Interdisciplinary Graduate Programme in the

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Master Thesis

CHARACTERIZATION OF THE MECHANISMS UNDERLYING THE EMERGENCE OF UP AND DOWN STATES IN A PREFRONTAL CORTEX MODEL MICROCIRCUIT

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Abstract

Up and Down states are oscillations between periods of prolonged activity (Up state) and quiescence (Down state) and are recorded both *in vivo* and *in vitro* in layer V prefrontal cortex (PFC) pyramidal neurons. Biophysical mechanisms that have been proposed to underlie this phenomenon include the balance of excitation and inhibition within local PFC networks along with certain intrinsic membrane mechanisms such as the afterdepolarization. Using a biophysical compartmental network model of PFC layer V pyramidal neurons that incorporates anatomical data, we investigated the role of synaptic input, intrinsic currents and local interconnectivity in the following features of Up and Down states: (a) the emergence of Up and Down states, (b) the duration of Up states, (c) the frequency of Up states and (d) the firing frequency during the Up state.

We found that Up and Down states could emerge in our model microcircuit, provided the existence of background synaptic activity. Among the various conditions we examined, statistically significant results were obtained when:

- Increasing the firing frequency of the background synaptic input or the number of activated background synapses.

- Blocking the NMDA current, while compensating for the reduced excitability by enhancing the AMPA current (no emergence of Up and Down states).

- Increasing the iNMDA-to-iAMPA ratio.

- Activating the dADP mechanism at a physiological value (4mV).

Our results indicate that the generation of Up states in PFC is likely to involve not only a balance of excitation/inhibition provided within a microcircuit but also single-neuron dynamics shaped by intrinsic mechanisms.

Interestingly, the duration of the Up state was significantly altered in three of the conditions tested, namely, increased frequency of the background excitation, the enhancement of the NMDA current and the activation of the dADP mechanism. These findings suggest that the transition to more prolonged depolarizations is carefully controlled by the same mechanisms that have been associated with persistent firing during working memory tasks.

Contents

1. Introduction

The goal of the present study was to investigate the role of synaptic input, intrinsic currents and local interconnectivity in the emergence of Up and Down states by using a biophysical compartmental network model of prefrontal cortex layer V neurons that incorporates electrophysiological and anatomical data. Before describing our model, presenting and explaining the results, we review the theoretical background associated with this study. We start by describing the basic functional and anatomical properties of the prefrontal cortex and the pyramidal cells. An emphasis is placed on the N-methyl-D-aspartate (NMDA) receptor and working memory because they are of relevance with the mechanisms of Up and Down states, as indicated both from our results and the literature.

A) The Prefrontal Cortex

The prefrontal cortex (PFC) plays central role in cognitive control and is the most extended part of the human brain. It receives converging information from many brain systems processing internal and external information and is interconnected with motor structures required for the execution of voluntary action. PFC uses current internal states, goals, memories and environmental information to exert a top-down control to guide our actions, thus controlling our hard-wired reflexive behaviors. It is the part of our brain responsible for our goal oriented behaviors, enabling us to maintain goal-relevant information online and available for processing – an ability called working memory. More importantly, the PFC is responsible for our ability to generalize a specific circumstance to new situations and is highly plastic and flexible, allowing for newly acquired information and experiences to be used in the guidance of our future actions. Depending on which part they occur, damages in the PFC can result in a dysexecutive syndrome (inability to coordinate components of behavior with respect to goals and task-specific constraints), disinhibition and lack of behavioral control, emotional impairments, difficulty in planning and impaired working memory (Miller & Wallis, 2008).

In the monkey brain the PFC is subdivided in three major areas: the dorsolateral, the ventrolateral and the orbital PFC (Barbas 2000; Petrides 2005). The rat PFC has two main subdivisions: a) a medial region (mPFC) with characteristics of the primate dorsolateral and medial PFC. It comprises of the frontal area 2, the dorsal and ventral anterior cingulated areas, the prelimbic and infralimbic areas and the medial orbital areas, and b) a lateral and ventral region (OFC) that resembles the primate orbitofrontal cortex. It consists of the dorsal agranual insular

area, the ventral anterior insular area, the lateral orbital area and the ventral orbital area (Cerqueira *et al.*, 2008; Uylings *et al.,* 2003).

➢ Anatomical organization

As the rest of the cortex, the PFC has a laminar structure of six layers:

I. The molecular layer is the one closest to the pial surface. It is acellular and occupied by dendrites and axons.

II. The external granule cell layer is comprised mostly of granule cells.

III. The external pyramidal cell layer has various cell types, many of which are pyramidal neurons.

IV. Internal granule cell layer as layer II has mainly granule cells.

V. Internal pyramidal cell layer mainly contains pyramidal neurons, usually larger than the ones found in layer III.

VI. Multiform layer has a variety of neurons and carries axons to and from the cortex.

The apical dendrites of neurons located in layers V-VI terminate in layers I-III, whereas the basal dendrites of neurons from layers III-IV terminate in layers V-VI. However a cortical area can be agranular or disgranular if layer IV is absent or underdeveloped respectively. The rat's rostral PFC is such a case (Amaral, 2000; Ongür & Price, 2000; Zikopoulos & Barbas, 2007).

Given the prefrontal's cortex role in decision making and guidance of actions, it is not surprising that it receives input from many brain areas. However it does not receive direct input from the sensory periphery, instead the information required is obtained from other cortical and subcortical structures. Among these are areas of the visual, somatosensory, olfactory, gustatory and premotor cortex, the hippocampus, the cerebellum, the thalamus, the supplementary motor area, the presupplementary motor area, the superior colliculus, the cingulate cortex, the amygdala, the basal ganglia and the perirhinal cortex (Carmichael & Price, 1995; Barbas, 2000). The different layers of the PFC receive inputs from specific areas. Projections from the thalamus mainly terminate on the basal dendrites, the somata, layers III and IV and to a lesser extent II and IV (Tobias, 1975; Zikopoulos & Barbas, 2007; Cerqueira *et al.*, 2008). The hippocampus sends its projections to layers II-IV in the ventral portion of the PFC, but to layers V-VI in the dorsal PFC (Jay & Witter, 1991; Thierry *et al.*, 2000). The amygdala has been found to project to layers II and V (Orozco-

Cabal *et al.*, 2006), I, II, V and VI (Bacon *et al.*, 1996) and II-VI (Gabbott, 2006). The densest terminals of the primary motor cortex are in layers I, III and IV. The somatosensory cortex terminates in all layers and the visual cortex in layers I-IV (Van Eden *et al.*, 1992). Naturally there are heavy interconnections between the lateral, orbitofrontal and medial prefrontal cortices, ensuring both feedforward and feedback communication in the network. The mPFC and oPFC projections originate from their deep layers and terminate in the upper layers or lateral (eulaminate) PFC, whereas the lateral PFC issue its projections from its upper layers and they terminate in the deep layers of the mPFC and oPFC (Barbas, 2000).

- **Morphological and electrophysiological properties of PFC's deep layers pyramidal neurons.**

Neurons in different brain areas are intermingled in a wide variety of neural processes participating in different cognitive phenomena. The diversity of morphology and electrophysiological profiles, as well as the distinct expression of ion channels along the axo-somato-dendritic axis and between neurons have established that the integration properties of individual neurons is a complicated issue (London & Häusser, 2005). The passive properties of dendrites (Rall, 1959; Cash &Yuste, 1999) as well as active ion channels that mediate non-linear dendritic events, such as backpropagating spikes (Stuart & Sakmann, 1994) enrich the output of the neuron and greatly alter the processing that neurons perform: Active inward and outward dendritic currents act so as to amplify or linearize synaptic inputs or modulate the temporal integration window (Segev & London, 2000), whereas dendritic spikes may trigger a burst of APs at the soma (Wong & Prince, 1978; Doiron *et al.*, 2003; Milojkovic *et al.*, 2004; Polsky *et al.*, 2009), act as coincidence detectors of signals at different dendritic compartments (e.g. back-propagating action potentials from the soma and subthreshold excitation of distal dendrites (Larkum *et al.,* 1999) and are also involved in long-term memory processes (Magee & Johnston, 1997).

Several studies suggest that pyramidal neurons are subdivided in different functional subdomains and integration of inputs in each dendritic subunit follows a sigmoidal transfer function and their output linearly summarizes in the soma (Poirazi *et al.*, 2003a; Poirazi *et al.*, 2003b; Polsky *et al*., 2004; Losonczy & Magee, 2006). Especially in the prefrontal cortex, based on their morphology and evoked responses to intracellular depolarizing pulses, four groups of pyramidal neurons in layers V-VI have been identified: regular spiking (RS), intrinsic bursting (IB), repetitive oscillatory bursting (ROB) and intermediate (IM) neurons (Yang *et al.*, 1996). Most importantly, regenerative events in single layer V prefrontal pyramidal neurons have been reported, due to the presence of a somatic mechanism that underlies a delayed afterdepolarization

(dADP) (Sidiropoulou *et al*., 2009). The current that underlies the dADP is a calcium-activated non selective cation current (CAN) after action potential bursting and activation of metabotropic receptors (Fowler *et al.*, 2007).

- **NMDA Receptors (NMDARs)**

In layer V pyramidal neurons from rat neocortex, the response to glutamate (Glu) is mediated by AMPA and NMDA receptors. The results of blocking NMDA-R with the application of its antagonist D-APV suggest that the effect of Glu is preferentially mediated by NMDARs near the soma and by AMPARs in more peripheral dendritic sites. The sensitivity to AMPARs remains constant along the dendrite. On the contrary the relative sensitivity to NMDARs decays to 51±4% at a distance of 250μm from the soma and drops to 21±7% at a distance of 700μm from the soma (Dodt *et al.*, 1998). Besides a variability in its dendritic distribution, NMDAR also has the following differences from AMPAR: a) a longer decay time constant – greater by one or two magnitudes order, b) a higher affinity for Glu and therefore faster saturation properties and c) a voltage dependency of its conductance in the presence of extracellular magnesium –at hyperpolarized potentials the receptor's channel is almost closed (Compte, 2006).

In the adult brain, functional NMDARs are formed from a combination of the subunits NR1 and NR2 (NR2A, NR2B). Many of NMDAR's functional properties, such as the induction of LTP or LTD, are determined by the NR2A, NR2B subunits (Zhao *et al.*, 2005). In addition, the NMDAR-mediated currents are not identical across the cortex due to the variability in the expression of the receptor's subunits NR2A and NR2B. PFC's layer V pyramidal cells of adult rats have a higher expression of NR2B compared to visual cortex or to young rats leading to NMDAR-mediated currents with a nearly double time constant (Wang *et al.*, 2008). It has been shown that following brief application of Glu, NMDARs containing the NR2B subunit deactivate slower than NR2A containing receptors. Moreover their EPSCs (excitatory postsynaptic currents) also show slower decay. As a consequence the NR2B subunit allows greater temporal integration of non-synchronous synaptic inputs (Erreger *et al.*, 2005). These data suggest that the slow kinetics of NMDA receptors are crucial for the proper function of synaptic transmission in the prefrontal cortex .

The thin dendrites of pyramidal cells, namely the basal, oblique and tuft dendrites show a specific electric signal: the NMDA spike. When 10-50 neighboring glutamatergic synapses are simultaneously activated in these dendrites they trigger a local dendritic regenerative plateau driven by NMDA receptors (NDMA plateau). This plateau has local amplitude of 40-50mV and can

last up to several hundred milliseconds. When the NMDA plateau potential is initiated in an apical tuft dendrite it can maintain a large part of the tuft in a sustained depolarized state. However if the plateau is generated in the proximal segments of basal dendrites, then it is capable of bringing the neuronal cell body in a sustained depolarized state that resembles a cortical Up state. The strong amplitude and duration of NMDA spikes makes them the ideal candidate for being the cellular substrate for multisite independent subunit computations, thereby increasing the computational power of cortical pyramidal cells (Antic *et al.*, 2010). In the prefrontal cortex, the dendritic plateau potential at the basal dendrites triggers and shapes the dendritic Ca^{2+} dynamics and distribution during suprathreshold glutamatergic synaptic input. Three classes of voltage-Ca^{2+} interactions have been observed in three different zones (proximal, on and distal to the input site) of the same dendrtitic branch, and depend on small amplitude depolarizations due to the action of NMDARs. At 100μm away from the synaptic input site Ca^{2+} plateaus are generated and have a tight temporal correlation with the dendritic plateau. However at the input site, the Ca^{2+} plateaus significantly outlast the local dendritic plateau (duration 0.5-2 sec), causing a brief down-regulation of the dendritic excitability (Milojkovic *et al.*, 2007).

