interaction between treatment and manipulation ($F_{(1, 16)} = 5.178$; $p = 0.037$). The significant main effect of position was followed by Tukey post hoc which showed a significant increase of expression of DCX in suprapyramidal blade of DG ($p = 0.015$). When the effect of treatment, manipulation and its interaction was tested separately in each part of DG (supra- and infrapyramidal blade, separately), Two-way ANOVA did not prove any effect of these factors. For illustration see Fig. 15 and 16.

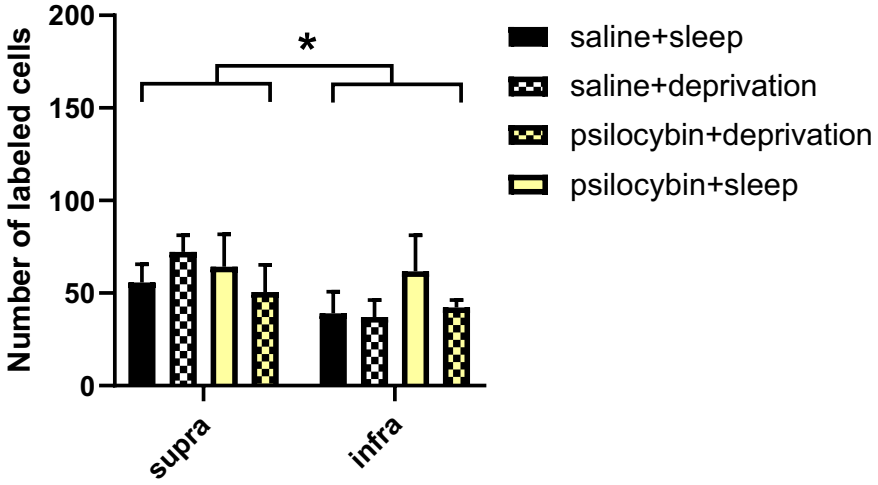

FIGURE 15: NUMBER OF DCX POSITIVE CELLS IN THE SUPRA- AND INFRAPYRAMIDAL BLADES OF THE DG. RESULTS WITHIN TREATMENT AND MANIPULATION IN GROUPS. DATA SHOWN ARE COLUMNS WITH MEANS± SD; N = 3 FOR ALL GROUPS. ANOVA FOLLOWED BY TUKEY POST HOC TEST. * $P < 0.05$.

