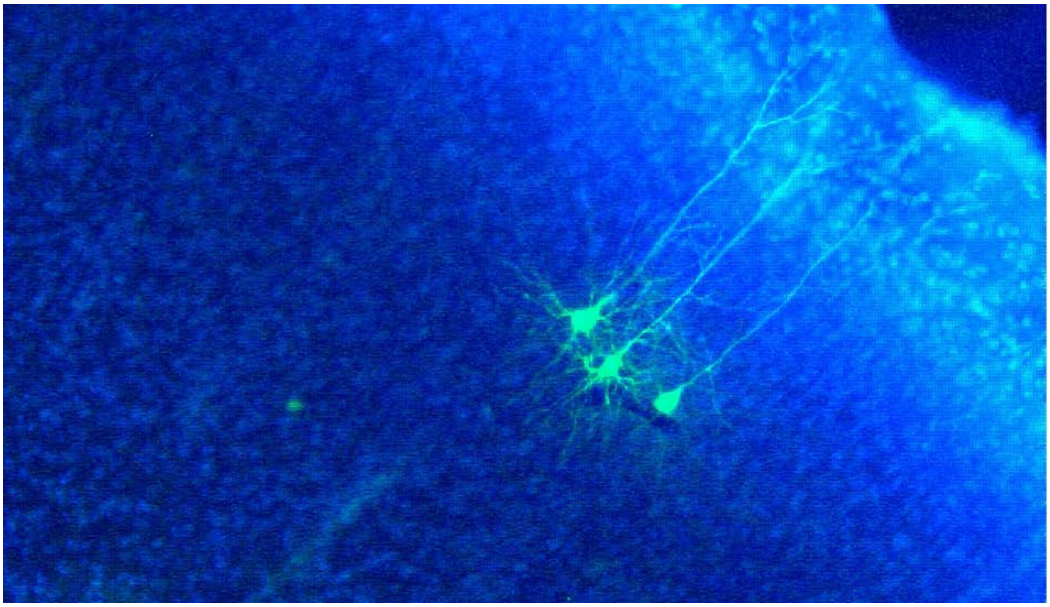

Emanuel Lima Oliveira

Circuit plasticity during Up-states in mouse barrel cortex

Mestrado em Investigação Biomédica,

Faculdade de Medicina

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UNIVERSIDADE DE COIMBRA

"A vida sem ciência é uma espécie de morte."

- Sócrates

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Investigação Biomédica, realizada sob a orientação científica do Professor Doutor Edward Mann (University of Oxford) e co-orientação institucional do Professor Doutor Henrique M.P. dos Santos Girão (Universidade de Coimbra)

Cover illustration: Patched pyramidal Cells from layer 2/3 (green) identified by fluorescent detection of biocytin. DAPI used to mark cell bodies (blue).

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RESUMO

O sono é caracterizado por ocorrências cíclicas de movimento rápido dos olhos (REM) e não-REM, pelo qual inclui “Slow wave sleep” (SWS, estágio 3 e 4) e o sono leve correspondentes aos estágios 1 e 2. Nos humanos, a primeira parte da noite (sono recente) consiste em SWS e o sono REM prevalece durante a segunda metade (sono tardio). Num evento de rede inteira, o sono suprime os sinais moleculares que mediam a remodelação sináptica relacionado com LTP mas estimula a sinalização dependente de LTD, parecendo ser mediado pelo SWS[4]. As oscilações lentas tem sido anteriormente observadas em diferentes áreas corticais, ocorrendo espontaneamente up-states e down, ambos *in vivo* e *in vitro.* Os espontâneos up-states ocorrem intrinsecamente dentro do córtex [5, 6]. Também conseguem ser observados *in vivo* em resposta a uma entrada sensorial [7] e conseguem ser ligados e desligados por estimulação sináptica *in vitro*[8].

O processo da consolidação de memórias pode produzir varias associações e alterações qualitativas na representação de memórias, em que o sono tem o potencial final de estabilizar os seus efeitos e melhorar a sua performance, sendo observado em tarefas de memória declarativas e procederias[9]. A hipótese de homeostasia propõe que a rede integrativa de pesos sinápticos, devido à codificação de informação durante o acordar, é compensada durante um subsequente SWS. De acordo com esta hipótese, o SWS serve para globalmente efetuar um “downscale” de pesos sinápticos ao longo do cortex, e assim aumentar o rácio sinal-para-ruído para reativar memórias e preservar a capacidade das redes corticais de armazenar nova informação, prevenindo a sua saturação[10].

Os endocanabinoides (ECs) são uma classe de neurotrasmissores sintetizados e libertados a partir da membrana pós-sináptica dos PNs corticais durante períodos de estimulação de atividade celular, como por exemplo, durante os up-states. No córtex, a

ativação de receptores CB1 diminuí a libertação de GABA e glutamato[11], sugerindo que este sistema neuromodulatório loca pode sintonizar a atividade das redes neuronais regulando os níveis de neurotransmissores excitatórios e inibitórios dentro dos circuitos corticais.

Dada a importância de consolidação de memórias durante o SWS, nós procedemos à investigação dos efeitos modulatórios dos recetores CB1 enquanto bloqueados pelo antagonista AM251. A presença de AM251 nas fatias de "barrel córtex" do ratinho resultaram numa diminuição de amplitude do estado up e, por sua vez, em acordo com a importância dos endocanabinoides na modelação das oscilações SWS.

Para investigar qual dos dois processos de plasticidade neuronal (LTP ou LTD) ocorre durante a plasticidade sináptica das redes neuronais presentes no "barrel córtex" durante a fase de SWS, os nossos resultados indicam que após uma experiência protocolo de "pairing" em up-states, são observados efeitos de LTD dada à redução significativa dos níveis de slope e força neuronal (EPSPs).

Em suma, é notavel mencionar o fato de afetar o sistema de endocanabinoides com o auxílio de antagonistas pode vir a ajudar no tratamento de, por exemplo, desordens de stress pós-traumático. Para além disso, estas descobertas permitem sugerir a importância de LTD durante o SWS no "barrel córtex" de ratinhos e, em termos de perspectivas futuras, poder-se-á proceder a experiências *in vivo*, de forma a revelar posterior informação valiosa sobre os mecanismos por detrás dos up-states em mamíferos e, potencialmente, estimular/inibir o processamento de memórias.

ABSTRACT

Sleep is characterized by the cyclic occurrence of rapid eye movement (REM) sleep and non-REM sleep, which includes slow wave sleep (SWS, stages 3 and 4) and lighter sleep stages 1 and 2. In humans, the first part of the night (early sleep) consists of SWS, whereas REM sleep prevails during the second half (late sleep). In a whole network event, sleep suppresses the molecular signals that mediate LTP-related synaptic remodelling but enhances LTD-related signalling, and this effect seems to be mediated by SWS[4]. Slow oscillations have previously been observed in different cortical areas as spontaneously occurring up- and down-states both in vivo and in vitro. Spontaneous up-states occur intrinsically within the cortex [5, 6]. They can also be observed in vivo in response to sensory input[7] and can be turned on and off by synaptic stimulation in vitro [8].

Memory consolidation can produce several associations and qualitative changes in memory representations, whereas sleep has the potential to outcome stabilizing effects and improve their performance, being observed in declarative and procedural memory tasks[9]. The synaptic homeostasis hypothesis proposes that the net build-up of synaptic weights, due to encoding of information during waking, is compensated during subsequent SWS. According to this hypothesis, SWS serves to globally downscale synaptic weights across the cortex, thus increasing the signal-to-noise ratio for reactivated memories and preserving the capacity of cortical networks to store new information by preventing their saturation[10].

Endocannabinoids (ECs) are a class of neurotransmitters synthesized and released from the post-synaptic membrane of cortical PNs during periods of enhanced cellular activity such as during up-states. In the cortex, activation of CB1 decreases release of

both GABA and glutamate[11] suggesting this local neuromodulatory system may tune network activity by regulating both excitatory and inhibitory neurotransmission within local cortical circuits.

Para investigar qual dos dois processos de plasticidade neuronal (LTP ou LTD) ocorre durante a plasticidade sináptica das redes neuronais presentes no “barrel cortex”, presente na fase de SWS, os nossos resultados indicam que após uma experiência protocolo de “pairing” em up-states, são observados efeitos de LTD dada à redução significativa dos níveis de slope e força neuronal (EPSPs) enfraquecida.

Given the importance of memory consolidations during SWS, we investigate the modulatory effects of CB1 receptors while being blocked by the antagonist AM251. The presence AM251 in a mouse barrel cortex slice resulted in an up-state amplitude decrease therefore agreeing with the importance of endocannabinoids in the modulation of SWS oscillations.

To investigate which of the two processes of neural plasticity (LTP or LTD) occur during synaptic plasticity in the barrel cortex circuitry, present in SWS, our results indicate that after conducting a pairing protocol experiment in up-states, it is observed LTD effects duo to a significant reduction of slope levels and weakening neural strength (EPSPs).

In sum, it is remarkable to mention that targeting the endocannabinoid system may aid in the treatment of disorder associated with impaired extinction-like processes, such as post-traumatic stress disorder. Overall these findings allow to suggest the importance of LTD during SWS in mouse barrel cortex and future projects can now proceed to *in vivo* experiments, in order to reveal further promising information about the underlying mechanisms of up-states in mammals and potentially enhance/inhibit the memory consolidation processing.