Thus the NMDA spikes may have a key role in cortical information processing in both awake (spatiotemporal binding, working memory) and asleep animals (Up and Down states, consolidation of memories) (Antic *et al.*, 2010). Application of an NMDA antagonist treatment at doses that impaired working memory potentiated the firing rate at most PFC neurons which was caused by an increase of irregularly discharged single spikes, and reduced significantly the organized bursting. These effects further suggest the implication of NMDARs in the transmission efficacy of cortical neurons and the regulation of cortical noise (Jackson *et al.*, 2004).

- **Working Memory**

When an animal is performing a working memory task, it is required to maintain for a period of time (delay period) the information from a relevant cue in order to successfully complete the task and receive the reward. During the delay period, neurons in some brain areas (eg. prefrontal cortex, posterior parietal cortex) display sustained elevated firing activity (10-50Hz). This delay-period activity is considered to be the neural correlate of working memory (Goldman-Rakic 1995). Several modeling studies have been performed to explain the underlying mechanisms of working memory. Most of them are based on the hypothesis that the sustained activity is maintained by a reverberating discharge within a network with strong recurrent excitatory connections. Another hypothesis is that of 'synfire chains', meaning that the activity circulates in loops of feedforward-connected subgroups of neurons with no direct feedback links between successive groups. There is

also the possibility that single neurons can maintain activity by membrane currents that allow cellular bistability. Finally we can also distinguish the models based on whether they use discrete attractor states to represent discrete memory items or continuous attractor states to represent continuous variables like space (Wang, 1999; Durstewitz *et al.*, 2000).

A main question concerns the mechanism responsible for the rate control of sustained activity, i.e. how is runaway excitation prevented and how firing rates are controlled in a network with strong recurrent excitatory connections. Wang addressed this issue in a modeling study in 1999. He supported that in addition to negative feedback mechanisms (like spike frequency adaptation, feedback shunting inhibition, and short-term depression of recurrent excitatory synapses) for a stable sustained state to be achieved, the recurrent excitatory synapses must be dominated by a slow component. The only current matching these requirements is the one mediated by NDMARs because of its slow gating kinetics that lead to synaptic saturation at low firing rates (Wang, 1999).

Similar results are presented in a model for spatial working memory by Compte *et al.* (2000). For a working memory function the PFC must display bistability between a resting state and a spatially structured state with high frequency firing rates. In this study, the bistability is accomplished by the stabilizing effect of NDMARs at recurrent excitatory synapses, and a dominance of the GABAergic contribution over the recurrent synaptic inputs. Specifically when varying the NMDAR/AMPAR ratio the network's dynamics change drastically: a) when NMDARs dominate over AMPARs then sustained activity is stable (20-40Hz) and the network dynamics are essentially asynchronous, b) if AMPARs have a higher contribution then the network oscillates with a constant phase and c) if AMPARs-mediated recurrent excitation is too large, then sustained activity is abolished. Neurophysiological studies have shown that the property of resisting distracting stimuli during working memory tasks might be specific to the PFC. The model network displays robustness against distractors with strong recurrent inhibition provided a low stimulus intensity and also when there is enhanced NMDAR-mediated recurrent excitation and feedback inhibition suggesting a possible implication of neuromodulators (Compte *et al.*, 2000).

The implication of NMDARs in working memory is further supported by the model of Lisman *et al.* (1998). According to their results, the selective excitation required for maintaining novel items in working memory is produced by the voltage dependence of NMDAR-mediated EPSPs with the following mechanism: when glutamate is released to an already active cell, the pre-existing depolarization allows the NMDAR channels to open. The inward current from the NMDAR prevents the normal repolarization and sustains the firing of the cell. On the contrary, if the glutamate is released to an inactive cell, the opening of the NMDARs would not be sufficient. However for this mechanism to hold true the contribution of AMPARs at recurrent synapses must be low. This

criterion can be met if the NMDAR/AMPAR ratio is made high through neuromodulation – a possible candidate is the dopaminergic modulation through D1 receptors (Lisman *et al.*, 1998).

Neuromodulation of working memory by dopaminergic input has since been well studied. Dopamine (DA) acts mainly through D1/D5 receptors in the PFC and increases during working memory tasks. Among its various effects the most important concerning working memory are: a) the modulation of excitatory synapses through the enhancement of persistent Na^+ and NMDAR conductances and a reduction of AMPAR conductances, b) modulation of a persistent Na^+ current (I_{NaP}) and a Ca^{2+}-dependent postsynaptic mglutamateR-mediated delayed afterdepolarization (dADP) and c) an enhancement of signal-to-noise ratio. The implementation of DA's effect in biophysically detailed models of the PFC resulted in enhanced robustness against distracting stimuli during the delay-period activity. However the effects of DA remain complex since they depend on time, agonist concentration and the receptor subtype activated, so further investigation is required. Acetylcholine (Ach) is another neuromodulator involved in working memory and might promote cellular bistability in PFC neurons, independent of synaptic input. Following application of muscarinic agonists in single PFC neurons *in vitro*, enhancement of an afterdepolarizing Ca^{2+}-activated mixed cationic current is observed and the neurons exhibit bistabillity (Yang & Seamans, 1996; Durstewitz *et al.*, 2000; Gorelova & Yang, 2000; Seamans *et al.*, 2001; Tseng & O'Donnell, 2005; Kroener *et al.*, 2009; Sidiropoulou *et al.*, 2009).

B) Up and Down states

Neocortical pyramidal neurons exhibit a state in which their membrane potential oscillates between prolonged periods of hyperpolarization (Down state) and plateaus of depolarization (Up state) where the neurons exhibit tonic firing that can last from 300ms up to a few seconds. Up and Down states are synchronous in pairs of cells, but the firing is asynchronous. They are observed *in vivo* during slow wave sleep (fig. 2) and under anesthesia (fig. 1) with certain anesthetics, whereas *in vitro* (fig. 3) they can both occur spontaneously or be induced with current pulses. During the Up state the neurons' firing characteristics resemble those of sustained activity, whereas during the Down state the neurons are either completely silent or fire at a very low frequency. Depending on the experimental protocol, some variation in the characteristics of Up and Down states (eg. duration of Up state and firing frequency during the Up state) is observed but the network always oscillates at slow rhythm that is smaller than 1 Hz.

In epochs of irregular EEG activity (waking animals and during Up states under anesthesia), intracellular recordings from cortical neurons have shown that the neurons are more depolarized

and have a smaller input resistance, continuous membrane potential fluctuations and fire spontaneously at rest. Thus they have been described as being at a 'high conductance state'. In waking animals the cortical neurons have a low input resistance (5-40MΩ) and a depolarized membrane potential (-60 mV ± 2-6mV), causing irregular and tonic firing (5-40Hz). During the Up state the input resistance is 9.3±4.3MΩ, whereas in the Down state it's much higher (39±9MΩ). Interestingly, when stimulating the brainstem ascending systems that maintain the waking state in anesthetized animals (with ketamine-xylazine or urethane), showing Up and Down states, a prolonged Up state with a desynchronized EEG is elicited. Moreover a similar pattern is seen in animals during the transition from slow-wave sleep to wakefulness. Therefore it might be possible that the Up states represent network states similar to wakefulness and thus have similar underlying mechanisms (Destexhe *et al.*, 2003).

So far the proposed mechanisms regarding the origin of these slow oscillations involve either an intracortical or an extracortical initiation (eg. from thalamus, hippocampus, ventral tegmental area, locus coeruleus etc.). Layer V pyramidal neurons have been identified as the strongest candidate for the origin of Up states in the neocortex and as described by Chauvette *et al.* (2010) three hypotheses have been proposed concerning the responsible mechanism for the initiation of an active state:

- *The "spontaneous release" hypothesis*

According to this hypothesis an Up state is initiated due to the spontaneous release of transmitter which occasionally results in the depolarization of some cells until they reach the firing threshold. This means that every neuron is capable of initiating an active state, however neurons receiving higher excitatory input (like layer V neurons) are more likely to be activated before the others.

- *The "layer V neuron" hypothesis*

In this proposed explanation, the transition from a Down to an Up state is caused by the intrinsic or synaptic properties of layer V pyramidal neurons. These mechanisms maintain layer V neurons in a more depolarized level which allows them to generate action potentials during the Down state, when other cortical neurons are silent. Then the activity propagates to other cortical layers.

- *The "selective synchronization" hypothesis*

According to the third hypothesis Up states are initiated by the selective synchronization of spatially structured neuronal groups made up of a small number of cells. In order for a synchronization to occur, some neurons must generate irregular spontaneous firing during the

Down states. However there is contradictory evidence on whether neurons fire or not during the silent states.

Regarding the transition from the Up to the Down state three mechanisms have been proposed:

a) During an Up state the intracellular levels of Ca^{2+} and Na^{+}, activate K^{+} currents, which eventually precipitates the network in a Down state. During this time the levels of Ca^{2+} and Na^{+} inside the cell decrease, thus allowing the cycle to start again and another Up state is generated. Specifically, it is hypothesized that it a slow Na^{+}-dependent K^{+} conductance underlies this cycle (Compte *et al.*, 2003; McCormick *et al.*, 2003).

b) The second mechanism takes into account the input that neurons receive. According to this hypothesis the lack of synaptic input underlies termination of the Up states (Timofeev *et al.*, 2001; Seamans *et al.*, 2003).

c) Finally, synaptic "fatigue" or synaptic depression has been proposed as another possible candidate for the transition to a Down state (Contreras *et al.*, 1996).

- ***in vivo* recordings of UP and DOWN states**

Up and Down states are recorded when the animal is sedated with certain anesthetics like ketamine - xylazine, ketamine - nitric oxide, halothene, urethane and chloral hydrate (fig. 1). The drug administered for anesthesia has been show to be the cause of variations in the frequency of the slow oscillation (Steriade *et al.*, 1993). On the contrary anesthesia with barbiturates depresses cortical excitability and doesn't result in the generation of Up and Down states (Destexhe *et al.*, 2003).

Intracellular recordings from PFC neurons in rats (anaesthetized with urethane and/or ketamine/ ketamine-xylazine) showed that stimulation of the nucleus locus coeruleus (LC) results in the generation of Up and Down states. The Up states' duration was 0.37±0.18 sec (range 0.16-0.89) and had a frequency of 1.4±0.3 Hz (Branchereau *et al.*, 1996). In another study, *in vivo* intracellular recordings were performed in rats under anesthesia with chloral hydrate, after electrical and chemical stimulation of the ventral tegmental area (VTA), from neurons in the medial and orbital PFC. Up and Down states were observed in both cases. Transitions to the Up state occurred at 1.01 ± 0.35 Hz, lasted 362 ± 160 ms and had a firing frequency of 4.0 ± 4.9 Hz (range: 0–20 Hz). No action potentials were recorded during the Down state. Interestingly, in the same study, activation of the hippocampal and thalamic afferents couldn't elicit a transition to the Up state. In addition, they simulated the release of dopamine (DA) by applying trains mimicking the

burst firing of VTA. This resulted in a prolonged Up state, which supports the theory for the existence of common mechanisms between the sustained activity recorded during working memory tasks and Up states. These findings suggest that although DA doesn't mediate the transition to the Up state, it helps in its maintenance (Lewis & O'Donnell, 2000).