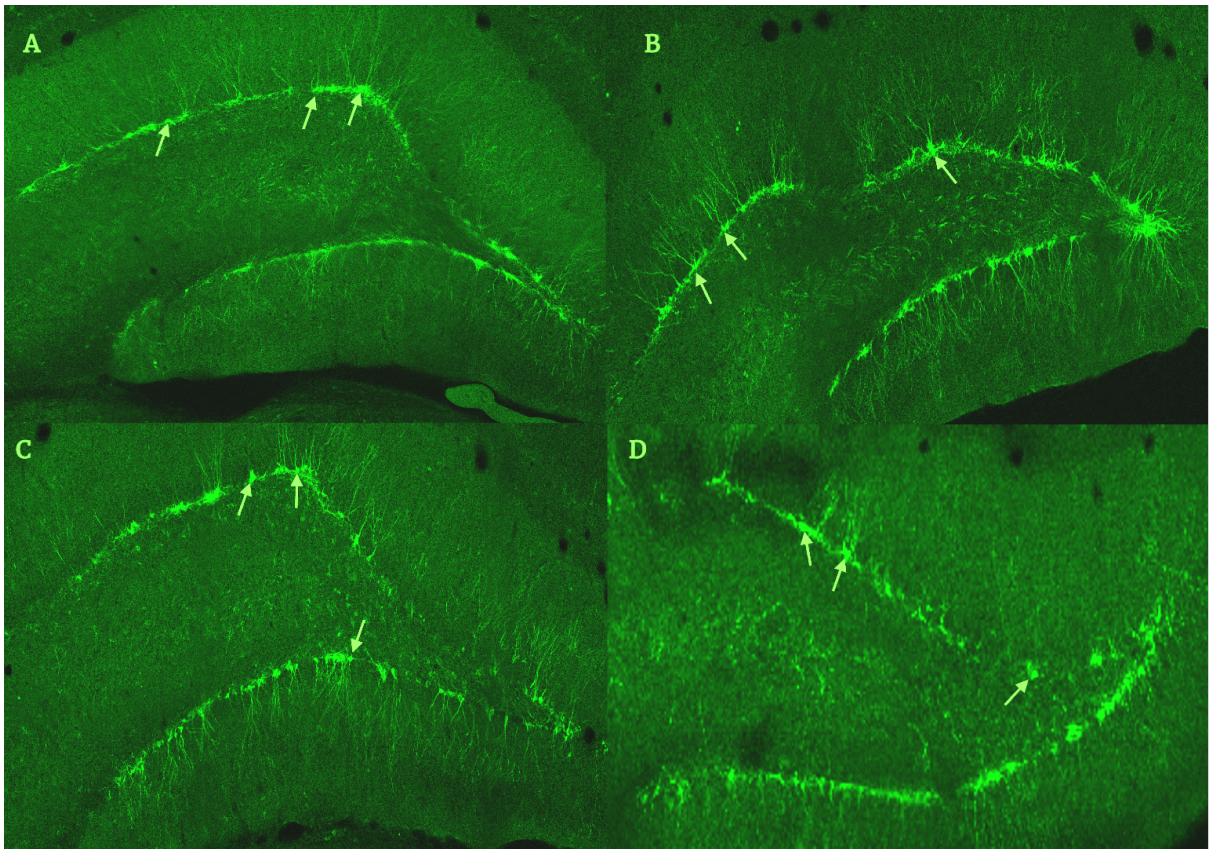

FIGURE 16: ILLUSTRATIVE IMAGES OF DCX LABELLING IN THE DG HIPPOCAMPAL SLICES. SALINE + SLEEP (A), SALINE + DEPRIVATION (B), PSILOCYBIN + SLEEP (C), PSILOCYBIN + DEPRIVATION (D). THE SUPRAPYRAMIDAL BLADE OF THE DG CONTAINED SIGNIFICANTLY MORE DCX-POSITIVE CELLS (ARROWS). THERE WAS NO SIGNIFICANT EFFECT CAUSED BY TREATMENT AND SLEEP MANIPULATION

Arc

Three-way ANOVA proved that the number of Arc-positive cells was significantly affected by all three examined factors including position ($F_{(1, 16)} = 78.790$; $p < 0.001$), treatment ($F_{(1, 16)} = 5.694$; $p = 0.030$), and manipulation ($F_{(1, 16)} = 6.546$; $p = 0.021$). The significant main effect of position was followed by the Tukey post hoc test which showed a significant increase of expression of Arc in suprapyramidal blade of DG ($p < 0.001$). Further, a significant interaction between treatment and manipulation ($F_{(1, 16)} = 10.690$; $p = 0.005$) was found. Analyses were therefore followed with Two-way ANOVAs separately in each part of the DG to further investigate this interaction (Fig. 17 and 18).