ABBREVIATIONS

aCSF	Artificial cerebralspinal fluid
AM251	N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
AMPA	Α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
Arc	Activity-regulated cytoskeleton-associated protein
CA1	Region I of hippocampus proper
EC	Endocannabinoid
Egr1	Early growth response 1
EPSP	Excitatory postsynaptic potential
GABA	Gamma-Aminobutyric acid
IEGs	Immediate early genes
LTD	Long term depression
LTP	Long term potentiation
M1	Motor cortex
ms	Mili-second
NMDA	N-methyl-D-aspartate
PGO	Ponto-geniculooccipital
PKA	Camp-dependent protein kinase A
POm	Posterior thalamic nucleus
PSD	Post-synaptic density
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SWS	Slow wave sleep
VGCC	Voltage-gated calcium channels.
VMP	Ventral posteromedial nucleus
vS1	Vibrissal somatosensory system

Chapter I - Introduction

1.1 - Sleep: Importance and phases

Sleep is a natural biological process capable of reducing responsiveness to external stimuli and loss of consciousness. This process follows regular intervals and is homeostatically regulated. If in deprivation or delay status it is possible to result in prolong sleep [12, 13] or, in more severe cases, cognitive impairments [14]. While studying for several weeks sleep deprived, it was observed temperature and weight dysregulation and, ultimately, infections and tissue lesions [15].

Sleep is a very common process between all vertebrates and sleep states are surprisingly similar in invertebrates, i.e. flies, bees and cockroaches [16].

In an evolutionary point of view, sleep is a dangerous process which positions a significant danger towards survival by reducing threatening stimuli and responsiveness. Though there is still a strong debate about sleep exact functions [17], it has been proposed for energy-saving function [18, 19], the restoration of energy resources and the repairing of cell tissue [20], thermoregulation [15], metabolic regulation [21, 22], and adaptive immune functions [23]. Yet, all of these functions could be reached by having a state of quiet wakefulness and couldn't still explain the reason of occurring loss of consciousness and responsiveness to external threats during sleep. These features can be considered related to brain processes in which targets the consciousness state [24, 25].

In sum, from detoxication of free radicals in the brain [26, 27], glycogen replacement [28], and, most promising, the involvement of sleep in memory and synaptic plasticity [29, 30].

Sleep is characterized by the cyclic occurrence of rapid eye movement (REM) sleep and non-REM sleep, which includes slow wave sleep (SWS, stages 3 and 4) and lighter sleep stages 1 and 2. In humans, the first part of the night (early sleep) consists of SWS, whereas REM sleep prevails during the second half (late sleep).To distinguish the SWS and REM sleep, it is important to identify specific patterns of electrical field potential oscillations and neuromodulator activity: SWS is hallmarked by slow high-amplitude EEG oscillations (slow wave activity, SWA), whereas REM sleep is characterized by fast and low-amplitude oscillatory brain activity [29, 31].

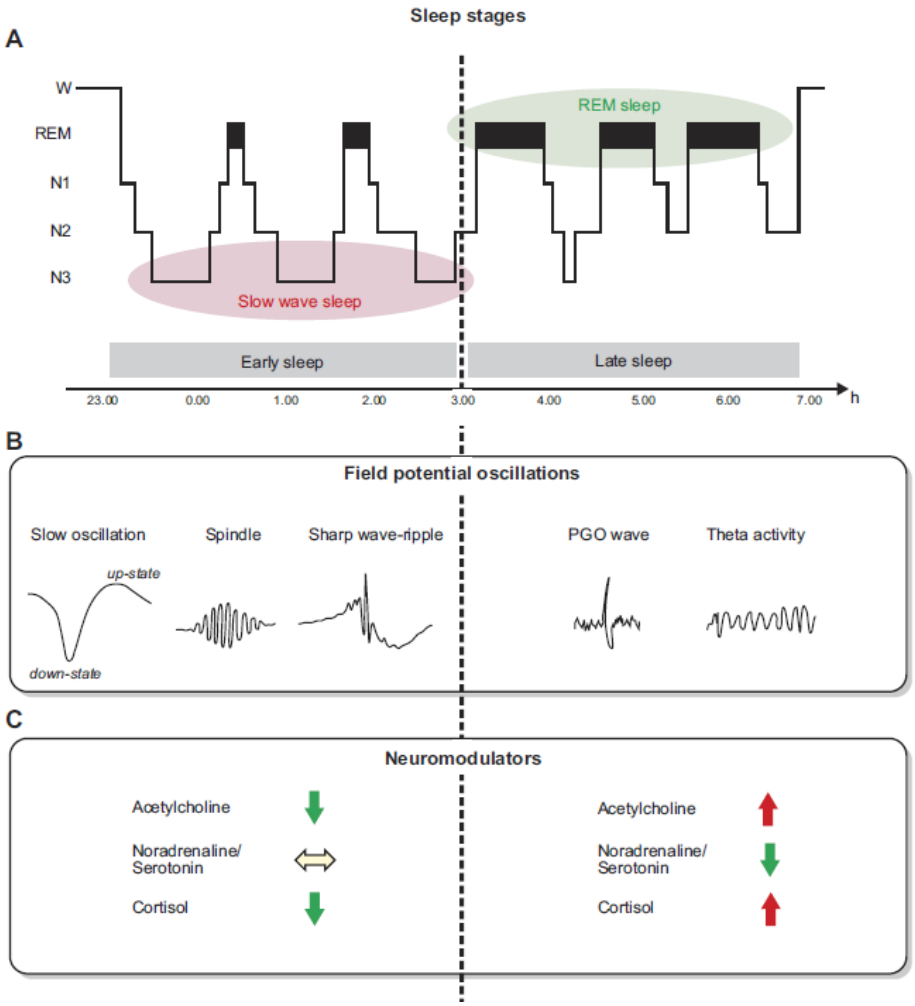

Figure 1 | **Typical human sleep profile and sleep-related signals**.

A: sleep is characterized by the cyclic occurrence of rapid-eye-movement (REM) sleep and non-REM sleep. Non-REM sleep includes slow-wave sleep (SWS) corresponding to N3, and lighter sleep stages N1 and N2. SWS is divided into stage 3 and stage 4 sleep. The first part of the night (early sleep) is dominated by SWS, whereas REM sleep prevails during the second half (late sleep). B: the most prominent electrical field potential oscillations during SWS are the neocortical slow oscillations (~0.8 Hz), thalamocortical spindles (waxing and waning activity between 10–15 Hz), and the hippocampal sharp wave-ripples (SW-R), i.e., fast depolarizing waves that are generated in CA3 and are superimposed by high-frequency (100–300 Hz) ripple oscillation. REM sleep, in animals, is characterized by ponto-geniculooccipital (PGO) waves, which are associated with intense bursts of synchronized activity propagating from the pontine brain stem mainly to the lateral geniculate nucleus and visual cortex, and by hippocampal theta (4–8 Hz) activity. In humans, PGO and theta activity are less readily identified. C: sleep is accompanied by a dramatic change in activity levels of different neurotransmitters and neuromodulators. Compared with waking, cholinergic activity reaches a minimum during SWS, whereas levels during REM sleep are similar or even higher than those during waking. A similar pattern is observed for the stress hormone cortisol. Aminergic activity is high during waking, intermediate during SWS, and minimal during REM sleep.

1.2 - Types of oscillations during sleep

During SWS, the most noticeable field potential oscillations are slow oscillations, spindles and sharp wave-ripples. In REM sleep originates ponto-geniculooccipital (PGO) waves and theta activity. The frequency of slow wave oscillations can reach ~0,8 Hz, whereas synchronize their neuronal activity into down-states of widespread hyperpolarization and neuronal silence and subsequent up-states, which are associated with depolarization and strongly increased, wake-like neuronal firing[32]. The rise of hyperpolarization results from activation of a Ca^{2+}-dependent K^+ current and inactivation of Na^+ current, which lowers excitability[33]. By triggering up-states depolarization (simultaneously in several neurons), can originate a summation of miniature EPSPs (from residual activity from encoding information), which is formed by persistent Na+ currents.

Spindle activity refers to regular electroencephalographic oscillations of ~10–15 Hz, which are observed in human sleep stage 2 as discrete waxing and waning spindles, but are present at a similar level during SWS (although here they form less discrete spindles)[34]. Spindles originate in the thalamus from an interaction between GABAergic neurons of the nucleus reticularis, which function as pacemakers, and glutamatergic thalamo-cortical projections that mediate their synchronized and widespread propagation to cortical regions[34]. Hippocampal sharp waves are fast depolarizing events, generated in the CA3, on which high-frequency oscillations (100–300 Hz) originating from an interaction between inhibitory interneurons and pyramidal cells in CA1 (so-called ripples) are superimposed[35]. Sharp wave-ripples occur during SWS and also during waking, and accompany the re-activation of neuron ensembles that are active during a preceding wake experience[36]. PGO-waves are driven by intense bursts of synchronized activity that propagate from the pontine brainstem mainly to the lateral geniculate nucleus and

visual cortex. They occur in temporal association with REM in rats and cats but are not reliably identified in humans. Theta oscillations (4–8 Hz) hallmark tonic REM sleep in rats and predominate in the hippocampus[35]. In humans, theta activity is less coherent[37].