One of the first reports of intracellular Up and Down states was made by Steriade *et al.* in 1993 using recordings from cortical association areas 5 and 7, motor areas 4 and 6, and visual areas 17 and 18 of cats. Pyramidal neurons in all areas from layers III-VI exhibited the slow oscillation. The Up state lasted for 0.8-1.5 sec and recurred rhythmically at less than 1Hz (Steriade *et al.*, 1993). In another study, intracellular recordings from primary visual cortical neurons in halothane-anesthetized cats revealed rhythmic slow oscillations between depolarized and hyperpolarized membrane potentials at a periodicity of once every 3.44 ± 1.37 seconds. The depolarized phase lasted an average of 1.08 ± 0.38 seconds. The membrane potential exhibited a bimodal distribution with peaks at –71 ± 3.6 mV (Down state) and –59.4 ± 4.9 mV (Up state) (Sanchez-Vives & McCormick, 2000). Destexhe and colleagues have also reported Up and Down states in pyramidal neurons of areas 5 and 7 (parietal cortex) from cats anaesthetized with ketamine-xylazine. During active periods the neurons fired at a frequency of 5–20 Hz. Up states usually lasted 0.4-2 seconds but in some occasions they lasted up to several seconds and the EEG displayed low-amplitude waves of fast frequency in the gamma range (Destexhe & Paré, 1999).

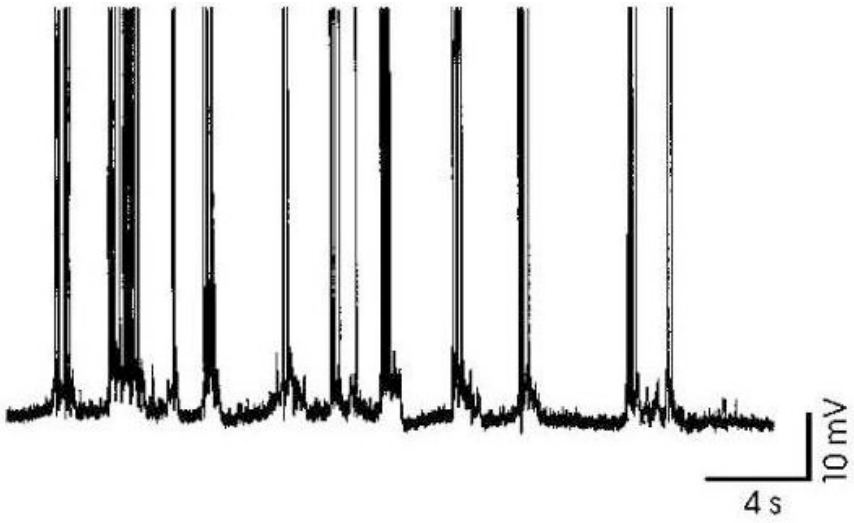


Figure 1. Intracellular recordings of Up and Down states in halothane-anesthetized cats from primary visual cortical neurons (Sanchez-Vivez & McCormick., 2000)

According to a recent study performed in cats, during slow wave sleep deep layer neurons (presumably layer V) tend to depolarize and fire before other cells. Interestingly the depth seems to affect their firing frequency since the highest firing rates were observed in cells recorded deeper than 800μm. There was clear evidence of a prominent synaptic buildup prior to the onset of Up states in the leading neurons, which comes in support of the spontaneous release hypothesis. During the Down state neurons remained silent. The experiments were also conducted while the

animals where under ketamine-xylazine anesthesia and yielded the same results (Chauvette *et al.*, 2010).

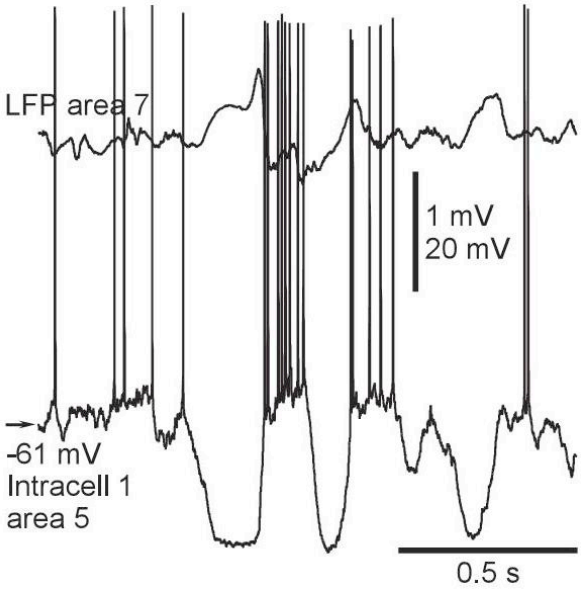


Figure 2. Intracellular and LFP recordings of Up and Down states during slow wave sleep in cats. (Chauvette *et al.*, 2010)

- ***in vitro* recordings of Up and Down states**

Up and Down states have also been recorded *in vitro* in the prefrontal cortex as well as other brain areas (fig. 3). Sanchez-Vivez and McCormick observed the spontaneous generation of Up and Down states in ferret visual and prefrontal cortical slices. For the Up and Down states to appear, the slices had to be maintained in a bathing medium with low Mg^{2+} concentration, to allow activation of the NMDA receptors. The oscillation was stable for the life of the slice, in phase with the extracellularly recorded multi-unit activity and its characteristics were nearly identical to those occurring *in vivo* (Firing frequency: 2–10 Hz, Up frequency: 3.44 ± 1.76 s, Up duration 0.72 ± 0.43 s). The slow oscillation propagated through all layers: it initiated in layer V and propagated to layer VI and finally to layers II and III. The peak of activity was larger in layer V neurons and lasted for a longer period. The depolarized state consisted of barrages of both EPSPs and IPSPs. Their results indicate that the initiation of the slow oscillation can occur spontaneously at the site with the shortest refractory period, but could start from any place within the cortical slice (Sanchez-Vives & McCormick, 2000).

Further investigating of the mechanisms that underlie these oscillations with extracellular and intracellular recordings made in slices of ferret prefrontal and visual cortices resulted in synchronous depolarization and hyperpolarization of neighboring neurons. Neighboring neurons depolarized and hyperpolarized in synchrony. The depolarization was shown to be mediated by synaptic potentials and was 4-10 mV. This activity initiated in layer V neurons, its Up frequency was 0.2±0.1 Hz, its Up duration was typically 0.5-3 sec (mean 1.7±0.3 sec) and its firing frequency

was 2-47Hz (mean 17.1 ± 11.1Hz). The transition period from an Up to a Down state varied and could occur within less than 200 ms. During the Down states ~56% of the cells didn't fire spontaneously, whereas the others discharged at an average rate of 3.6 ± 3.0Hz. Blocking of NMDA and non-NMDA receptors resulted in the abolishment of Up states. On the contrary blocking of $GABA_A$ receptors transformed the Up states into epileptiform like activities (McCormick *et al.*, 2003). Because the sustained activity of the Up state was associated with a bombardment of excitatory and inhibitory synaptic potentials and additionally it was unaffected by a hyperpolarization of the cell, it was proposed to be generated through network mechanisms and in particular, through the recurrent excitation between neighboring pyramidal cells, which in turn is precisely controlled by the local inhibitory interneurons (McCormick *et al.*, 2003; Shu *et al.*, 2003).

Intracellular injections of depolarizing current pulses during the Up and Down states revealed that the Up state increases the neuronal responsiveness. This was seen as facilitation in the generation of action potentials, especially for pulses with smaller amplitudes. This facilitation was even more pronounced if the membrane potential was increased. Furthermore, an increase in the variation of the interspike interval was observed after injection of same pulses during an Up state (McCormick *et al.*, 2003). Single-shock electrical stimulation could initiate an Up state, while the same shock was also able to terminate the Up state - depending on the strength of the stimulus and the time since the beginning of the activity. Increasing the strength of the stimulus while initializing an Up state decreased the Up duration, whereas when terminating the Up state it shortened the delay after which the Up state would terminate (Shu *et al.*, 2003). The observation that excitation can switch off an active state, supports either a hypothesis of massive inhibitory recruitment by external input during the Up state, or a reset mechanism that is synchrony-based (Compte, 2006).

Cossart *et al.* (2003) reported the occurrence of synchronized Up state transitions in spatially organized groups of neurons consisting of only ~ 9 cells, in unstimulated slices from visual cortices (maintained in standard artificial cerebrospinal fluid). Since they differ from 'global' Up and Down states and resemble more closely the sustained activity, they are referred as 'cortical flashes', however they all share common properties on a single-cell activity level. During the peaks of synchrony, 71% of active cells were pyramidal neurons, whereas the rest were interneurons and could repeatedly involve the same components of the network. The duration of the Up states was 60ms-30s and the firing frequency was 21±9 Hz during the Up states and in 44% of the neurons 0.64±0.18 Hz during the Down states (the rest were silent). In the presence of AMPA- and NMDA-receptor antagonists Up states were still observed in a few cells and were abolished only after the subsequent addition of picrotoxin ($GABA_A$ antagonist) (Cossart *et al.*, 2003).

Seamans *et al.* (2003) performed an interesting study both *in vivo* and using organotypic cultures, focusing on the way 'neuromodulatory' inputs from VTA, CA1 and septum to layer V PFC pyramidal neurons initiate Up states and sustained activity. Their results indicate that the Up state begins with an EPSP-IPSP sequence, followed be the depolarizing plateau. Furthermore pharmacological investigation showed that both NMDA and non-NMDA receptors are required for the generation of Up states, but the depolarizing plateau of Up states is mediated by NMDA currents, which act as a bridge for the AMPA mediated EPSPs. In addition the inhibitory events appear to be responsible for the fast membrane fluctuations. As for the transition to a Down state, since it was associated with a marked decrease in synaptic activity, they proposed that Up states are terminated by a lack of synaptic inputs. They also addressed the issue of using ketamine – an NMDA antagonist - as an anesthetic and the contribution of NDMA receptors in the appearance of Up and Down states. They showed that the dosage of ketamine used in anesthesia doesn't completely block NMDA receptors, since a supplemental injection of ketamine decreased significantly the duration and amplitude of the Up state (Seamans *et al.*, 2003).

	Up Frequency	**Firing Frequency**	**Up Duration**
Sanchez-Vivez, 2000	0.3±0.2 Hz	2-10 Hz	0.72±0.43 sec
McCormick, 2003	0.2±0.1 Hz	17.1±11.1 Hz	1.7±0.3 sec

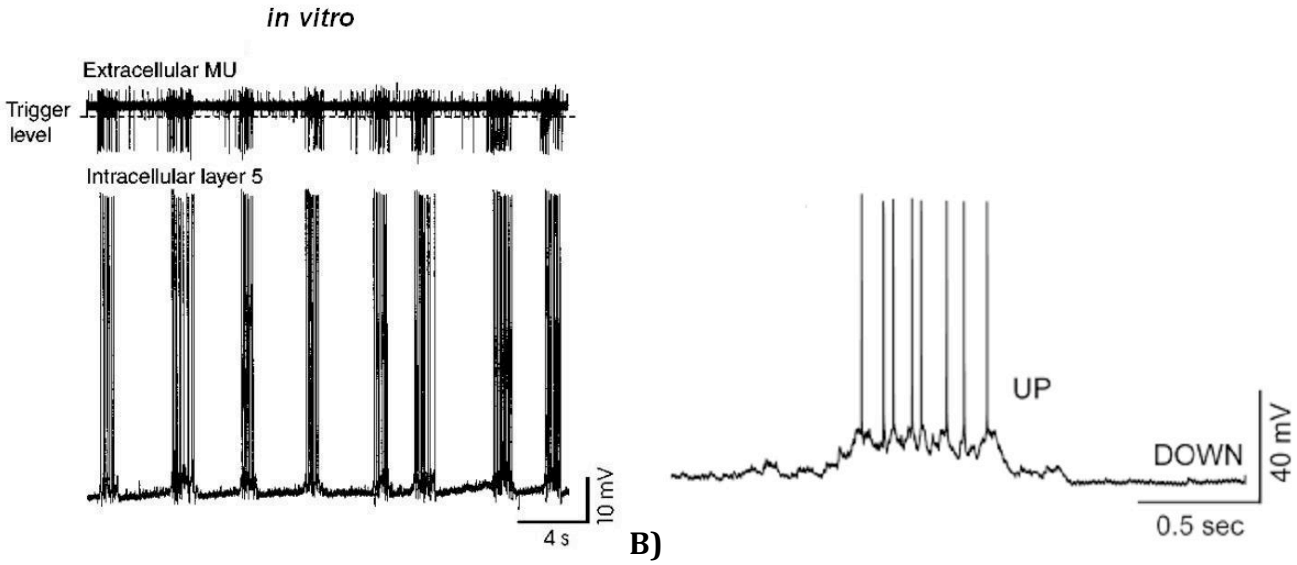


Figure 3. **A)** Simultaneous extracellular and intracellular recordings of Up and Down states in ferret cortical slices maintained *in vitro* (Sanchez-Vivez *et al.*, 2000) **B)**. Intracellular recordings from layer V pyramidal neurons of ferret prefrontal cortex (McCormick *et al.*, 2003).