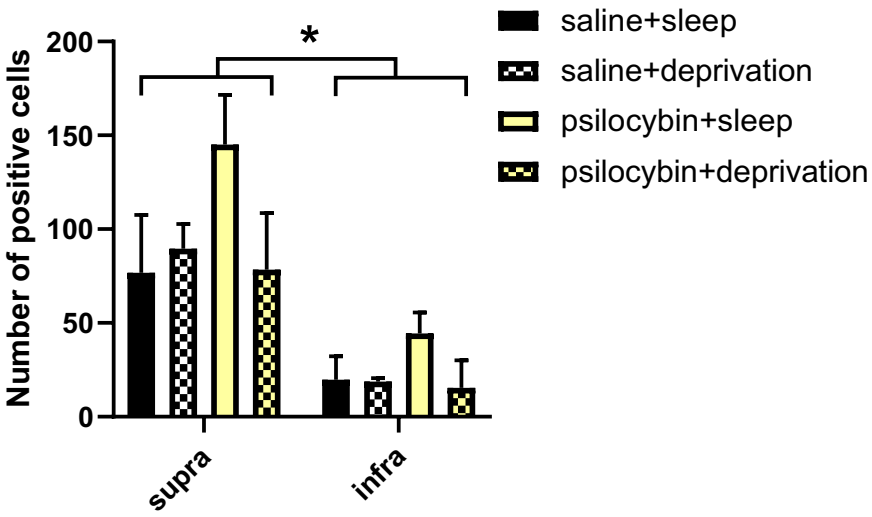

FIGURE 17: NUMBER OF ARC-POSITIVE CELLS IN THE SUPRA- AND INFRAPYRAMIDAL BLADES OF THE DG. RESULTS WITHIN TREATMENT AND MANIPULATION IN GROUPS. DATA SHOWN ARE COLUMNS WITH MEANS ± SD; N = 3 FOR ALL GROUPS. ANOVA FOLLOWED BY TUKEY POST HOC TEST. * P < 0.05.