1.3 - Impact of sleep in memory

Greater improvements on memory have been potentially associated with well and consistent sleep. It has been determined that 8 hours of night sleep and 1-2 hours of short naps increases memory retention. Surprisingly, procedural memories are particularly consolidated in prolonged sleep durations[38]. There are also some studies which indicate a better optimization when there is a short delay between learning and sleep[39].

For memories to gain access for the sleep-dependent consolidation mechanisms, it is important to highly consider the encoding conditions. It is likely to consider that declarative memories at typically explicit and procedural memories can be characterized as both implicit and explicit processes. In several data it has been suggested that explicit encoding of a memory favours access to sleep-dependent consolidation[40].

The importance of sleep has been tightly related with several benefits in forming memories more difficult to encode, and even greater for memories with a significant behavioural meaning. Hence, it is suggested that sleep consolidation of memories can be related for planning and future actions (D. S., I. Wilhelm, u. Wagner, J. b., unpublished observations). It is also to consider that a motivational tag in memories gain a superior performance when re-testing after sleep[41]. Thus, the memorization process can be enhanced by relevance and behavioural effort, integrating intentional and motivational aspects of a task/environmental relevant elements. A great neural circuitry

communication between the prefrontal cortex and the hippocampus might be determined to bring a memory in a sleep-dependent memory consolidation system[42].

Memory consolidation can produce several associations and qualitative changes in memory representations, whereas sleep has the potential to outcome stabilizing effects and improve their performance, being observed in declarative and procedural memory tasks[9].

It is still unknown whether sleep passively protects memories from decay and interference or actively consolidates fresh memory representations44. However, there is a strong evidence for an active consolidating influence of sleep from behavioural studies, which determines that sleep has the potential to change qualitatively memory[43]. For example, in one study, subjects learned single relations between different objects which, unknown to the subject, relied on an embedded hierarchy[43]. When learning was followed by sleep, subjects at a re-test were better at inferring the relationship between the most distant objects, which had not been learned before. Likewise, after sleep subjects more easily solved a logical calculus problem that they were unable to solve before sleep or after corresponding intervals of wakefulness. Of note, sleep facilitated the gain of insight into the problem only if adequate encoding of the task was ensured before sleep[44].

From the behavioural test results described above it was observed the capacity of re-organizing newly encoded memory representations, coupled with associations and extraction of invariant features from complex stimuli. Sleep also promotes the transformation of implicit into explicit knowledge, as was shown in an SRTT which was implicitly trained but in which explicit knowledge about the underlying sequence was examined during the re-test[45]. Following post-training sleep, subjects were better at

explicitly generating the SRTT sequence. The study results suggest that procedural and declarative memory systems interact during sleep-dependent consolidation.

It has also been proposed that disengagement of memory systems is an essential characteristic of sleep-dependent consolidation49. Experimental results showed that declarative learning of words immediately after training of a procedural skill can block off-line improvement in that skill if the subject does not sleep between learning and re-testing, but not if the subject sleeps between learning and re-testing[46]. This suggests that memory systems compete and reciprocally interfere during waking, but disengage during sleep, allowing for the independent consolidation of memories in different systems. Thus, the can be related to the synchronous functionality of different sleep stages (REM sleep and SWS), as discussed below.

Memory formation has been considered as a process in which neuronal activity in specific circuits promotes synaptic changes. A process which occurs off-line after encoding relies on the re-activation of neuronal circuits that were initially “used” in the encoding of the information. This would promote both the gradual redistribution and re-organization of memory representations directed to long-term storage (system consolidation) and the synaptic changes that are necessary to stabilize memories (synaptic consolidation). The conditions that enable these two processes during sleep differ strongly between SWS and REM sleep.

Several studies determined the re-activation of neural circuits, whereas the analysis of the spatio-temporal patterns of neuronal firing occurring in the hippocampus during exploration of a novel environment are re-activated in the same order during subsequent sleep[47]. These events mostly arise during SWS and also the first hour after learning[48], and typically only in a minority of recorded neurons[47]. Moreover, unlike re-activations that occur during wakefulness, re-activations during SWS almost always

occur in the order in which they were experienced[49]. Compared with activity during encoding phases, re-activations during SWS seem to be noisier, less accurate and often happen at a faster firing rate. They are also observed in the thalamus, the striatum and the neocortex[13]. First evidences of the importance of SWS were the re-exposure to the odour during SWS, but not REM sleep, demonstrated an enhancekment of spatial memories and induced stronger hippocampal activation than during wakefulness, indicating that during SWS hippocampal networks are particularly sensitive to inputs that can re-activate memories.

In addition to system consolidation, consolidation involves the strengthening of memory representations at the synaptic level (synaptic consolidation)[50]. Long-term potentiation (LTP) is design as a key mechanism, but it is unclear whether memory re-activation during sleep promotes the redistribution of memories by inducing new LTP (at long-term storage sites not involved at encoding) or whether re-activation merely enhances the maintenance of LTP that was induced during encoding. LTP can be induced in the hippocampus during REM sleep but less reliably so during SWS[51]. LTP induction in the hippocampus or neocortex during SWS is probably temporally restricted to the up-states of the slow oscillation and its concurrent phenomena of ripples and spindles[52]. Indeed, in neocortical slices, stimulation that mimicked neuronal activity during SWS could induce long-term depression (LTD)[53] or LTP[52] depending on the pattern of stimulation (rhythmic bursts or spindle-like trains, respectively).

In a whole network event, sleep suppresses the molecular signals that mediate LTP-related synaptic remodelling but enhances LTD-related signalling, and this effect seems to be mediated by SWS[4]. This observation, however, does not preclude that LTP occurs during sleep (during SWS or REM sleep) in specific regions, for example in those that were engaged in memory encoding prior to sleeping. In rats, both induction of

hippocampal LTP and exposure to a novel tactile experience during waking increased the expression of the plasticity-related immediate early genes (IEGs) Arc and Egr1 (which are implicated in LTP) during subsequent sleep, mainly in cortical areas that were the most activated by the novel experience, and this effect seemed to be mediated by REM sleep. Findings in visual cortex in cats and humans have observed that sleep-dependent plasticity depends on the activation of glutamatergic NMDA (N-methyl-d-aspartate) receptors and associated cAMP-dependent protein kinase A (PKA), and on AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor activation, that is, the postsynaptic machinery that is crucial for the induction and maintenance of LTP9[54] (Figure 3). These findings indicate that local, off-line re-activation of specific glutamatergic circuits supports both LTP induction and maintenance, and the molecular processes underlying synaptic consolidation. Moreover, these processes probably occur preferentially during REM sleep, although they are likely to be triggered by the re-activations that occur during prior SWS. Evidence about how LTP induction and maintenance is linked to specific sleep stages is presently scarce, but based on the available data it is tempting to speculate that SWS supports the re-activation of new memories (system consolidation) and thus, could initialize LTP and therefore relevant to networks for synaptic consolidation during subsequent REM sleep (Figure 2).

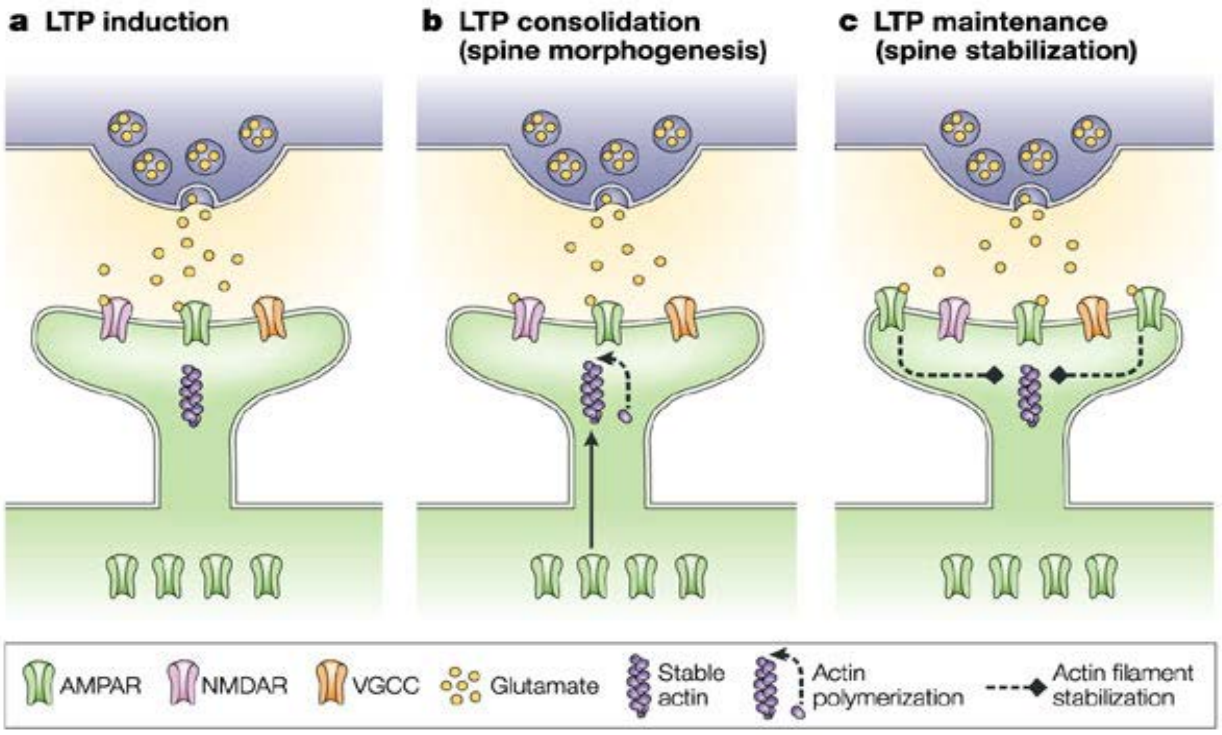

Figure 2 | **Long-term potentiation (LTP) and behavioural experience induce glutamate receptor trafficking into spines.**
a | LTP induction leads to activation of N-methyl-D-aspartate receptors (NMDARs). b | Ca2+ influx through NMDA receptors leads to trafficking of glutamate receptors into spines. c | Increase in AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs) in dendritic spines could enhance the responsiveness to glutamate and could contribute to glutamate-induced maintenance of spines by stabilizing actin filaments. VGCC, voltage-gated calcium channels.