C) Other computational models of Up and Down states

Using the results from *in vivo* and *in vitro* experiments, Compte *et al.* created a biologically realistic network model consisting of 1024 pyramidal cells and 256 interneurons, to reproduce the slow rhythmic activity (<1Hz). The model neurons are interconnected with biologically plausible synaptic dynamics, but without any autapses. The only sources of noise in the network are the random distribution of some intrinsic cellular parameters (which renders the population heterogeneous) and the random connectivity. The model pyramidal cells have a somatic and a dendritic compartment with many currents incorporated. Interneuron models are more simplified, having a single compartment and only the spiking and leak currents.

This model generates Up and Down states with an Up state firing frequency of ~20-30Hz. Up states are generated through the increase of spontaneous activity caused by the recurrent excitation. Down states are controlled by the Na^+-dependent K^+ current (I_{Kna}), a slow negative feedback mechanism. The control of firing rate of Up states is thought to be achieved both through a dynamic balance of synaptic excitation and inibition, and intrinsic K^+ currents. An interesting result is that by increasing the permeability to K^+ ions through leakage channels, a network that oscillates irregularly can obtain a more periodic oscillation at a lower frequency due to the decrease in the intrinsic excitability of pyramidal cells. In addition, the inverse manipulation increases the Up state duration and decreases the duration of the Down state. Their results support the generation and propagation of a wave from spike discharges of single neurons. They also confirmed the small effect of the NMDA receptor's conductance in the propagation of the wave, as well as the previous predictions of Golomb's and Ermentrout's model for the existence of two propagating waves with different speeds: the pyramidal cells' slow wave and a second one (preceding the slow wave) caused by the firing of interneurons (Golomb & Ermentrout, 2001). The model's speed of wave propagation is three to four times lower than the experimental data, and it predicts a strong relationship between the wave velocity and the distance of long-range horizontal connections. (Compte *et al.*, 2003)

In 2006 Holcman and Tsodyks in an effort to identify the dynamics of Up and Down states analyzed a simplified stochastic dynamical system that models a large recurrent excitatory network of spiking neurons with activity-dependent synaptic depression. Their model consists of two equations that describe the mean firing rate of a homogenous population of neurons (first equation) and the inhibitory connections (second equation). Only certain values of the synaptic weights resulted in the appearance of two stable attractors: one that corresponds to the state of

zero activity and is associated with the Down state, and another that corresponds to higher activity and is associated with the Up state.

Their model shows that the transitions between the attractors are randomly generated by the noise activity. The time spent to a Down state is comparable to the mean time it takes for the synapses to recover from depression. During a Down state depression recovers exponentially until an Up state is generated. The transition from an Up to a Down state occurs when the noise pushes the dynamics outside the Up state region. An increase of the noise amplitude results in a decrease of the Up duration and more transitions between the two states. According to this model the synapses are depressed most of the time and therefore the noise activity produces synaptic depression, the role of which is to decrease the duration of Up states (Holcman & Tsodyks, 2006).

Parga and Abbott used a network of 4000 integrate and fire neurons (17% of which were inhibitory and the rest excitatory) extended by adding a nonlinear membrane current, to study the mechanisms responsible for the transitions between Up and Down states and the network's responses to sensory stimulation. Each neuron receives on average 25 synapses all excitatory synapses include both AMPA and NMDA components whereas $GABA_A$ receptors were assigned to 55% and $GABA_B$ receptors to 45% of inhibitory synapses. The network was simulated by applying conductance pulses to 17% of the excitatory neurons for 10ms.

This network exhibited spontaneous synchronous transitions between Up and Down states. The two states are generated due to the interaction between the nonlinear membrane current (an intrinsic property) with the synaptic activity. They found that the heterogeneity in the neuron parameters generates a subpopulation of neurons that spontaneously reactivates the network after it switches to a Down state. The transition to a Down state was caused by a network oscillatory mechanism in which the inhibition following excitation destabilized the Up state. The duration of an Up state depended on the synaptic time constants and could be controlled by the type of synaptic receptors. The outcome of a response generated to sensory stimulation depends on the state of the network at the time of stimulus application and is higher when the network is in the Down state. Finally by varying the external noise they were able to induce different states ranging from a constant Down state to a perpetual Up state (Parga & Abbott, 2007).

Using a model of nonlinear integrate and fire neurons able to display intrinsic properties like low-threshold spike, regular spiking or fast-spiking, Destexhe investigated the oscillatory and asynchronous irregular dynamics of thalamic, cortical and thalomocortical networks. Of interest to the Up and Down states, is the role of spike-frequency adaptation (SFA).

In the thalamocortical network with strong SFA the network's state changed from asynchronous irregular to Up and Down states. This observation might explain the action of certain neuro-modulators (e.g. acetylcholine) who are responsible for the SFA by blocking/reducing K^+ conductances and is similar to the transition from slow-wave sleep (where Up and Down states occur) to awake (desynchronized activity in the EEG). The generation of the Up states in this model is caused by the input the cortical cells receive from the thalamus, whereas the Down states occur as a result of the activity's termination. Additionally in a two-layer cortical network, in which a small sub-network generated asynchronous irregular states, Up and Down states were generated and self-sustained solely from intrinsic dynamics (Destexhe, 2009).

In a recent study Ngo *et al.* introduced a simple model that specifically tries to reproduce and explain the experimental results of Shu *et al.* (2003) - initiation and termination of an Up state by injecting positive electrical pulses with specific intensity and interstimulus interval. Their model is based on the Compte *et al.* hypothesis that the Up and Down states are caused by a dynamic balance of recurrent excitation and inhibition and controlled by the I_{KNa} current. They are using an integrate and fire model were the neuron only has two states, an active and an inactive/ground state. Each neuron is described with only three equations and receives the same input. The model focuses on the dynamics of slow oscillations, so effects important for times scales close to a single spike were not included.

Initially they used a single neuron model, and showed that the parameter expressing the build-up of inhibitory currents during an Up state was key in explaining the experimental results. Then they extended the model to a network of 10000 neurons with an excitatory all-to-all coupling and noise following a Gaussian distribution. They concluded that the experimental results could be explained with a network in which Up states could be caused either by internal noise and the coupling of neurons or by external stimulation, while during the Down states the network exhibits homogeneously distributed random activity (Ngo, 2010).

After reviewing some of the most important models for Up and Down states we can see that they don't always reach the same conclusions, they use integrate and fire neurons to simulate the pyramidal neurons of the cortex and they often simulate large networks and focus on the dynamics of propagation of the slow wave. However, there are still important points that are left untouched and we try to address these with this study. Because the activity of pyramidal neurons does not follow an integrate and fire pattern, we chose to develop a biophysical compartmental model of pyramidal neurons with characteristics that simulate more accurately the actual neurons (see Materials and Methods for details). Our model consists of 5 neurons, therefore we do not look into the propagation of the slow wave. We were more interested on investigating the effects of the

parameters that have been suggested to influence Up and Down states – intrinsic currents, synaptic mechanisms, effect of reverberation. For instance we've seen that the NMDA receptors have been implicated in both Up states and working memory, an issue that has not been addressed with models so far. In this study we investigate the role of NMDA receptors on the generation and characteristics of Up states, and by adding the dADP mechanism to closer mimic the activity during working memory tasks, we look for a link between Up states and sustained activity. Finally another important issue we cover in this work that has not been addressed, is the impact of the reverberation. The intrinsic connectivity of the cortex is unique and has been suggested as necessary for the generation of Up and Down states, so we investigate under various conditions what happens to the Up and Down states phenomenon when this connectivity is severed.

2. Material and Methods

A) Brief description of the model.

We used a previously described model (Papoutsi *et al.*, 2009) of a microcircuit of layer V pyramidal neurons of the prefrontal cortex implemented in the NEURON simulation environment (Hines & Carnevale, 1997). The microcircuit (fig. 4) simulates the basic architecture of a layer V microcolumn and consists of 4 pyramidal neurons and one fast-spiking interneuron (in the prefrontal cortex interneurons constitute 25-35% of the neuronal population (Dombrowski *et al.*, 2001)). The model pyramidal neurons consist of five compartments: soma, axon, basal, proximal apical and distal apical dendrite. The interneuron model has two compartments: an axon and a soma. A key feature of our model is its ability to reproduce the electrophysiological profile of layer V PFC neurons.

Pyramidal neuron model: The model neuron included ionic and synaptic biophysical mechanisms, known to be present in these cells. The electrophysiological properties (both passive and active) of the pyramidal neuron model were validated against experimental data. Also, all synaptic currents (iAMPA, iNMDA, $iGABA_A$ and $iGABA_B$) of the microcircuit have been validated against both electrophysiological and anatomical data. The model neurons contain a biologically plausible delayed afterdepolarization (dADP) mechanism and ratio of iNMDA-to-iAMPA at the basal dendrite equal to 1, as reported in by Wang *et al.* (2008). Moreover it can generate NMDA spikes similar to the ones reported experimentally (Schiller *et al.*, 2000). The resting membrane potential was set to -65mV (Papoutsi *et al*. submitted).

Interneuron model: The interneuron model was adapted from a previously published, fast spiking (FS) neuron, (ModelDB, accession number 82784, (Hines *et al.*, 2004)). The interneuron included biophysical mechanisms for Na^+ current (I_{Naf}), and two types of K^+ currents (I_{Kdr}; I_D).

Model microcircuit: Each of the four morphologically simplified pyramidal model neuron was connected with the other three neurons in a recurrent circuit, as well as the interneuron. The interneuron sent inhibitory synapses to each pyramidal neuron. The latencies of the excitatory connections targeting the interneuron and of inhibitory connections targeting the pyramidal cells were drawn from Gaussian distributions respectively. The recurrent connections of pyramidal model neurons were of the same strength, targeted the basal dendrites and followed a normal distribution. Autapses were also implemented in both the pyramidal cells and the interneuron of our model. All details on connectivity (number of synapses/location/delays) have been validated according to anatomical and electrophysiological data (Papoutsi *et al*. submitted).

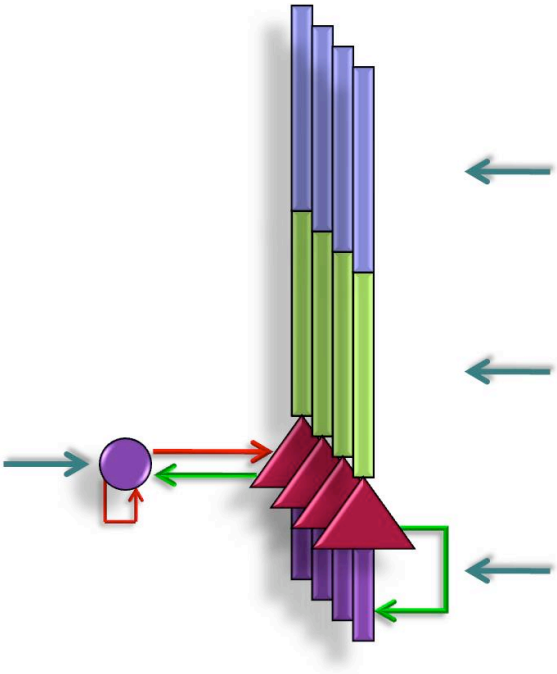


Figure 4. Structure of the layer V PFC microcircuit.
Red triangle: soma of the pyramidal neuron model,
Purple circle: the two-compartment interneuron model,
Purple line: basal dendrite of the pyramidal neuron model,
Green line: proximal dendrite,
Blue line: apical dendrite,
Green arrows: local excitatory synapses,
Red arrows: local inhibitory synapses,
Blue arrows: excitatory background activity from other brain areas.

In order to simulate as closely as possible the *in vivo* conditions, an artificial, white noise like, current was injected in all neuronal models that resulted in an experimentally observed fluctuation of the membrane potential of both pyramidal and interneuron models (Papoutsi *et al*. submitted).