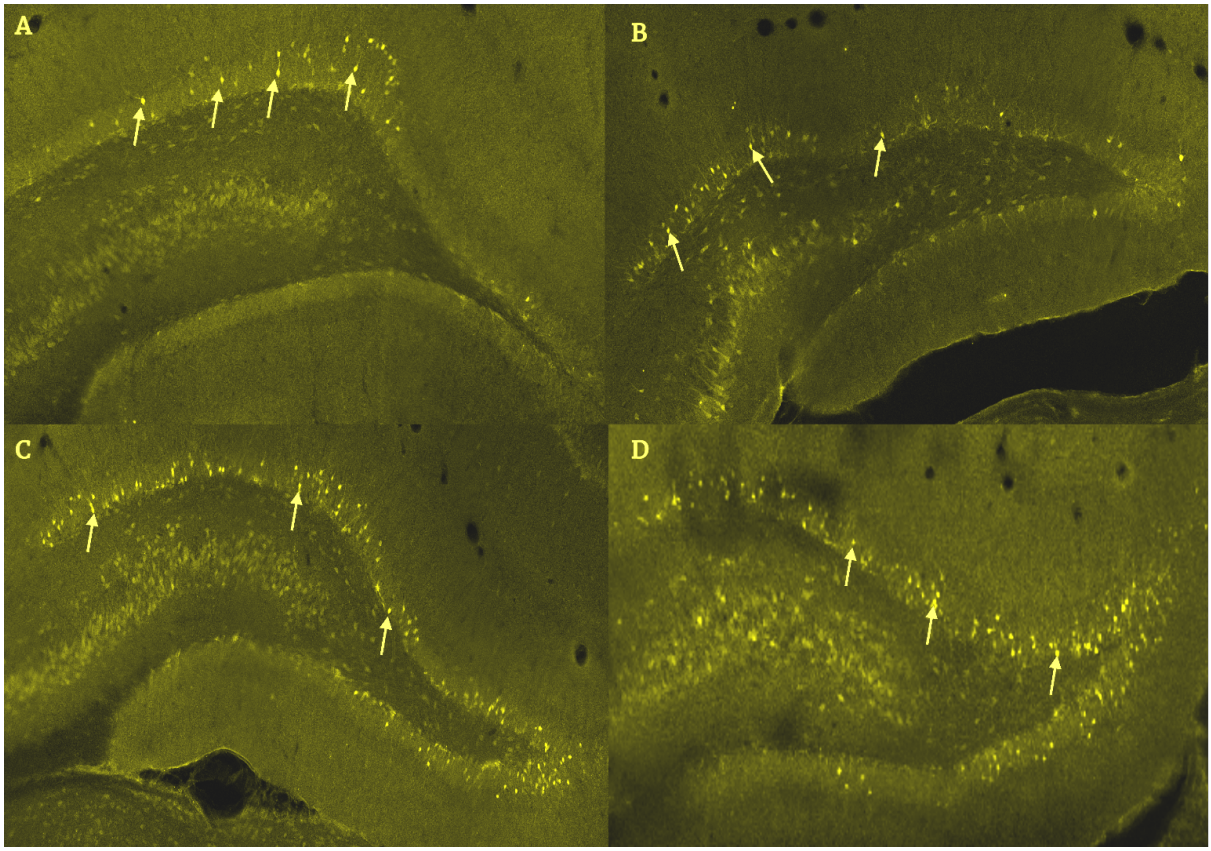

FIGURE 18: ILLUSTRATIVE IMAGES OF ARC LABELLING IN THE DG HIPPOCAMPAL SLICES. SALINE + SLEEP (A), SALINE + DEPRIVATION (B), PSILOCYBIN + SLEEP (C), PSILOCYBIN + DEPRIVATION (D). THE SUPRAPYRAMIDAL BLADE OF THE DG CONTAINED SIGNIFICANTLY MORE ARC-POSITIVE CELLS (ARROWS)

Suprapyramidal blade

In the suprapyramidal blade, Two-way ANOVA proved that the number of Arc-positive cells was significantly affected by the interaction of treatment and manipulation ($F_{(1, 8)} = 6.931$, $p = 0.030$), whereas the tested factors itself didn't cause any significant effect. According to Tukey post hoc test, the number of positively stained cells tended to be higher when animals were treated with psilocybin and allowed to sleep undisturbed (sleep + psilocybin group) than in any other group of animals, but the trend was non-significant ($p = 0.05$-0.1) (Fig. 17 and 18).

Infrapyramidal blade

According to Two-way ANOVA, the number of Arc-positive cells in the infrapyramidal blade was significantly affected by sleep manipulation ($F_{(1, 8)} = 5.438$, $p = 0.048$). The interaction between treatment and manipulation only showed a trend towards

significance ($F_{(1, 8)} = 4.664$, $p = 0.063$), whereas the treatment itself didn't cause any significant effect. The Tukey post hoc test revealed that the number of positively stained cells tended to be higher when animals were treated with psilocybin and allowed to sleep undisturbed (sleep + psilocybin group) than in any other group of animals, but the trend was non-significant ($p = 0.052 - 0.1$) (Fig. 17 and 18).

11.4 Vigilance state analysis from EEG recordings

Wakefulness

One-way RM ANOVA showed significant effect of repeated treatment + manipulation ($F_{(3, 12)} = 376.500$, $p < 0.001$). According to Tukey post hoc test, the animals that were sleep-deprived spent a significantly longer time awake than those that were left to sleep undisturbed ($p < 0.001$). Furthermore, when the animals were treated with psilocybin and slept undisturbed (psilocybin + sleep) they spent a significantly longer time awake than those that were treated with saline and slept undisturbed (saline + sleep) ($p < 0.05$, Fig. 19).

SWS

One-way RM ANOVA revealed significant effect of repeated treatment + manipulation ($F_{(3, 12)} = 321.5$, $p < 0.001$). According to Tukey post hoc test, SWS was significantly reduced when animals were sleep-deprived compared to those that slept undisturbedly ($p < 0.001$). For illustration see Fig 19.

PS

One-way RM ANOVA revealed a significant effect of repeated treatment + manipulation ($F_{(3, 12)} = 48.420$, $p < 0.001$). According to the Tukey post hoc test, PS was significantly reduced when animals were sleep-deprived compared to when they slept undisturbed ($p < 0.05$ – $p < 0.001$). Furthermore, animals that were treated with psilocybin and slept undisturbed (psilocybin + sleep) had significantly more PS than compared to those that were treated by saline and slept undisturbed (saline + sleep) ($p < 0.001$). For illustration see Fig. 19.