1.4 - The two-stage model of memory consolidation

Awake behaviour is associated with acute processing of stimuli and activity-dependent plasticity in neural circuits during learning, while sleep appears to be critically involved in consolidation of newly encoded memories[29]. Indeed, numerous behavioural studies have established that sleep not only improves performance in memory tasks, but also that it is actively involved in memory consolidation[29].

It has been hypothesised that SWS and REM sleep act sequentially to promote long-term storage of new memories[55]. According to the active system consolidation hypothesis up-states during SWS drive the repeated reactivation of newly encoded memories, which eventually leads to their integration into pre-existing networks for long-term storage[56].

Such active system consolidation has been suggested to occur on the background of global synaptic downscaling. The synaptic homeostasis hypothesis proposes that the net build-up of synaptic weights due to encoding of information during waking is compensated for during subsequent SWS. According to this hypothesis, SWS serves to globally downscale synaptic weights across the cortex, thus increasing the signal-to-noise ratio for reactivated memories and preserving the capacity of cortical networks to store new information by preventing their saturation[10]. REM sleep, in turn, has been suggested to mediate a process termed synaptic consolidation, in which those memories that were previously reactivated during SWS are locally consolidated and thereby strengthened[31] (Figure 4). However, there is currently insufficient evidence directly confirming the proposed mechanisms, making the role of sleep in memory consolidation an active area of research.

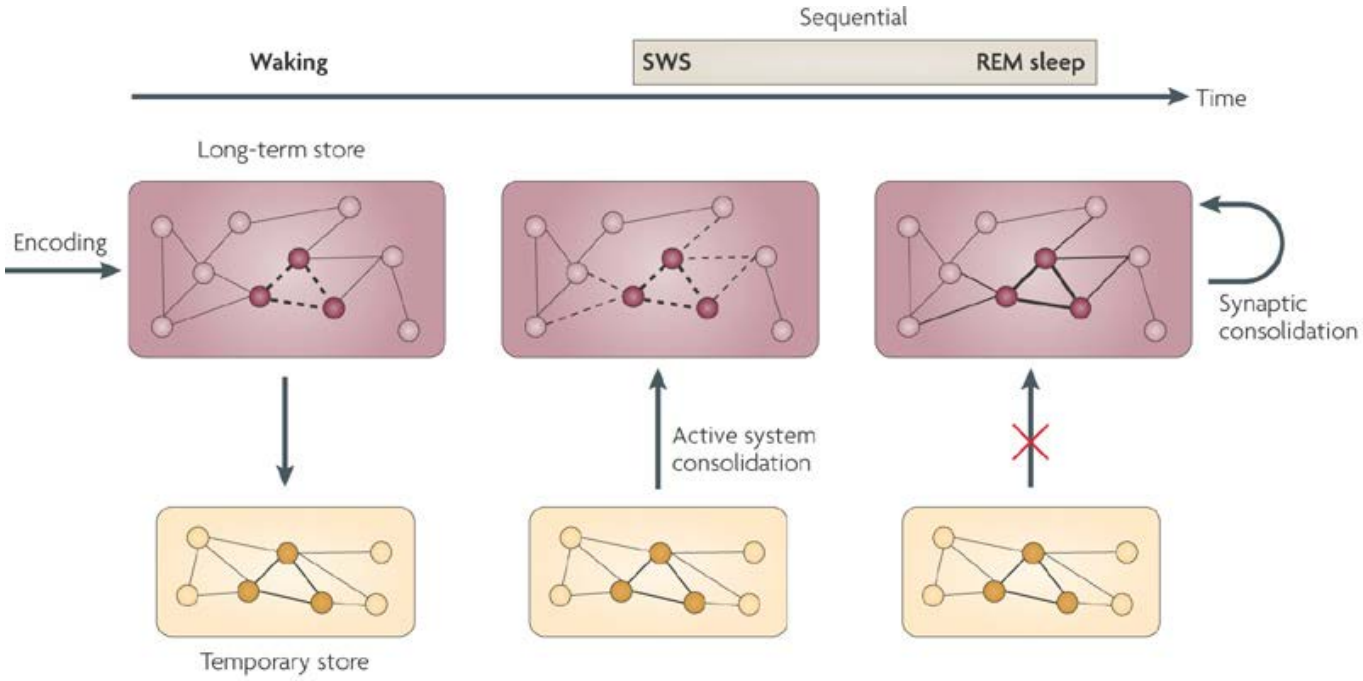

Figure 3 | **Sequential contributions of SWS and REM sleep to memory consolidation in a two-stage memory system**.

During waking, memory traces are encoded in both the fast-learning, temporary store and the slow-learning, long-term store (in the case of declarative memory these are represented by the hippocampus and neocortex, respectively). During subsequent slow wave sleep (SWS), active system consolidation involves the repeated re-activation of the memories newly encoded in the temporary store, which drives concurrent re-activation of respective representations in the long-term store together with similar associated representations (dotted lines). This process promotes the re-organization and integration of the new memories in the network of pre-existing long-term memories. System consolidation during SWS acts on the background of a global synaptic downscaling process (not illustrated) that prevents saturation of synapses during re-activation (or during encoding in the subsequent wake-phase). During ensuing rapid eye movement (REM) sleep, brain systems act in a 'disentangled' mode that is also associated with a disconnection between long-term and temporary stores. This allows for locally encapsulated processes of synaptic consolidation, which strengthen the memory representations that underwent system consolidation (that is, re-organization) during prior SWS (thicker lines). In general, memory benefits optimally from the sequence of SWS and REM sleep. However, declarative memory, because of its integrative nature (it binds features from different memories in different memory systems), benefits more from SWS-associated system consolidation, whereas procedural memories, because of their specificity and discrete nature, might benefit more from REM sleep-associated synaptic consolidation in localized brain circuits.

1.5 - Sleep-specific field potential oscillations

Sleep stages are characterized by specific electrical field potential rhythms that temporally coordinate information transfer between brain regions and might support Hebbian and spike-time-dependent plasticity.

Field potentials associated with SWS. Neocortical slow oscillations, thalamo-cortical spindles and hippocampal ripples have been associated with memory consolidation during SWS. The neocortical slow oscillations (of <1 Hz), by globally inducing up- and down-states of neuronal activity, are thought to provide a supra-ordinate temporal frame for the dialogue between the neocortex and subcortical structures that is necessary for redistributing memories for long-term storage[57]. The amplitude and slope of the slow oscillations are increased when SWS is preceded by specific learning experiences[58]. Thalamo-cortical spindles seem to prime cortical networks for the long-term storage of memory representations. Repeated spindle-associated spike discharges can trigger LTP[59] and synchronous spindle activity occurs preferentially at synapses that were potentiated during encoding[60]. Selective disruption of ripples by electrical stimulation during the post-learning rest periods in rats impaired formation of long-lasting spatial memories[61], suggesting that ripples have a causal role in sleep-associated memory consolidation. Interestingly, there is a fine-tuned temporal relationship between the occurrence of slow oscillations, spindles and sharp wave-ripples during SWS that coordinates the bidirectional information flow between the neocortex and the hippocampus. A consistent finding in humans, cats, rats and mice is that spindle activity and ripples increase during the up-state and become suppressed during the down-state of a slow oscillation[62]. Sharp wave ripple complexes are also temporally coupled to sleep spindles[57], with individual ripple events becoming nested in individual spindle

troughs[62]. It has been suggested that such ripple-spindle events provide a mechanism for a fined-tuned hippocampal-neocortical information transfer, whereby ripples and associated hippocampal memory re-activations feed exactly into the excitatory phases of the spindle cycle[57]. In this scenario, the feed-forward control of slow oscillations over ripples and spindles enables transferred information to reach the neocortex during widespread depolarization (during the up-state), that is, a state that favours the induction of persistent synaptic changes, resulting in the storage of the information in the cortex. The extent to which the grouping effect of the slow oscillation on hippocampal activity is associated with transfer of memory-specific information in the opposite direction (from cortex to hippocampus), is currently unclear.

1.6 - Barrel cortex

Mice are an attractive model to study mammal sensory processing and somatosensoring integration[63] since, amongst others reasons, they share with us a reasonable number of basic brain features[64]. Thus, in this review, we are going to predominantly focus on the rodents' somatosensory circuits. Particularly, the vibrissal somatosensory system (vS1) is extensively chosen for being relatively simple and easy to manipulate.