In the present study we extended the microcircuit model to include low frequency synaptic activity, mimicking background noise. Specifically, we constructed two pre-synaptic processes - one projecting to the 4 pyramidal neurons and one to the interneuron – that provided background excitation at random time intervals according to a Poisson process, along the somatodendritic axis of the pyramidal model neurons and to the interneuron. The ratio of iNMDA-to-iAMPA was validated against the experimental data of Dodt *et al.*, (1998) and varied along the basal, proximal and apical dendrites of the pyramidal neurons. In particular, we kept as a reference point the iNMDA-to-iAMPA ratio at the basal dendrites and reduced the conductance of the NMDA receptor (while the conductance of AMPA receptors remained unaltered), to reproduced the reported decrease in the NMDA amplitude along the apical dendrite (Dodt, Frick et al. 1998)

B) Analysis of Up and Down states

Data analysis was performed using MATLAB (MathWorks, Inc.) and IgorPro (Wavemetrics, Inc.). For each simulated condition 10 trials were conducted, each lasting 30 seconds. Due to the stochastic mechanisms we implemented in our model, the resulting traces from each trial were different. However the same number of a trial would always produce the same trace, allowing us to

directly compare the effects of altering a parameter between two conditions (for example see fig. 7B). Kruskal-Wallis test analysis was conducted for each parameter, between each condition, the control and where required between conditions.

Up and Down states were categorized using the thresholds suggested by Shu *et al.,* (2003): a depolarization plateau above -60 mV, lasting for at least 500ms was classified as an Up state. Periods exhibiting a depolarization plateau that lasted less than 500ms were not used for the analysis. Periods were the model neuron was silent and the membrane potential was below -60 mV, fluctuating close to the resting level (fig. 5) were classified as a Down state, regardless of its duration. In various conditions we investigated: (a) the emergence of Up and Down states, (b) the frequency of Up states, (c) the firing frequency during Up states and (d) the duration of Up states.

For the identification and analysis of the Up and Down states we used the following methodology:

1. We filtered the traces from all trials in MATLAB using the low-pass Butterworth filter in order to remove action potentials and reveal the underlying depolarizing plateaus during the Up states (fig.6).
2. In the resulting matrix we identified depolarizing activity by (i) selecting the first time that the membrane potential was above the -60mV threshold (ii) removing prior values (iii) selecting the first time the membrane potential was below -60mV. In conditions were the dADP mechanism was activated, thus the cell was more depolarized, the threshold was set to -61mV to ensure a more accurate selection of depolarizing plateaus by the filter.
3. We calculated the number of spikes (from the data of the unfiltered traces) during the previously identified depolarizing plateaus, by counting the instances that the membrane potential crossed the value of 0mV.
4. Depolarizing plateaus lasting less than 500ms were discarded, leaving only Up states and their corresponding duration.
5. We calculated the total number of Up states from all trials to find the Up states frequency.
6. Using the number of spikes we calculated the firing frequency.
7. Statistical analysis of the results using the non-parametric Kruskal-Wallis test.

All the code written in MATLAB for analyzing and visualizing purposes can be found in the Appendix.

3. Results

The primary goal of this study was to investigate the possible interactions between single-cell intrinsic properties and synaptic integration in the generation of Up and Down states. Stimulation of the model microcircuit was simulated by activation of excitatory mechanisms along the proximal apical, the distal apical and the basal dendrite. For each condition, 10 trials of 30 seconds were carried out. In total we simulated 27 conditions (table 2) in which we varied different parameters of our model one at a time and in combination to examine the impact of: i) the excitatory background activity onto pyramidal neuron models, ii) the number of activated background synapses, iii) NMDA current amplitude, iv) various intrinsic currents and v) the network connectivity on the emergence and characteristics of UP and Down states.

We found that Up and Down states could emerge in our model microcircuit, provided the existence of background synaptic activity which simulated the current-evoked or spontaneously occurring synaptic bombardment observed in slices of the PFC. Although most of the conditions were able to modify the frequency of Up states and the firing frequency during an Up state, it was the dADP, the iNMDA-to-iAMPA ratio and the background activity that were capable of increasing the duration of an Up state. The impact of connectivity was examined in combination with most of the conditions and is presented at the end of this section.

1. Control condition

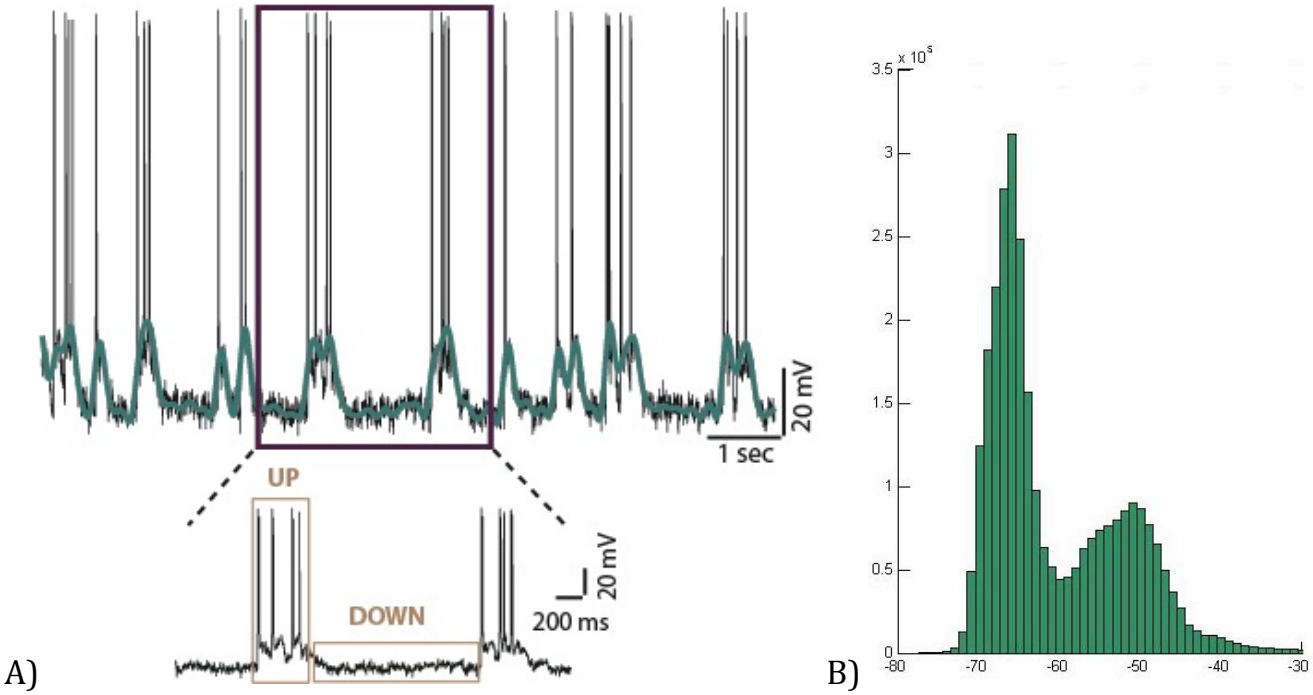
The characteristics of the Up state for the control condition were the following:

(a) duration: 689ms ± 170ms

(b) Up state frequency (occurrence of Up states): 0.17Hz ± 0.05Hz

(c) firing frequency during the Up state: 16.7 Hz ±3 Hz.

The minimum duration for an UP state was set to 500ms (according to McCormick *et al.*, 2003). Up states with smaller durations were present in our model but were not included in the data analysis. The values of our model closely resemble the ones reported in 2003 by McCormick *et al.* (duration: 1.7±0.3s, Up frequency: 0.2±0.1Hz, firing frequency: 17.1±11.1Hz). The membrane potential fluctuated mainly around two values: -65mV and -50mV corresponding to the Down and Up state respectively (fig. 5B). As can be seen in fig. 5A the Down states were more stable (smaller fluctuations in the membrane potential) than the Up states and were close to the resting membrane potential (-65mV). No spiking activity was observed during the Down states.



A) B)

Figure 5. A) Representative trace (black) of Up and Down states. Blue trace shows the signal after it has been filtered with the Butterworth filter. Orange boxes indicate an Up and a Down state that meet the categorization criteria (500ms duration and above -60mV depolarization plateau).
B) Histogram from all simulations of the control condition showing the time spent (1 ms bins) at each membrane potential. Two membrane states are distinguishable around -65mV (Down state) and -50mV (Up state).

Control condition parameter values (which are varied in the following experiments):

- presence of interconnectivity: yes
- number of background excitatory synapses = 180
- number of background inhibitory synapses = 180
- frequency of pyramidal cells' background excitatory activity = 4 Hz
- frequency of interneuron's background excitatory activity = 16.8 Hz
- iNMDA-to-iAMPA ratio = 1
- sAHP, I_D, I_{CaL} : unblocked
- dADP : inactivated
- amplitude of the current injection that resulted in membrane noise for the pyramidal neurons = 0.035 nA
- amplitude of the current injection that resulted in membrane noise for the interneuron = 0.06 nA

2. Characterizing the role of the excitatory background synaptic input that the pyramidal neurons receive

After validating our model and establishing the optimal combination of parameters for the emergence of Up and Down states we investigated the role of several network mechanisms, in determining the emergence and shaping the firing characteristics of Up and Down states. First we varied the frequency of the excitatory background synaptic activity received by the pyramidal neurons (control value = 4 Hz). The results of the variability in the firing characteristics of Up and Down states are summarized in the following table (***: p value <0.001, - : no statistically significant difference).

Table 1: Effect of the firing frequency of background excitation on Up and Down state characteristics

Frequency of background activity (Hz)	Up Frequency (Hz)	Firing frequency (Hz)	Up Duration (ms)
4 (control)	0.17 ± 0.05	16.7 ± 3	689 ±170
2	0.01 ± 0.02 ***	14.64 ± 2.63 -	580.75 ± 66.43 -
5	0.46 ± 0.08 ***	21.43 ± 3.71 ***	742.64 ± 236.51 -
10	0.53 ± 0.07 ***	33.48 ± 6.22 ***	1520.5 ± 996.85 ***
16.4	0.46 ± 0.06 ***	43.59 ± 7.66 ***	1897.55 ± 1194.61 ***

Decreasing the frequency of background excitatory synaptic activity by 50% (2 Hz) had a severe impact on the emergence of Up states, with significantly less than one Up state appearing every 10 seconds. The firing frequency and Up states duration were also slightly decreased, but this wasn't statistically significant. Gradually increasing the frequency of excitation – 5, 10 and 16.4 Hz – also increased the firing frequency in the microcircuit. When the background frequency was 10Hz (purple trace in fig. 6A) or 16.4Hz the duration of the Up state was also significantly longer. The smaller Up states frequency when the background frequency was 16.4 Hz compared to 10 Hz is easily explained when we take into account the fact that in the former case, the Up duration has increased enough to bridge neighboring Up states. However the timing of Up and Down states was entirely different, as can be seen in fig. 6A, which wasn't the case for simulations were other parameters were changed (see for comparison fig. 6B).

3. Varying the strength of the synaptic background excitation

Next we investigated the effects of varying the strength (number of activated synapses) of excitatory background input (control: excitatory = 180, inhibitory = 180). The results are shown in Table 2 (***: p value<0.001, **: p value<0.05, -: no statistically significant difference). In an effort to simulate the effect of an external stimulus (as opposed to spontaneous background activity) we increased the number of background excitatory synapses to the pyramidal neurons to 280, while leaving the number of synapses to the interneuron unchanged (blue trace in fig. 6B). We observed a significant increase in the firing frequency – which can be clearly seen in the inset of fig. 6B - and the Up frequency. Although the change in the Up duration wasn't enough to be considered important, it is the main reason for the higher occurrence of Up states, because it prolonged some subthreshold Up states enough to exceed the 500ms criterion.