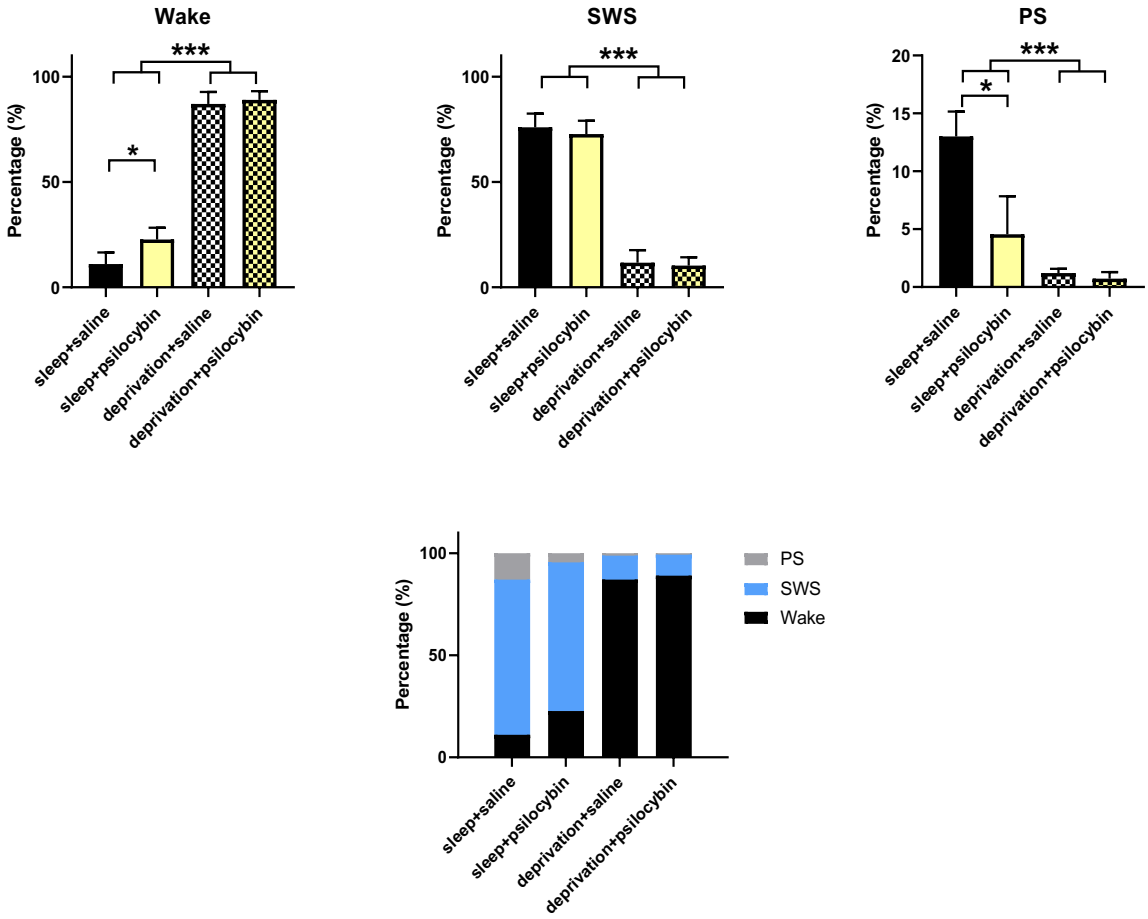

FIGURE 19: PERCENTAGE OF WAKEFULNESS (A), SWS (B) AND PS (C) DURING EEG RECORDING. RESULTS WITHIN REPEATED TREATMENT AND MANIPULATION IN ANIMALS. DATA SHOWN ARE COLUMNS WITH MEANS ± SD; N = 5. (D) DATA SHOWN ARE PARTS OF A WHOLE FOR EACH REPEATED TREATMENT AND MANIPULATION – VIGILANCE STATE ANALYSIS. RM ANOVA FOLLOWED BY TUKEY POST HOC TEST. * $P < 0.05$, *** $P < 0.001$.