Rats and mice are nocturnal animals and, subsequently, cannot rely much in visual information. Therefore, they use whisker movements to collect relevant information from the surrounding environment by sensing slight perturbations when in contact with objects [2, 3]. Whiskers' bases contain specialized peripheral sensory mechanoreceptors[64], which convert movements in action potentials patterns by mechanogated ion channels activation (Figure 4)[2, 63]. Those spike trains are then transmitted by the trigeminal

nerve, allowing the animal to perceive sensory signals as location, intensity and duration of tactile stimuli[64], and subsequently to discriminate objects[2]. Then, the sensory signals make excitatory glutamatergic synapses within the trigeminal nuclei, in the brain stem[63]. From where they are retransmitted to the appropriate thalamic nuclei, making their second synapse, also glutamatergic[65]. Finally, they are spread to their homologous whisker-specific cortical columns (barrels) in the Layer 4 of the primary somatosensory cortex[2, 65].

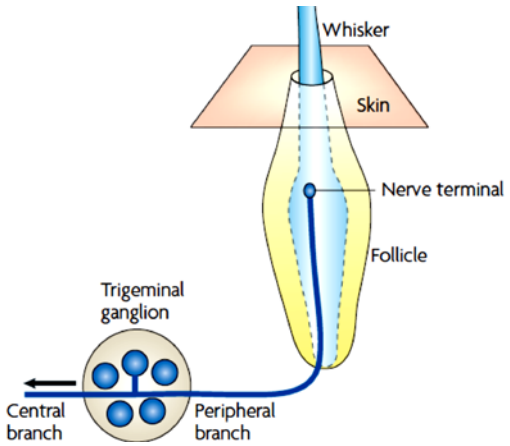

Figure 4 | **Whisker mechanoreceptor terminal.**

The mechanoreceptors, present at the base of each whisker's follicle, encode information about its deflection and respective direction, velocity and duration. Those sensory fibers carry the information to its cell bodies, located at the trigeminal ganglion, from where it is transmitted to the trigeminal nuclei, in the brain stem. (Figure reproduced from [2].)

The trigeminal nuclei receives vibrissal information through three parallel pathways: lemniscal, extralemniscal and paralemniscal[66] (Figure 5). Although the functional differences between this three pathways is still not very clear, current knowledge suggests that they are, respectively, responsible for touch (contact and movement) information; contact timing; movement and object location[2, 67-69].

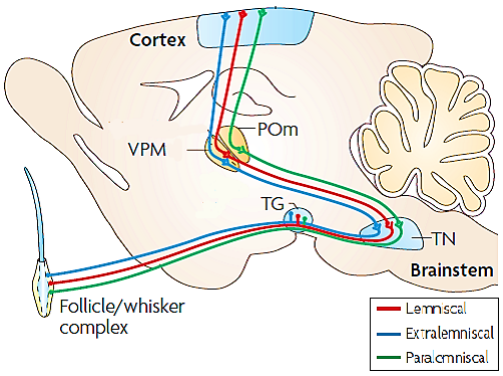

Figure 5 | **Schematic representation of lemniscal, extralemniscal and paralemniscal pathways**.

The sensitive information encoded by the peripheral sensory afferents are transmitted to the trigeminal nuclei (TN), in the brain stem, passing through the trigeminal ganglia (TG), and, from here transmitted to the thalamic ventral medial posterior nucleus (VMP) or to the posterior thalamic nucleus (POm), where it is processed before arriving into the somatosensory cortex. This transmission can be carried by three different neuronal pathways: lemniscal, extralemniscal and paralemniscal. (Figure adapted from [2].)

The lemniscal and extralemniscal signals relays to the ventral medial posterior nucleus of the thalamus (VMP) and are then retransmitted to the tactile region of the primary (S1) and secondary (S2) somatosensory cortex. While the paralemniscal pathway is connected with the posterior thalamic nucleus (POm), that in turn retransmits information to the S1, S2 and motor cortex (M1)[70]The thalamus is considered a relay station of somatosensory information[71], subsequently, thalamocortical projections are crucial in outside world sensations perception, by conducting those information to the somatosensory cortex[72].

The vibrissal primary somatosensory cortex has been shown to be essential for simple stimuli detection and discrimination by either passive or active whiskers[73]. The functional somatotopic columns, in which it can be divided, represent different points in the receptive field with the same layout as the whisker on the snout (FIG. 10-b)[3, 74], what facilitates the decoding of the stimulated whisker location[75]. They have a laminar structure and, although more than one layer can simultaneously process the same whisker deflection, responses are layer and cell-specific[76]. Each of the cortical six layers contains distinct sets of excitatory neurons involved in specific whisker-circuits[63, 64, 77], allowing delineation of functional organization and plasticity[63]. In fact, a map relating single whiskers and the respective processing cortical columns has already been formulated (FIG. 10-a)[2]. Hereupon, the barrel cortex is a great model to relate sensory stimuli, specific neuronal responses and subsequent behaviour[78]. Perceived whiskers sensory information is used to refine motor control (Figure 6). Therefore, there are essential interactions between the sensory pathways, particularly from the vS1, and the motor cortex. Which, in turn, integrates the sensory inputs with motor outputs, leading to an enhanced coordination of the whisker movements, in order to improve the tactile sensory perception[79].

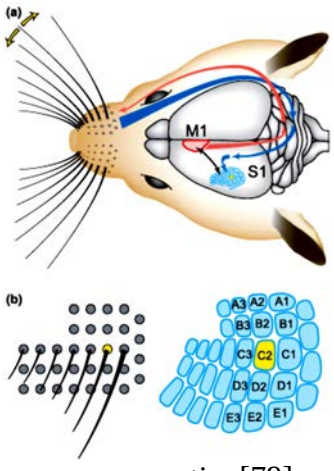

Figure 6 | **Barrel cortex columns-whiskers associative map**.

(a) Sensory and motor pathways are represent by blue and red lines, respectively. During active sensory perception, the neuronal signals originated by whiskers' deflection are transmitted by trigeminal nerves until the trigeminal nuclei, in the brain stem. From here, they are transmitted to the thalamic somatosensory nuclei and then to the appropriate barrel in the primary somatosensory cortex. In response, the motor cortex projects information to the brain stem nuclei, which controls whisker motor neurons. (b) The layout by which whiskers are organized in the snot is similar with the barrels distribution in the vS1 cortex. (Figure reproduced from [3].)

In fact, during perceptual decision-making, unilateral inhibition of anterior lateral motor cortex biased responses to the ipsilateral side. It was demonstrated that, in one side, the barrel cortex showed stimulus-specific activity during sensation, whereas motor cortex showed choice-specific preparatory activity and movement-related activity, associated with motor planning and movement. This suggests serial information flow from sensory to motor areas during perceptual decision making[80].

Also, studies in Layers 2/3 of the motor cortex, with lick/no lick discrimination tasks, have shown that sensory discrimination learning leads to strengthening of connections between sensory and motor neurons populations. Local potentiation of excitatory or inhibitory connections may occur between neurons of similar or different response types, respectively (Figure 7)[1]. During initial tasks learning, it also occur changes in the synaptic inputs through enhanced spine growth[81]. Thus, Layer 2/3 neurons in the motor cortex might participate in sensorimotor integration and learning [1, 82].

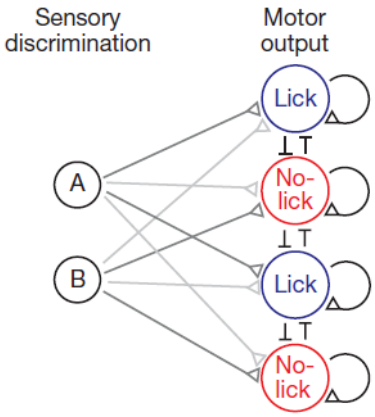

Figure 7 | **Learning-related coupling of neuronal ensembles.**

Sensory information is selectively detected by neurons A or B. Learning leads to strengthening (black lines) of local excitatory (triangles) or inhibitory (lines) circuits. (Figure reproduced from [1].)

CHAPTER II - Objectives

The overall aim of the project is to characterise underlying plasticity mechanisms associated with up- /down-states in the barrel cortex in vitro. In particular, experiments in slices of barrel cortex will address whether up-/down-states differentially affect synaptic plasticity in vitro. This is to establish directly whether SWS is necessary to consolidate changes in synaptic strength. The primary somatosensory barrel cortex (S1 barrel cortex) was chosen as a model system for this project. Each whisker is represented in the barrel cortex by its respective cortical column. Individual columns are somatotopically arranged according to the whiskers on the snout and can be anatomically distinguished in layer 4 of the cortex as barrel-like structures [63, 83]. These barrel structures allow for experiments to be carried out in the context of a clear anatomical map with well-defined circuitry. Furthermore, the barrel cortex has the advantage that it is accessible in vivo for investigating state-dependent plasticity using relatively simple sensory stimulation paradigms, as well as in vitro for examining up-/down-states in slice preparations.