Table 2: Effect of synaptic excitation strength on Up and Down state characteristics

Background synapses to pyramidal neurons	Background synapses to interneuron	Up Frequency (Hz)	Firing frequency (Hz)	Up Duration (ms)
180 (control)	180 (contol)	0.17 ± 0.05	16.7 ± 3	689 ±170
280	180	0.28 ± 0.05 ***	25.29 ± 4.62 ***	703.54 ± 195.55 -
40	45	0.07 ± 0.05 **	7.28 ± 1.30 ***	662.27 ± 158.02 -

We also wanted to see how our model network would behave when receiving a weaker synaptic background excitation. We gradually lowered the number of synapses which resulted in a gradual decrease of the Up state's frequency and firing frequency (data not shown). The weakest input that was able to produce some Up states was with 40 excitatory background synapses to each pyramidal neuron and 45 excitatory background synapses to the interneuron (*herby termed "reduced synapses" condition*). The obtained values for both firing frequency and Up state occurrence are statistically different than the control's, although the membrane potential continued to show bistability and was depolarized occasionally for more than 500ms. It should be noted that many of the potential Up states (those that didn't reach 500ms) were highly unstable due to very fast fluctuations of the membrane potential, which brought it close to the resting level only for a brief moment and then back to the depolarized state.

Seeing that a higher frequency in the background activity and stimulation of fewer synapses have opposing effects, we combined the two conditions: frequency of excitatory background activity = 10Hz under the "reduced synapses" condition. The characteristics of the Up states under these conditions were the following: a) Up frequency: 0.63 ± 0.12 Hz, b) firing frequency: 11.74 ± 2.23 Hz, c) Up duration: 1012.66 ± 576.46 ms. Compared to the condition were the input frequency

was 10 Hz and the number of activated synapses as in the control (180), the difference of the firing frequency and Up duration are statistically significant (p values < 0.001). These values deviate considerably from the control, thus providing stronger validation of the initial parameters. Additionally we see that the combined effect of both conditions doesn't result from a simple subtraction. On the contrary, it reveals a higher impact for the frequency of excitatory background activity on the Up states' frequency and duration, whereas for the number of synapses the impact is greater on the firing frequency.

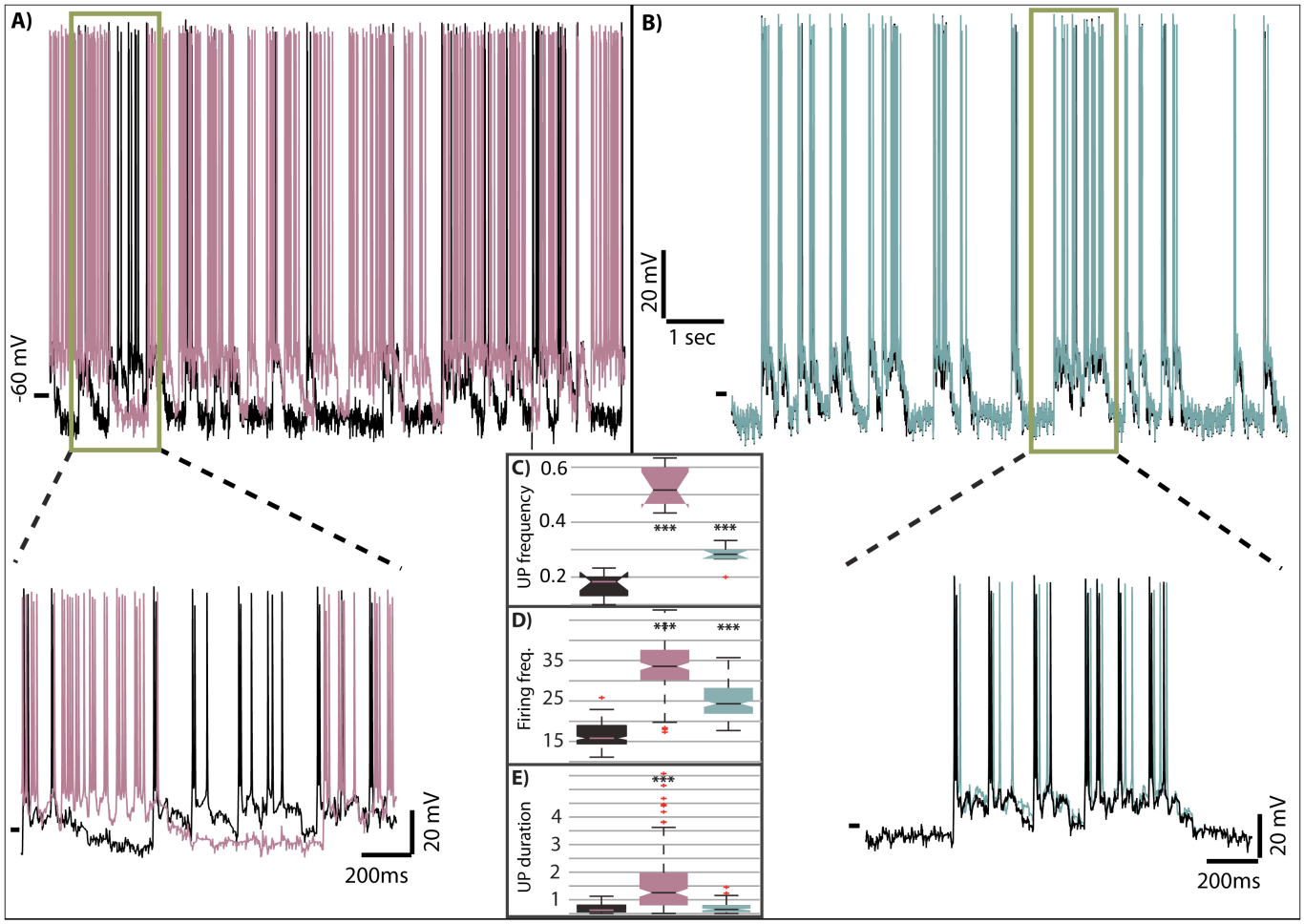


Figure 6. A) A representative trace of the control condition (black) (frequency = 4Hz) overlaid with a trace were the only parameter differing was the a frequency of the excitatory background input to the pyramidal cells which was increased to 10 Hz (purple). This change resulted in a different response, were the pyramidal neuron had more Up states with higher firing frequency and duration, which also occurred in different times. See for comparison the blue trace in B, were the timing of Up states is identical between the two conditions.
B) A representative trace of the control condition (black) (180 activated excitatory synapses) overlaid with a trace were we activated 280 synapses (blue). Up states' frequency and firing frequency increased significantly, but there was no effect in the duration of the Up state.
C-E) Box plots of the occurrence of Up states (Hz), firing frequency during Up states (Hz) and duration of Up states (sec) for all three conditions. Data for each condition is from 10 runs, each lasting 30 seconds. Red dots: outliers ***: p values < 0.001

4. Role of NMDA receptors in Up state emergence

Given the role of NMDARs in the generation of the dendritic plateau and their possible involvement in the generation of Up states (as suggested by Antic *et al.,* 2010) we investigated the role of NMDARs in our model. We deactivated the NMDARs in the entire network and compensated the resulting loss in excitability by increasing the weight of AMPARs, so that the amplitude of the depolarization would be of the same amplitude with the control. This manipulation resulted in a complete abolishment of Up states (purple trace in fig. 7). The network continued to exhibit spiking activity, but the depolarizing plateau - characteristic of an Up state - was absent, supporting the hypothesis that it is generated through the action of NMDARs. Additionally the number of spikes was lower, which is explained by a reduced excitability due to the absence of the depolarizing state. However firing was restricted in times where there would have been an Up state if the NMDARs were active, suggesting that NMDARs are necessary for the maintenance of an Up state but it's the network's synaptic activity that is responsible for their generation.

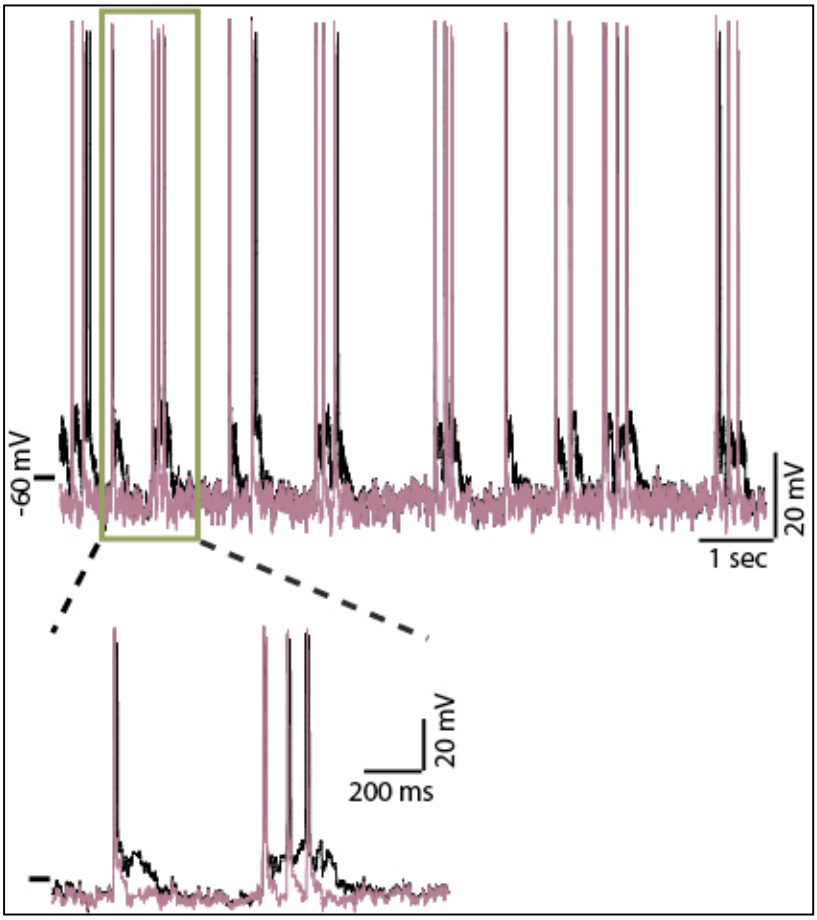


Figure 7. A representative trace of the control condition (black) (AMPA and NMDA currents are both active) overlaid with a trace were NMDA current was deactivated and the decrease of excitability was compensated by increasing the AMPA current (purple). Deactivating the NMDA current resulted in a complete loss of Up states. The neuron continued to fire single spikes, but the depolarizing plateau is absent, which resulted in fewer spikes.

5. Role of NMDA amplitude in Up and Down states properties

It has been proposed that the underlying mechanisms of Up and Down states might be similar to those of working memory and the iNMDAR-to-iAMPAR ratio is considered to be one of those

mechanisms. In particular neuromodulation through dopamine enhances the NDMA current (iNMDA) up to 50%. Thus, we next increased the iNMDA-to-iAMPA ratio in our model (control ratio = 1) to 1.25 and 1.5 (purple trace in fig. 8). The effects on the Up states are summarized in the following table (***: p value<0.001, **: p value<0.05, -: no statistically significant difference).

Table 3: NMDA current amplitude influences UP state characteristics

iNMDA-to-iAMPA ratio	Up Frequency (Hz)	Firing frequency (Hz)	Up Duration (ms)
1 (control)	0.17 ± 0.05	16.7 ± 3	689 ±170
1.25	0.29 ± 0.05 ***	18.27 ± 3.27 **	728.52 ± 194.76 –
1.5	0.49 ± 0.08 ***	20.94 ± 4.16 ***	1202.24 ± 1020.38 ***

In both conditions tested all three Up state parameters increased. Although with an iNMDA-to-iAMPA ratio = 1.25 the increase in Up states' duration wasn't significant, when we set it to 1.5, Up states could last for several seconds. Concerning the timing of an Up state's emergence, as can also be seen in fig. 8, it always coincided with Up states that would emerge if the ratio was 1. When also taking into account the conclusions from the deactivation of the NMDAR, then our results further enhance the hypothesis that the role of the NMDAR is to maintain the depolarizing plateaus, as well as increase the possibility of spiking by making the neuron more excitable.