12 DISCUSSION

We have failed to establish whether the combined action of psilocybin administration and sleep manipulation had a significant effect on memory consolidation in behavioural tasks. Sleep manipulation and psilocybin also appeared to have little effect on neurogenesis and Arc expression in the hippocampus. We were successful in validating the efficacy of the gentle handling method utilized for sleep deprivation and optimizing the biotelemetric implantation paradigm used to acquire the relevant data.

12.1 OPR

Our findings indicate that neither psilocybin nor sleep manipulation appeared to influence memory consolidation in the OPR task and we also failed to find any significant effect when testing the potential interactions of treatment and sleep manipulations. On the other hand, we proved that the animals had spent more time exploring the displaced object in the evening sessions and had therefore presumably memorized its previous location. The exploration times in the morning session were relatively uniform across all groups. The sole group that showed no difference in exploratory activity between the morning and evening was the deprivation + saline group. This effect was not ruled significant, however.

Our results contradict the expected effects of sleep manipulation and 5-HTR activation. Sleep after the OPR task was previously proved to enhance memory consolidation (Binder et al., 2012). Others have also shown that a period of SD immediately after the OPR task impaired memory consolidation and subsequent retrieval (Binder et al., 2012; Ishikawa et al., 2014; Palchykova et al., 2006). This is in line with the idea that sleep plays an essential role in hippocampus-dependent memory consolidation. It was also previously proved, that selective 5-HT_{2A}R activation with TCB-2 enhanced object memory consolidation (Zhang et al., 2013). The enhancement of memory was dose-dependent and was blocked by the administration of 5-HT_{2A}R antagonist MDL 11,939.

Since rats are typically most active during their dark phase, the fact that the experiment had been carried out during the light phase may have harmed performance (Hawkins & Golledge, 2018). The lack of significant effect in our findings could be attributed to different phases of circadian rhythms which have been shown to impact performance in

memory tasks (Binder et al., 2012). However, the most likely factor is the small sample size.

12.2 MWM

Average escape latency and trajectory length

Our analysis revealed that sleep manipulation significantly affects the average escape latencies to the platform. This effect also showed a tendency to interact with the time of measurement. Even though post hoc did not confirm any significant difference specifically between animals which was let sleep undisturbed and sleep-deprived animals, it still can be interpreted as SD tended to impaired memory consolidation during the delay period between the times of measurements. This is consistent with various sources demonstrating post-learning SD can impair learning and spatial memory consolidation in rodents in various paradigms such as the MWM (Saygin et al., 2017; Yang et al., 2008; Zhang et al., 2017), object recognition (Palchykova et al., 2006), radial arm water maze (Zagaar et al., 2012), and novel arm recognition task (Hagewoud, Havekes, et al., 2010). A similar pattern was found for trajectory length; however, the effect was not deemed significant for sleep manipulation or interaction between manipulation and time of measurement. None of the other factors appeared to have a significant impact on MWM task performance. This is consistent with Rambousek et al.(2014) who found that although psilocin impaired memory retrieval after 4 days of MWM pre-training it did not impact memory consolidation. Zhang et al. found no significant difference in swimming duration in release and target quadrant between mice that received TCB-2, TCB-2 with MDL 11,939 and control (2017). The TCB-2 group swam slower in the target quadrant and displayed a delayed entry into the quadrant which was in line with reduced locomotion found in an open field setup (Zhang et al., 2017). Similarly, psilocybin was found to have little effect on spatial working memory in humans (Carter, Burr, Pettigrew, Wallis, et al., 2005). It should be noted that the lack of significant effect may have also been a consequence of the small sample size used in the experiment (n=10).

1st evening swim

By analyzing the 1st evening swim, we aimed to evaluate whether the animals have successfully memorized the position of the platform from the morning sessions. This should therefore be a measure of whether memory consolidation has taken place.