The layer 4 barrels are relatively stable after being formed early in development[63]. Indeed, plasticity at the thalamocortical synapse, which forms the layer 4 barrel structures, has only been found in the first postnatal week[84]. However, intracortical synaptic pathways remain plastic during adolescence and in some cases into adulthood, which is defined as an age of 6 months in rodents. Long-term potentiation (LTP) and long-term depression (LTD) have been described in layer 2/3 of the barrel cortex in a variety of studies[85]. However, it has not yet been established how plasticity in barrel cortex pathways is modulated by slow oscillations. Experiments have already been carried out in Ed Mann's laboratory investigating how up-/down-states modulate

synaptic plasticity in vitro in slices of entorhinal cortex. Here, up-states were paired with electrically-evoked synaptic events. Interestingly, stimulation protocols in which up-states were paired with subthreshold excitatory postsynaptic potentials (EPSPs) induced a long-lasting depression, whereas pairing of suprathreshold spikes with up-states resulted in a relative potentiation of synaptic connections (unpublished observations). Whether this is a general principle across the cortex has not yet been established. One of the aims of the proposed project is to use such stimulation protocols in layer 2/3 of the barrel cortex to investigate whether up-states modulate plasticity in a similar way. In addition, experiments will address the implications of such mechanisms for cortical processing in vivo. Synaptic plasticity in the barrel cortex is thought to underlie experience-dependent plasticity of sensory maps in vivo, which can be induced by whisker deprivation. When a whisker is trimmed, deflection of the whisker after regrowth shows depression of a response in the corresponding barrel. In addition, the cortical area responding to deflection of surrounding spared whiskers is increased[85]. Individual receptive fields in the barrel cortex can thus be depressed or potentiated in an experience-dependent manner using such sensory deprivation paradigms. It has, however, not yet been established whether sleep contributes to this type of plasticity. A critical role of sleep in experience-dependent cortical plasticity has indeed been found in the visual system. During a critical period, monocular deprivation in cats by occluding one eye results in a reduction of cortical responses to that eye. It has been found not only that sleep enhances such ocular dominance plasticity, but also that sleep deprivation during SWS following occlusion of the eye blocked this enhancement[86]. It is conceivable that sleep similarly affects experience-dependent plasticity of receptive fields in other sensory systems, such as the barrel cortex.

CHAPTER III- Materials and Methods

3.1 – Materials and Experimental Procedures

AM251 (CB1 Antagonist)

AM251 was dissolved in DMSO and subsequently diluted to working concentrations in ASCF containing 0.5% BSA as a carrier. In all cases, final DMSO concentrations were below 0.1%.

Protocol 1 - Preparation of Artificial Cerebrospinal Fluid (acsf)

Preparation of Stock Solutions

1. In a 1L beaker with ~70% total volume (700mL/1L) ddH2O and in continuous stirring, add the compounds indicated on the tables below for each of the stock solutions. To maximize measurement precision wash the weight boat with ddH2O above the solution beaker.

Stock I

Compounds	mM	mWt	g/l
CaCl2.6H2O	2	219.08	4.38
Glucose	10	180.0	18.00
$MgSO_4.7H_2O$	2	246.5	4.93
KCl	3	74.5	2.24
NaCl	126	58.5	73.70
$H_2NaPO_4.2H_2O$	1.25	156.0	1.95

Stock II – Sodium Bicarbonate buffer

Reagent	mM	mWt	g/l
NaHCO3	24	84.0	20.16

2. When all the compounds are completely dissolved, fish the magnetic bar and transfer the solution for 1L Erlenmeyer beaker and complete it until the 1L mark with ddH2O.
3. To maximize measurement precision wash the magnetic bar with ddH2O above the beaker; additionally wash the beaker with ddH2O and use this to complete the solution up to 1L.
4. Spin down the solution, transfer it to a 1L flask and store it at 4 ºC.
5. The stock solutions should be made fresh every week.

Preparation of aCSF

1. To prepare 1L aCSF, measure 100 ml of Stock I and 110 ml of Stock II with a graduated cylinder and to an Erlenmeyer beaker and fill it up to 1 litre with ddH2O.
2. Spin down the solution and transfer it to a 1L flask.
3. Cool down the solution by placing it on ice and oxygenate it with carbogen gas for at least 30 min before starting the dissection (or 15 min if cold ddH2O is used).
4. Prepare another 1L of aCSF to incubate the slices and keep it oxygenating at room temperature (RT).
5. Clean all the gas supplying tubes with deionized water before introducing them in the aCSF flasks.
6. 5. Cover both flasks with parafilm to avoid contamination with dust.

Protocol 2 - Anesthesia & Removal And Dissection Of The Brain

1. Before starting, prepare the following materials and place them in a double sheet of cleaning paper:
   - **Decapitation tools**: decapitation scissors, dissection scissors, forceps, narrow spatula, large spatula, scalpel holder nº22 and respective blade, beaker with a large glove, beaker for dissection, beaker to put the brain after dissection, cutter, gloves.
   - **Dissection tools:** super glue, scalpel holder nº22 and respective blade, glass pipette with rubber bulb, tape, petri dish on top of black plastic bag, cutting template, single edge blade, incubation chamber, chamber cover, brush to use with glue, clean brush.
   - Additionally, clean a double-edge razor blade with acetone and deionized water and put it in the vibratome (use the slicer adjuster); put the slicing chamber on the -20 ºC freezer and the slicing block on ice.
   -
2. Right before starting, place the slicing chamber in the vibratome, fill it with it cold aCSF and connect a carbogen tube to oxygenate it. Turn on the vibratome. Additionally, dry the dissection block and place a piece of tape on it (use normal scissors).
3. Place cleaning paper in the bottom of the anesthetic chamber and check if there is enough anesthetic (isoflurane). Open the oxygen gas bottle connected to the anesthetic chamber.
4. Open the 15 days pups transport box with the cutter.
5. Fill the beakers and the Preti dish with cold aCSF. Fill the incubation chamber with RT aCSF and connect an oxygenation tube to it.

6. Turn on the oxygen around 2 L/m and isoflurane at 4-5% until anaesthesia is induced. Control level of anaesthesia by observing the respiration rate and check with pedal reflex. If there is no reflex, turn off the oxygen and isoflurane flow, turn on the gas absorber and proceed to the decapitation.
7. 4. Hold the animal at the level of the front paws and cut the head with a quick and precise cut using the decapitation scissors. This should be done above the beaker for dissection filled with cold aCSF. Put the body in the beaker with the glove.
8. Cut the skin longitudinally with the scalpel, retract the skin flaps to the sides and then to the base of the head. This will allow you to visualize the skull and help you to hold the head during the dissection.
9. The use of a scalpel is optional in very young animals as the skin pills very easily.
10. By using fine scissors, (1) make a posteroanterior cut in the midline of the skull (1) and two subsequent medio-lateral cuts (2) above the eyes. In this way, two skull flaps are formed, which may be reflected laterally with forceps (3) to expose the brain. These bony flaps should be pulled away, taking care not to damage the brain surface.
11. Gently retract the brain away from the skull base with a small spatula (Figure 9).

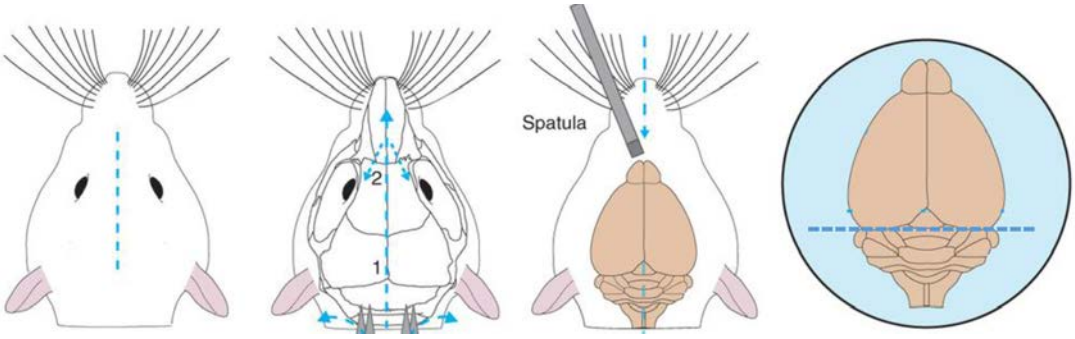

Figure 8 | **Step procedures of brain removal**

12. **For cortical slices:** place the brain in a Petri dish; transfer it to the cutting template with a large spatula and very carefully dry it with paper tissue to reduce movement;

with a clean brush adjust the brain to be in the position shown in Figure 10; using the template, cut the brain while holding it with the brush.

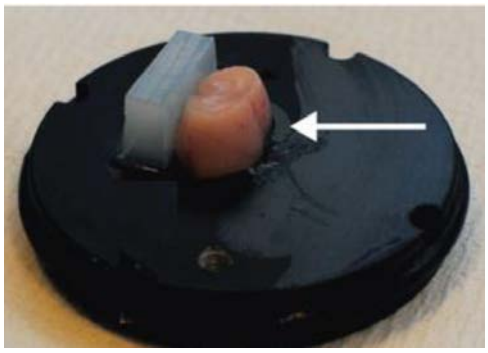

Figure 9 | **Brain positioning to further obtain cortical slices**

13. Take the brain by placing the scalpel blade under its base (use a brush to help, if necessary) and gently dry the base of the brain with tissue. Put a piece of tape in the slicing block and super glue on top of it. Then place the base of the brain in top of the glue (Figure 6).
14. Place the slicing block in the slicing chamber filled with cold aCSF in continuous oxygenation. For thalamocortical slices, the right hemisphere should be facing the cutting blade. For coronal slices, the dorsal surface of the brain should be facing the cutting blade (white arrow on Figure 6).
15. Put the blade in the first cutting position and move the slicing chamber up until the tip of the blade is inside aCSF. Program the slicer to 350 μm and 0.28 mm/s. Then set up the beginning and end of the slice. Press ‘START’ and then, right after the blade starts vibrating, press ‘PAUSE’, move the slicing chamber up until the level where you want to start slicing and press ‘START’ again.
16. One or two slices before the slices you are interested in, change the velocity to 0.16 mm/s.