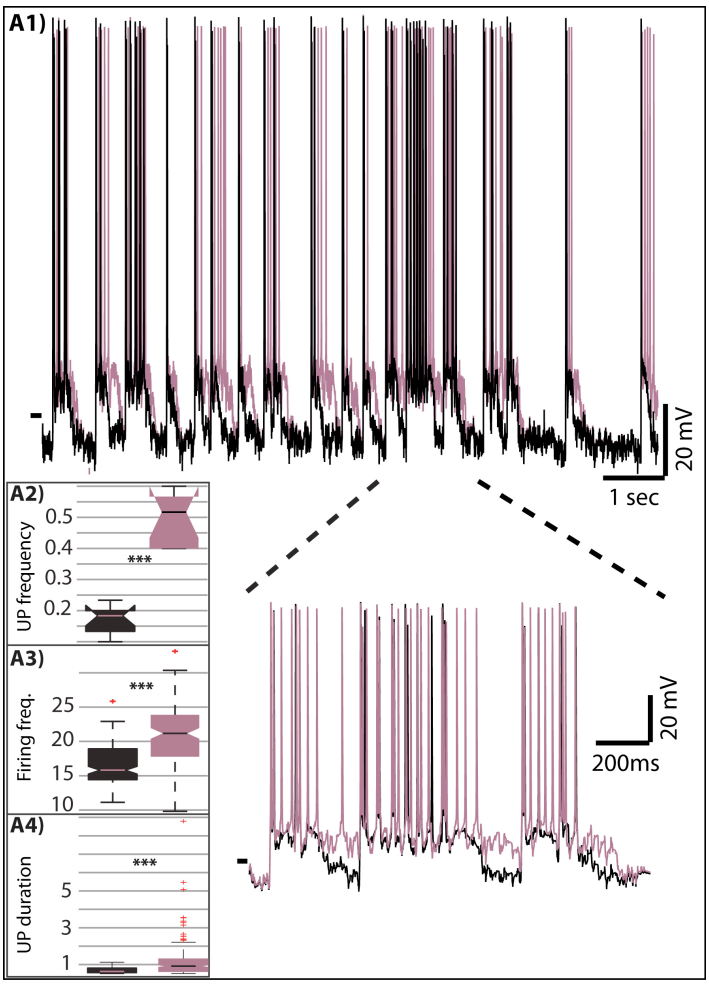


Figure 8. A1) A representative trace of the control condition (black) (iNMDA-to-iAMPA ratio = 1) overlaid with a trace were iNMDA-to-iAMPA ratio = 1.5 (purple). All Up states' parameters increased significantly. The inset shows a clear view of the prolongation of the depolarizing plateau, bridging Up states and causing the increase of their duration.

A2-A4) Box plots of the occurrence of Up states (Hz), firing frequency during Up states (Hz) and duration of Up states (sec) for the two conditions. Data for each condition is from 10 runs, each lasting 30 seconds. Red dots: outliers. ***: p values < 0.001

6. Impact of intrinsic mechanisms

Since the intrinsic membrane mechanism makeup regulates synaptic excitability in layer V pyramidal neurons, we investigated the impact of single-cell properties in the emergence of Up and Down states. First we observed that a 20% increase in the amplitude of the membrane fluctuations did not affect the Up states properties (Up frequency: 0.17 ± 0.05 Hz, firing frequency: 16.42 ± 2.6 Hz, Up duration: 699.01±174.79 ms, p values > 0.05). Next we blocked the conductance of L-type voltage-gated Ca^{2+} channels (I_{CaL}), the slow Ca^{2+}-activated potassium channels (sAHP) and the D-type potassium channels (I_D), since these currents have been show to interact with synaptic potentials.

➢ Blocking of ICaL / ID / sAHP.

The results of the simulations are summarized in Table 4 below.

Table 4: Effects of current blockade on Up state properties

Deactivated intrinsic currents	Up Frequency (Hz)	Firing frequency (Hz)	Up Duration (ms)
I_{CaL}	0.14 ± 0.06 -	17.84 ± 3.37 -	688.31 ± 172.18 -
I_D	0.41 ± 0.07 ***	65.97 ± 5.75 ***	705.66 ± 193.66 -
sAHP	0.23 ± 0.06 *	27.91 ± 5.16 ***	694.7 ± 203.06 -
Control	0.17 ± 0.05	16.7 ± 3	689 ±170

***: p values < 0.001, *: p value < 0.05, - : no statistically significant difference

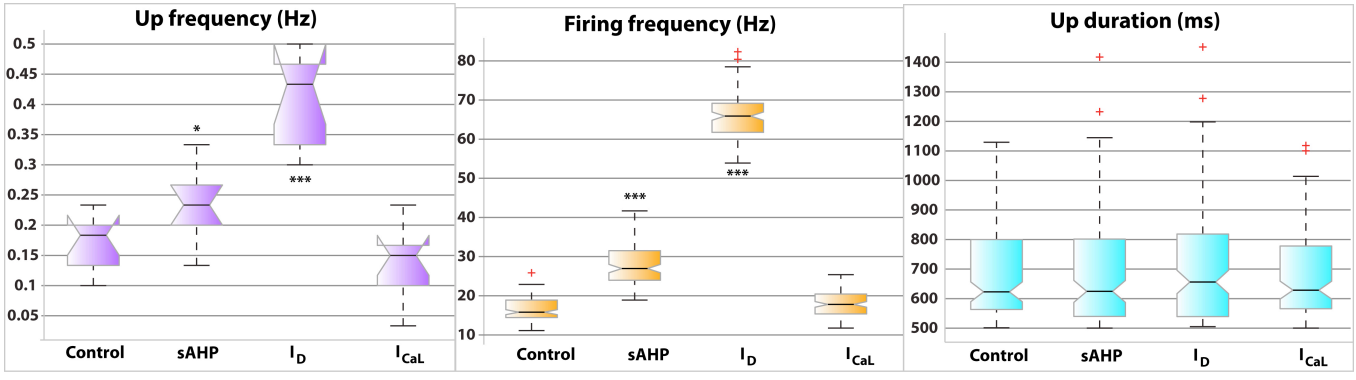


Figure 9. Box plots of the occurrence of Up states (Hz), firing frequency during Up states (Hz) and duration of Up states (ms) for three conditions compared with the control. I_{CaL}: blocking the conductance of L-type voltage-gated Ca^{2+} channels, sAHP: blocking the conductance of the slow Ca^{2+}-activated K channels, I_D: blocking the conductance of the D-type K channels. Data for each condition is from 10 runs, each lasting 30 seconds. Red dots: outliers

Deactivation of the L-type Ca^{2+} voltage channels had no statistically significant effect in any of the Up states' characteristics. Deactivation of the slow Ca^{+2}-activated K^+ channels and the D-type K^+ channels increased the Up frequency and the firing frequency. However none of these manipulations had any effect on the duration of the Up state. We should point out that the deactivation of the I_D led to the biggest increase of the firing frequency compared to all manipulations and to epileptiform-like activity.

- **Activation of the dADP mechanism**

Finally, we activated the dADP mechanism at a physiological value (purple trace in fig.10 A1-A4), inducing a 4mV depolarization at the soma, because it has been proposed to underlie sustained firing in pyramidal neurons. All three parameters of the Up states were significantly enhanced: a) Up frequency 0.53±0.05 Hz, b) firing frequency: 27.11±7.58 Hz, c) Up state duration: 1302.29±781.36 ms (p values < 0.001). As expected from activating a depolarizing mechanism, the membrane potential was also more depolarized by ~4mV. The timing of Up states emergence compared to the control was again unaffected, suggesting that the dADP isn't responsible for the emergence of Up states, but can influence their characteristics.

As a further test, we combined the dADP activation with the condition of reduced synapses (purple trace in fig.10 B1-B4). The results were counter-intuitive: a) Up frequency 0.14±0.05 Hz, b) firing frequency: 19.11±12.64 Hz, c) Up state duration: 5.6±7.3 sec. The p values when comparing against dADP activated and control number of synapses were: a) < 0.001, b) < 0.001, c) < 0.05. The p values when comparing against dADP deactivated and reduced synapses were: a) < 0.01, b) < 0.001, c) < 0.001. In every run, after a few seconds (5-15) the neurons exhibited sustained activity that lasted for the remaining duration of the run. This is the reason for the reduced Up states frequency and large durations. These findings suggest that a sufficient , this simulation shows that a sufficient number of synapses (excitatory background and/or inhibitory) need to be active in order to maintain the bistability. Lack of sufficient inhibitory input may be the reason for entering into a sustained firing state.

Since the previous conditions revealed a way for entering into a prolonged firing state, we next investigate the combination of activated dADP and increased iNMDA-to-iAMPA ratio, that corresponds to the dopaminergic modulation exerted during working memory tasks. The results are shown in Table 5 (***: p value < 0.001).

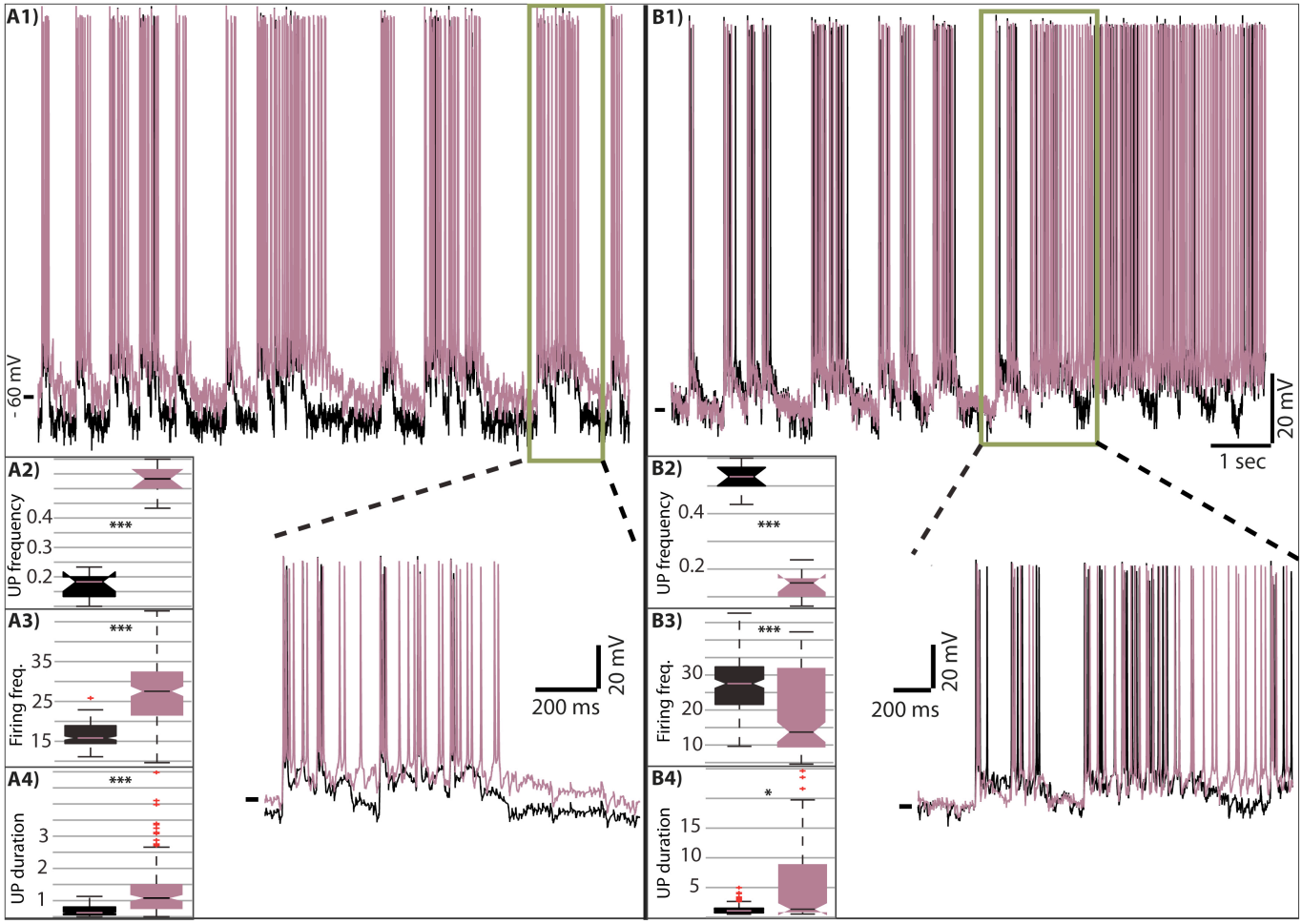


Figure 10. A1) A representative trace of the control condition (black) (dADP deactivated) overlaid with a trace were the dADP was activated (4mV) (purple). Activation of dADP increased significantly all Up states parameters, including the average membrane potential for both Up and Down state. As is clear from the inset, the depolarizing plateau lasted much longer, leading to an increase of both the duration and the occurrence of Up states.
B1) A representative trace were the dADP is activated (4mV) and 180 excitatory and 180 inhibitory background synapses are active (black, same condition as the purple in A1), overlaid with a trace were the dADP is activated (4mV) and 40 background excitatory & 45 inhibitory synapses are active (purple). Reducing the number of synapses while the dADP was activated reduced the firing frequency and the Up states frequency. However, after the first 12 seconds the neuron exhibited sustained activity for the remaining duration of the run, resulting in the observed increase of the Up states' duration.
A2-A4, B2-B4) Box plots of the occurrence of Up states (HZ), firing frequency during Up states (Hz) and duration of Up states (sec) for all three conditions. Data for each condition is from 10 runs, each lasting 30 seconds. Red dots: outliers. ***: p values < 0.001, *: p value < 0.05

Table 5: Effect of dopaminergic modulation (dADP and NMDA enhanced) on Up state properties

iNMDA-to-iAMPA ratio	Up Frequency (Hz)	Firing frequency (Hz)	Up Duration (ms)
1	0.53 ± 0.05	27.11 ± 7.58	1302.29 ± 781.36
1.25	0.18 ± 0.09***	36.67 ± 12.59***	5182.92 ± 6820.08***
1.5	0.04 ± 0.01***	51.96 ± 14.27***	24492.68 ± 10769.49***

In both conditions (iNMDA-to-iAMPA = 1.25, fig.11 A1-A4 and iNMDA-to-iAMPA = 1.5, fig.11 B1-B4) the firing frequency and the duration of the Up states increased dramatically. When the ratio was 1.25 the Up states could last up to more than 10 seconds, whereas when it was 1.5 the neurons' exhibited sustained activity that could sometimes last for nearly the entire duration of the run.