The results of the 1st evening swim proved to be somewhat difficult to interpret as the escape latencies and trajectory lengths varied greatly and the data were not normally distributed. Despite this, we managed to find a significant between-group difference in escape latencies of animals that received saline and were sleep-deprived (saline + deprivation) compared to saline + sleep animals with the latter showing shorter escape latencies. Similarly, SD has been shown to impair spatial memory in a probe trial in works that were previously published (Guan et al., 2004; McCoy et al., 2013; Saygin et al., 2017). In other MWM setups, a probe trial is typically administered after the learning phase of memory acquisition. The platform is then removed, and the rat is placed in the arena for a given amount of time. The time spent searching in the target quadrant in which the platform had originally been present is measured (Vorhees & Williams, 2014).

In the future, a probe trial analysis approach could reveal more conclusive results for our purpose, as it has been shown that SD resulted in reduced time spent searching in the target quadrant (Karabulut et al., 2019; Salari et al., 2015).

12.3 IHC

DCX

Based on the results of our IHC labelling, none of the manipulations and treatment combinations appeared to significantly influence the number of DCX-positive cells in the DG. When considering each portion of the DG as a separate unit (supra- and infrapyramidal blades) we also failed to find any significant differences in DCX expression across groups. This may be attributed to the temporal characteristics of DCX expression. DCX is a marker of NPCs differentiating into neurons, in the DG (Gleeson et al., 1999). Since the brains were harvested immediately after the behavioural task there may have not been a sufficient time window for detectable differentiation to take place. Jha et al. found no significant effect of neither blockade nor activation (with ketanserin and DOI/LSD respectively) of $5HT_{2A/C}$ receptors(2008).

We did however find that the suprapyramidal blade of the DG appeared to contain a significantly higher number of DCX-positive cells in all groups compared to the infrapyramidal blade. This finding is in line with previous works which had shown suprapyramidal granule cells appear to be preferentially activated after experience and exposure to novel environments resulting in greater expression of relevant proteins (Chawla et al., 2005; Erwin et al., 2020; Guenthner et al., 2013; Jha et al., 2008; Ramírez-Amaya et al., 2005).

Arc

Just as in the first case we found that the suprapyramidal blade of the DG contained a significantly higher number of Arc immunoreactive cells. This is once again consistent with previous findings focused on the expression of Arc as well as other IEGs (Chawla et al., 2005; Erwin et al., 2020; Guenthner et al., 2013; Ramírez-Amaya et al., 2005) In the suprapyramidal blade the number of Arc-positive cells was shown to be increased in the group that received psilocybin and was left to sleep undisturbed compared to any other group, however, the difference was closely non-significant. We observed the same pattern in the infrapyramidal blade, where the number of immunoreactive cells was again increased by sleep and psilocybin treatment, but the difference was not statistically significant.

The data previously published on Arc expression and its relation to sleep and sleep deprivation showed mixed results. Arc mRNA was upregulated following novelty exposure in the frontal cortex and hippocampus of mice (MA et al., 2011). Pre-treatment with a 5-HT_{2A}R antagonist inhibited the up-regulation of Arc mRNA in the frontal cortex but not the hippocampus. When compared with undisturbed sleep, SD has been shown to induce higher Arc expression in the hippocampus in rodents (Cirelli & Tononi, 2000; Thompson et al., 2010). However, others have reported the opposite; that is, sleep increases the expression of Arc in the DG and SD having little to no effect (Delorme et al., 2019). A single dose of psilocybin has been shown to induce a minor but statistically significant decrease in Arc expression (Jefsen et al., 2021b). Using other serotonergic hallucinogens, it has been demonstrated that single doses of LSD (Nichols & Sanders-Bush, 2001) and DOI (Benekareddy et al., 2013; Q et al., 2000) induced robust expression of Arc in neocortical areas of the rat brain, but Arc levels in the hippocampus remained relatively unchanged. Our findings on Arc expression appear to be in line with

some of the effects of other related 5-HT2AR agonists. Arc levels were primarily affected in neocortical areas while remaining relatively stable in the hippocampus. However, the lack of significant findings could also be attributed to the small sample size (n=3).