17. Take the slices with a glass transfer pipette (with a rubber bulb) to a petri dish above the black plastic bag (the contrast will help you to see the slices). With the scalpel remove the major portions of the slice you are not interested in - this will reduce the movement of the slice during the electrophysiology experiments.
18. Transfer the slices for the storage chamber, previously filled with warm aCSF and in continuous oxygenation. It is important that there are no air bubbles below the net on the chamber, otherwise the slices will not receive proper oxygenation. Remove any existent air bubbles with a clean brush.
19. In the end, cover the slices storage chamber with a petri dish (if slices have no fluorescence) or with a chamber cover (if slices have fluorescence) and leave the slices to recover for, at least, ~1 hour.
20. **Cleaning and tiding up:** close the oxygen gas bottle connected to the anesthetic chamber; put all the animal waste on the glove, tie it and put it in the appropriate shelf in the -20 ºC freezer; remove all the blades (scalpel, single and double edge) and put them in the appropriate container; very carefully clean the area of the slicer blade holder with deionize water and dry it; wash all the other tools with deionized water and leave them to dry. Clean the anesthetic chamber and all the dissection area with Virkon. Retract the slicer (‘DOWN’ and ‘BACK’), turn it off and cover it. Clean all the no longer necessary gas supplying tubes with deionized water.

CHAPTER IV – Results

4.1 - AM251 induces an increase in Up-states amplitude in mice barrel cortex slices

Up-states arise from coordinated signalling between glutamatergic and GABAergic synapses and are modulated by systems that affect the balance between inhibition and excitation. The possibility to modulate the performance of this synchronous events by interfering with AMPA-, GABA- and NMDA- currents could lead to several alterations of initiation and maintenance [87]. Findings suggest that extra-cortical neuromodulators are not requested to arise up-states, i.e. monoaminergic inputs. However, there is still a lack of knowledge whether within and between pyramidal neurons (PNs) and interneurons in the cortical microcircuitry may act synergistically with intrinsic neuromodulatory systems to regulate network activity.

Endocannabinoids (ECs) are a class of atypical neurotransmitters synthesized and released from the post-synaptic membrane of cortical PNs during periods of enhanced cellular activity such as during up-states[88]. Therefore ECs could be considered as an intrinsic neuromodulatory system. ECs bind to the presynaptic cannabinoid 1 (CB1) receptor [89] that mediates most of the physiological effects of cannabinoids in the CNS[90, 91]. In the cortex, activation of CB1 decreases release of both GABA and glutamate[11] suggesting this local neuromodulatory system may tune network activity by regulating both excitatory and inhibitory neurotransmission within local cortical circuits.

Findings in whether CB1 antagonists have a direct effect in neocortical manifestations in vitro suggests that the peak of amplitude was significantly reduced, especially when administrating AM251 [92].

Network up-states were recorded in single cells using whole-cell current-clamp experiments in slices of barrel cortex from adolescent mice (P15-21). Recordings were targeted to layer 2/3 pyramidal neurons in acute slices of barrel cortex. Neurons were generally in a down-state under the conditions used here. However, up-state transitions could be evoked by synaptic stimulation in the layer 4 barrel. Up-states were most reliably evoked using aCSF containing 1.6 mM Ca^{2+} and 1 mM Mg^{2+}. Under these conditions, up-states occurred with an average duration of 2.05 ± 0.43 s (n = 7 cells), not including failures of electrical stimulation to evoke an up-state.

After conducting a similar experiment in mouse barrel cortex slices, it was also observed an impactful decrease in up-state amplitude during the administration of AM251 (Figure 11). As for up-state duration, it was not observed any significant changes. Recordings were made using a single electrode protocol while patching a pyramidal cell in Layer 2/3 and stimulating the layer 4 region in order to evoke up-states.

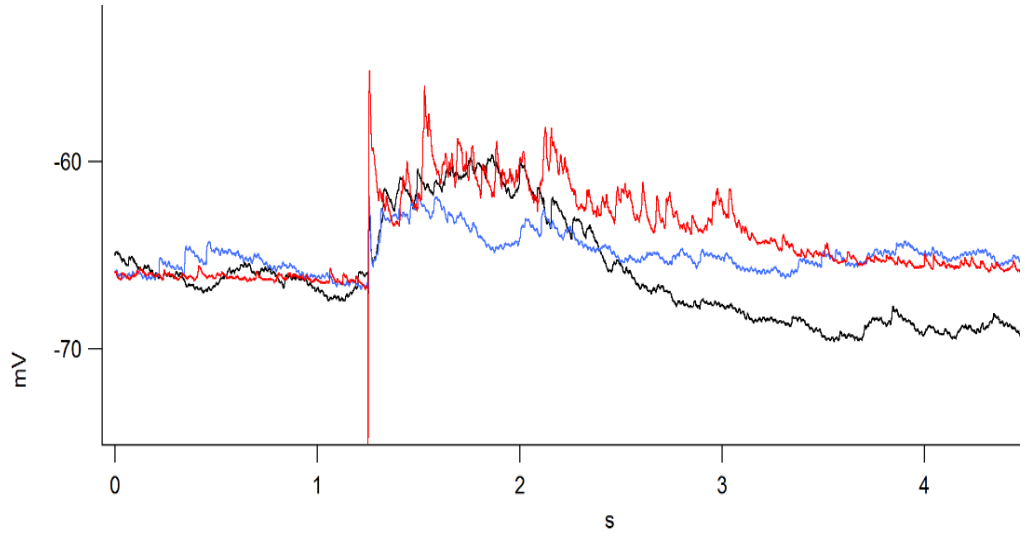

Figure 10 | **AM251 reduces up-state amplitude via a CB1-dependent mechanism**.

Average traces showing baseline up-states before (black), during (blue) and after/wash (red) drug administration. Each experimental phase duration corresponded for 15 minutes.

4.2 – Paired-recording protocols induces Long term depression in mice barrel cortex slices

Since the neocortex circuits might be modulated via CB1-dependent mechanisms, and the above results demonstrated that blocking CB1 receptors decreases up-state amplitude in mouse barrel cortex, it might be suggested an experimental procedure where plasticity is induced in the network circuitry and observe whether occurs a potentiation or depression in synaptic plasticity. This can be achieved by defining a stable baseline, analysing EPSPs and, most importantly determine the average slope before and after pairing. By firstly evoking up-states through layer 4 stimulation in the barrel cortex and patching a pyramidal cell located in layer 2/3, we proceeded to stablish a baseline for approximately 10 minutes and obtain successful EPSPs. Furthermore, it was executed a USD Plasticity protocol using a pairing control. This is basically a sequence of peak burst

occurred in the beginning of each resultant up-state (~50 ms), during the oscillatory train. For the barrel cortex region it is suggested a 20 pairing sets due to its resilience in performing synaptic plasticity. The stimulation protocols in which up-states were paired with subthreshold excitatory postsynaptic potentials (EPSPs) induced a long-lasting depression (Figure 12) (n=9 cells). To determine whether the pairing sets might have a significant impact in the network circuitry itself and even without evoking up-states, it was established a control experiment with barrel cortex slices with the potential of evoking up-states. Results showed that the pairing control did not induced Long Term Depression without evoking up-states (Figure 13) (n=7 cells).

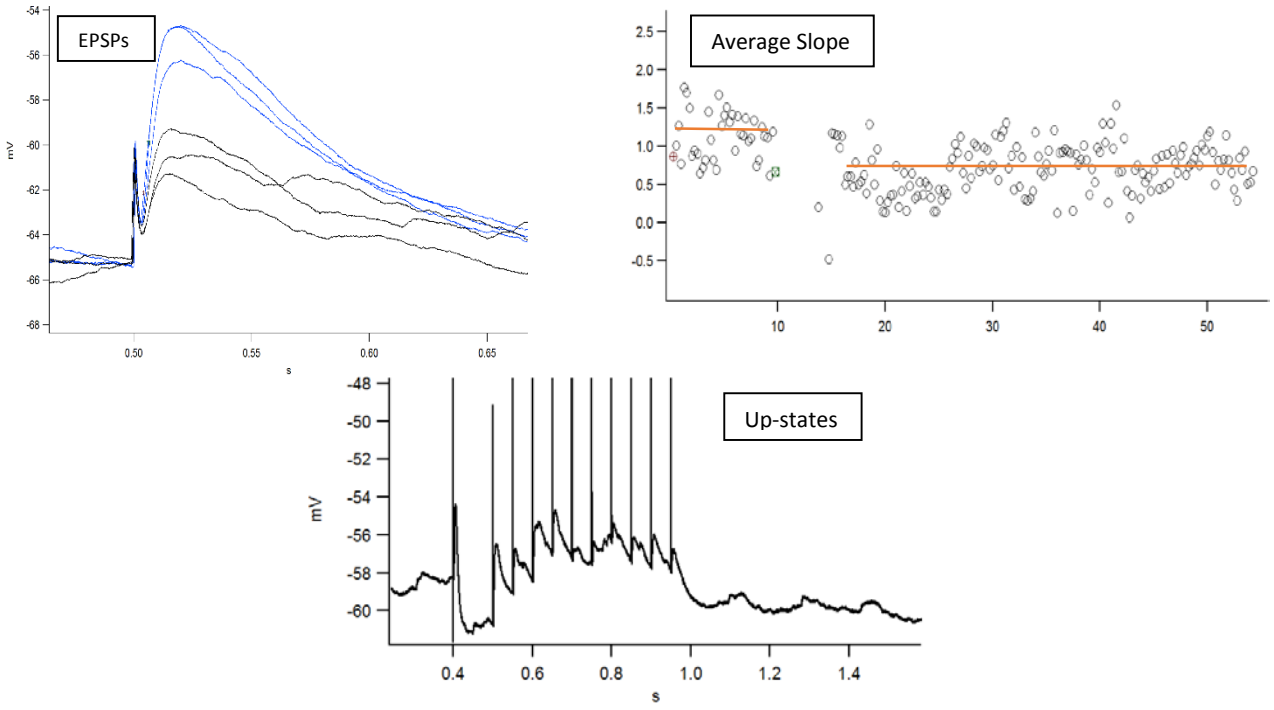

Figure 121| **Paired-recording protocols induces LTD**.