12.4 EEG

Vigilance state analysis

Using the telemetric EEG module, we successfully assessed the vigilance states in repeatedly treated animals. According to expectations, the animals that were subjected to SD spent a significantly longer time in a wakeful state compared to those that slept undisturbed. Furthermore, the animals that received psilocybin and slept undisturbed spent a significantly longer time in wakefulness compared to their control group (saline + sleep). SD significantly reduced the amount of SWS and PS in all sleep-deprived animals compared to those which slept undisturbed. Furthermore, the animals that received psilocybin and slept undisturbed had significantly less PS compared to animals that received saline and slept undisturbed.

Our findings are in line with existing data on the effect of serotonergic hallucinogens on sleep architecture. An acute dose of psilocin disrupted sleep in mice reduced the amount of SWS and PS and lead to prolonged wakefulness following administration (Thomas et al., 2021). Experiments on rats with other serotonergic hallucinogens like LSD(Depoortere & Loew, 1971), DOI(Monti et al., 1990; Monti & Jantos, 2006), and mescaline (Colasanti & Khazan, 1975) have yielded similar results.

12.5 5-HT_{2A}R EXPRESSION IN THE HIPPOCAMPUS

Since we examined the effect of serotonergic agonists on memory consolidation, it would be only logical to investigate the expression of 5-HT_{2A}R in the hippocampus. We indeed originally intended to examine these effects. At first, we tried to analyse the expression of the receptor with IHC, but after unsuccessful attempts with 2 different 5-HT_{2A}R-specific antibodies, we decided to abandon this approach. The lack of specific binding contradicted the robust literature on 5-HT_{2A}R expression in the hippocampus, which lead us to believe that the antibodies were faulty. Furthermore, we also collected

hippocampal tissue samples which were intended for RT-qPCR analysis but due to financial reasons and time constraints the results will have not made it into this thesis.

Recently a study conducted on pigs showed that a single dose of psilocybin increased synaptic density and decreased 5-HT_{2A}R density in the hippocampus (Raval et al., 2020). Azizi et al. administered 24h SD using the gentle handling method with effects persisting for 48 h after session, then receding to original levels. We have found no relevant sources investigating the effects of SD on 5-HT_{2A}R expression. Western blot analysis showed elevated levels of 5-HT_{1A}R detected in the CA1 hippocampal region of SD rats compared to control. When measuring population excitatory postsynaptic potentials in CA1, they discovered a dose-dependent depression of the response in all groups. However, the 5-HT treatment produced a steeper dose-response curve in SD rats (Azizi et al., 2017).

Several studies have linked the downregulation of 5-HT_{2A}R to the development of tolerance to various psychedelics after repeated exposure. Buckholtz et. al reported that daily LSD administration resulted in selective reduction of 5-HT_{2A}R (1985, 1988). Daily administration of several 5-HT_{2A} agonists also lead to a similar decrease in receptor binding while an acute dose produced no change in binding (Buckholtz et al., 1988).

13 CONCLUSION

We have not succeeded in finding a clear interaction between sleep and psilocybin administration and their effect on spatial memory consolidation in the rat. The process of memory consolidation and learning appeared to be impaired by sleep deprivation, but psilocybin had little effect on it. In the least, we were able to show that psilocybin did not lead to any memory impairments. Psilocybin and learning did not influence DCX expression, but this was likely due to the short temporal window for new neuron differentiation to take place. Psilocybin showed a trend towards increasing Arc expression which could, in turn, lead to an increase in neuroplasticity at hippocampal sites relevant to memory formation. We have successfully validated the efficacy of an ongoing biotelemetric paradigm and of the method for SD. Furthermore, we were able to observe the influence of psilocybin on sleep architecture. In recent years, psychedelic

research has been steadily on the rise due to the promising results of serotonergic psychedelics as a therapeutic agent for depression, anxiety, and other clinically relevant psychiatric diseases. Such preclinical research offers a great opportunity to further our understanding of how these substances mediate their effects. Since psychedelics are also being intensely researched for their proposed beneficial influence on cognition and general mental well-being, our telemetric paradigm will be of great use in future experiments that require long-term monitoring. To name just one, the paradigm is already being implemented in our facility for the research of chronic application of psychedelics and micro-dosing effects.

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