In the EPSPs graph, it is observed significant slope changes before (blue) and after (black) pairing. The average slope between the timescale 0-10 units and 20-55 units changed from 1,3 to 0.6, meaning it occurred LTD after pairing. A sample trace of up-states during the pairing phase show that the protocol was ordered to trigger up-states.

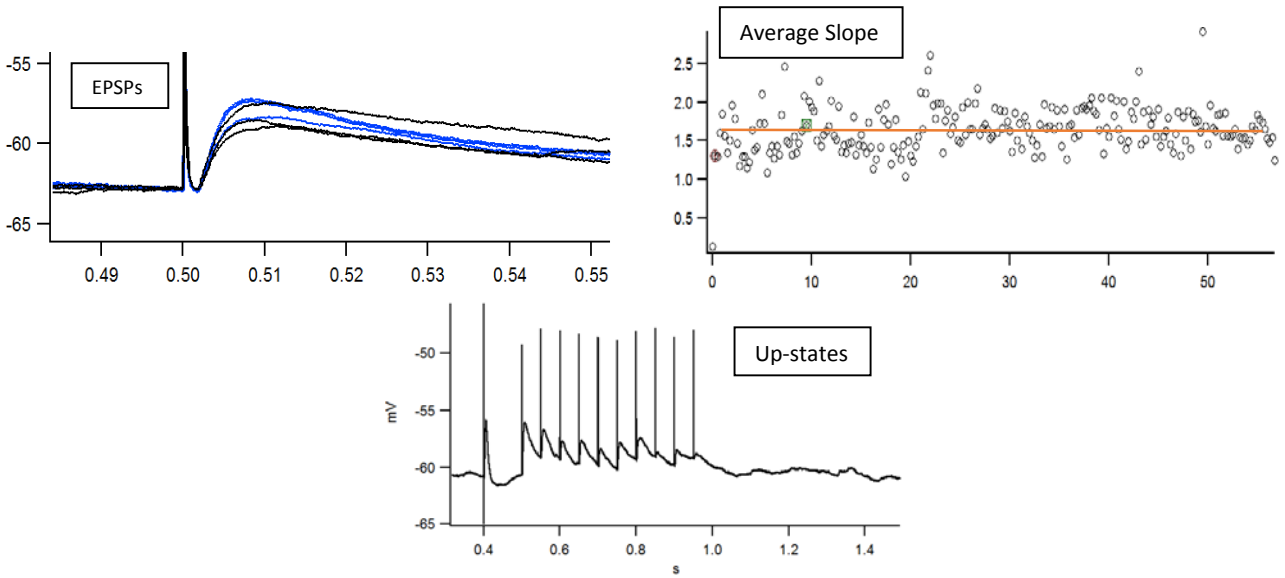

Figure 11 | ***Paired-recording protocol control does not induce LTD***.

In the EPSPs graph, there is no significant changes in slope before (blue) and after (black) pairing. The average slope maintained approximately 1.6, meaning it did not occurred LTD after pairing. A sample trace of up-states during the pairing phase show that the protocol was not ordered to trigger up-states.

CHAPTER V - Discussion

While these experiments seem to be rather simple to apply, the duration of each datasets normally ensures 1 day each. Being important to have every variable unchanged during the setup of the experiment, i.e. mouse age, successful anaesthesia & removal and dissection of the brain, room temperature, slice bath temperature, aCSF ionic concentration, perfect position of the electrodes in the correct barrel cortex regions, equipment performance, quality of the electrodes, and, most importantly, cell viability if successfully patched and evoking up-states through stimulation of layer 4, brings a tremendous effort on obtaining datasets with desired quality. By requiring tremendous practice, plasticity datasets are normally rather few.

From the findings above, it was given a reinforcement of understanding the underlying mechanisms of circuitry during up-states. Whole-cell current-clamp recordings from layer 2/3 pyramidal cells revealed that up-states can be evoked by electrical stimulation in the corresponding layer 4 barrel using aCSF containing 1.6 mM Ca^{2+} and 1 mM Mg^{2+}. While being suggested that up-states are modulated via CB1-dependent mechanisms [92], it was observed throughout the first experiment – 4.1. AM251 induces an increase in Up-states amplitude in mice barrel cortex slices – a significant decrease in amplitude when administrating a CB1 antagonist AM251. By blocking the receptors, it is suggested that the neural networks responsible for memory consolidation may be effected by reducing its firing burst.

During the experiment, AM251 did in fact decreased the amplitude of Up-states while being administrated along with aCSF. However, during the wash phase, the up-state amplitude increased, but not reaching the same value as initially established in the baseline. This is a surprising occurrence since AM251 has a high affinity to CB1 receptors

and, in theory, it would be very difficult to “wash” the drug and see improvements on upstates. It might be interesting to produce a longer experience and observe whether there is a maintenance of reduced up-state amplification throughout the administration and wash phase or a surprisingly similar recovery.

Being interestingly revelling the *in vitro* experiments, a similar protocol could be done in mice and, furthermore, can be subjected through quantification of their capacity of memorization in a Morris water maze and other behavioural tasks. These findings can lead to the knowledge of a potential drug responsible to reduce the capability of consolidating memories, i.e. post-traumatic stress disorders [92, 93].

Since synaptic plasticity events are a crucial factor for memory consolidation, in the second experiment - 4.2. Paired-recording protocols induces Long term depression in mice barrel cortex slices – it was observed the neural network force being significantly reduced after pairing up-states in barrel cortex slices. This can be demonstrated in Figure 12 EPSP graphic. While forcing the triggering of up-states and add a small peak burst in each upstate action potential along the oscillation train (Figure 12 Up-states graphic), the resulting slope after this phase was a significant lower slope (1.3 to 0.6), meaning that long term depression surprisingly occurred. To understand if this was a network impact or if the pairing setting managed somehow to reduce the neural network strength (EPSPs), we conducted a control experiment. In other terms, there was no attempt to trigger up-states during the pairing phase. For confirmation, the results showed no effects when comparing with the barrel cortex plasticity experiment.

CHAPTER VII - Conclusion

SWS and REM sleep have complementary functions to optimize memory consolidation. During SWS − characterized by slow oscillation-induced widespread synchronization of neuronal activity − active system consolidation integrates newly encoded memories with pre-existing long-term memories, thereby inducing conformational changes in the respective representations. System consolidation (which preferentially affects explicitly encoded, behaviourally relevant information) acts in concert with global synaptic downscaling, which serves mainly to preclude the saturation of synaptic networks. Ensuing REM sleep − characterized by de-synchronization of neuronal networks, which possibly reflects a disengagement of memory systems − might act to stabilize the transformed memories by enabling undisturbed synaptic consolidation. Slow oscillations have previously been observed in different cortical areas as spontaneously occurring up- and down-states both in vivo and in vitro. Spontaneous up-states occur intrinsically within the cortex [5, 6]. They can also be observed in vivo in response to sensory input[7] and can be turned on and off by synaptic stimulation in vitro [8].

In summary, sleep is a pair of special modes in the brain: SWS and REM. Both of these states are closed to outside inputs and work in tandem to prepare and clean the learning and memory system while retaining important information for later use–all so that the brain can be maximally focused on learning and adapting to its surroundings during the next period of awake activity. It is also remarkable to mention that targeting the endocannabinoid system may aid in the treatment of disorder associated with impaired extinction-like processes, such as post-traumatic stress disorder.

CHAPTER VI - Future perspectives

Future experiments would investigate the role of slow-wave sleep in experience-dependent plasticity in the barrel cortex. This would be done by optogenetically inhibiting up-states locally in the barrel cortex during a period of whisker deprivation, after which the responses to deflection of the trimmed and spared whiskers will be measured. Initial experiments will be aimed at establishing a suitable protocol for optogenetic inhibition of up-states. This is to establish a suitable stimulation protocol to determine the frequency and duration of optical stimulation that inhibits up-/downstates most efficiently. Following this, inhibition of slow oscillations by optogenetics will be assessed in vivo using multiunit recordings or patch-clamp recordings from the barrel cortex of anaesthetised mice. Furthermore, Beltramo et al. have already demonstrated the feasibility of an optogenetic approach to inhibit up-states for sensory cortical areas [94].

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