After seeing the results of the 1.25 ratio, in order to evaluate the stability of the sustained activity that emerged, we run an additional simulation where we increased the frequency of the excitatory background activity that the interneurons receive from 16.8 Hz to 19.4 Hz. The parameters for the Up states were the following: a) Up frequency 0.22±0.08 Hz, b) firing frequency: 35.15±11.00 Hz, c) Up state duration: 4.2±4.8 sec. Although there were small differences, none of them was statistically significant.

These findings suggest that the NMDA current and the dADP may serve as the underlying mechanisms that can transform Up and Down states into prolonged (sustained) firing, such as the one observed during working memory tasks.

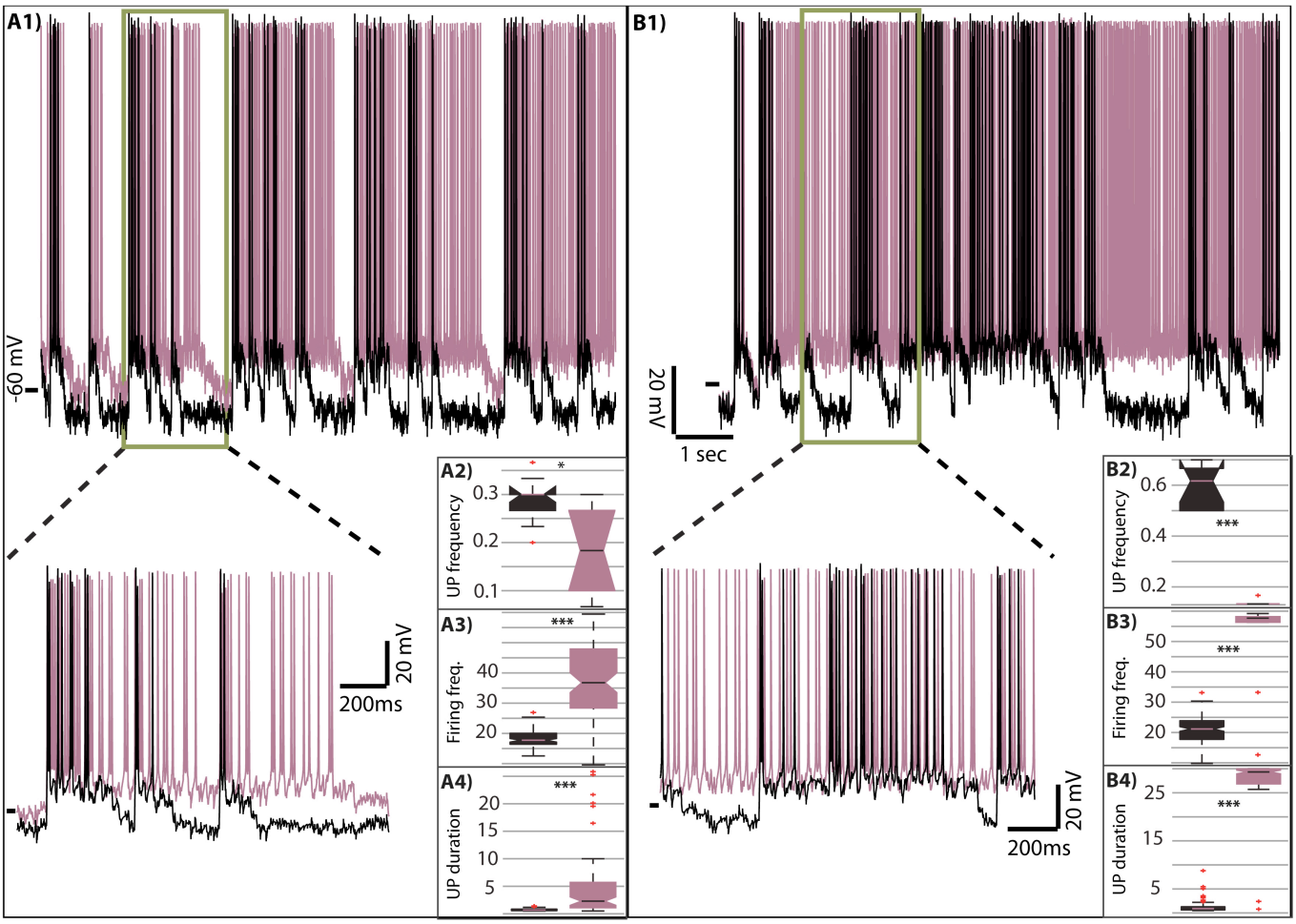


Figure 11. A1) A representative trace from a run with dADP deactivated and iNMDA-to-iAMPA ratio = 1.25 (black) overlaid with a trace were the dADP is activated (4mV) and the iNMDA-to-iAMPA ratio = 1.25 (purple). dADP activation resulted in great increase of both firing frequency and duration. Actually the depolarizing plateau was so prolonged that bridged many Up states at a time, resulting in a decrease in their occurrence.

B1) A representative trace from a run with dADP deactivated and iNMDA-to-iAMPA ratio = 1.5 (black) overlaid with a trace were the dADP is activated (4mV) and the iNMDA-to-iAMPA ratio = 1.5 (purple). Activation of the dADP in this case resulted in sustained activity, with high firing frequency that lasted for the entire duration of the run.
A2-A4, B2-B4) Box plots of the occurrence of Up states (Hz), firing frequency during Up states (Hz) and duration of Up states (sec) for the four conditions. Data for each conditions is from 10 runs, each lasting 30 seconds. Red dots: outliers. ***: p values < 0.001, *: p value < 0.05

7. Impact of microcircuit interconnectivity

We also examined the impact of interconnectivity between the neurons of the microcircuit on Up states properties. To do so we removed all connections that each pyramidal neuron received from the other pyramidal cells and the interneuron, thus keeping only the background synaptic excitation.

To our surprise, in the absence of interconnectivity (purple trace in fig. 12) and with all other parameters as the in the control condition, we observed the emergence of Up and Down states in individual pyramidal neurons, with characteristics similar to the ones of the network. We obtained the following values for the Up state were: a) Up frequency: 0.23 ± 0.06 Hz (p value = 0.049), b) firing frequency: 26.66 ± 4.56 Hz (p value < 0.001), c) Up duration: 692.21 ± 161.48 ms (p value > 0.05). The differences include an enhanced Up state frequency and firing frequency, but no change in the duration of the Up state. This enhancement of excitability in individual pyramidal neurons is most probably due to the lack of inhibition that was provided by the interneuron to the microcircuit. This suggests that inhibition contributes to the rate control of the network.

In the absence of interconnectivity and blocking the conductance of L-type voltage-gated Ca^{2+} channels (I_{CaL}) the characteristics for the Up states were: a) Up frequency: 0.2 ± 0.06 Hz (p value > 0.05), b) Firing frequency 32.43 ± 6.24 Hz (p value < 0.001) and c) Up duration: 655.07 ± 156.96 ms (p value > 0.05). In this case the only significant difference when comparing with either individual condition was the elevated firing frequency.

We found no difference between the intact network and the absence of interconnectivity when we blocked NMDARs and compensated the reduced excitability by increasing the weight of AMPARs.

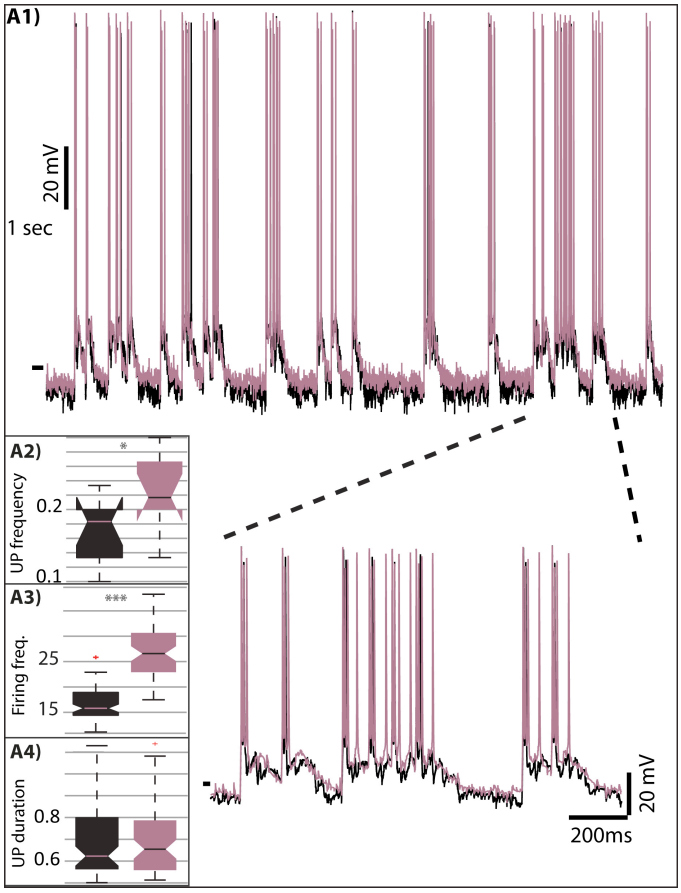


Figure 12. A1) A representative trace from the control condition where all pyramidal neurons are interconnected (black) overlaid with a trace from one of the pyramidal neurons in the microcircuit, in the absence of interconnectivity (purple). The average membrane potential is slightly more depolarized. A small increase in the Up states' frequency is observed, and a significant increase in the firing frequency. The Up states' duration remains unaffected.

A2-A4) Box plots of the occurrence of Up states (Hz), firing frequency during Up states (Hz) and duration of Up states (sec) for the two conditions. Data for each conditions is from 10 runs, each lasting 30 seconds. Red dots: outliers. ***: p value < 0.001, *: p value < 0.05

Next, we reduced the active background excitatory synapses from 180 to 40. Although, as we previously saw, weak background excitation was able to induce some Up states in the microcircuit, it failed to do so in isolated pyramidal neuron models. Specifically, although there was some firing activity there never was any depolarizing plateau. Thus the interconnectivity is essential for the emergence of Up and Down states when the background activity is low.

We also investigated the impact of interconnectivity when the dADP mechanism was activated (producing a 4mV depolarization in the soma). The resulting Up states had the following characteristics: a) Up frequency: 0.16 ± 0.07 Hz, b) firing frequency: 26.27 ± 11.04 Hz, and c) Up duration: 6.04 ± 5.8 sec. The p values when comparing against activated dADP in the intact network are: a) < 0.001, b) > 0.05, c) < 0.001. The p values when comparing against deactivated dADP in absence of interconnectivity are: a) < 0.05, b) > 0.05, c) < 0.001. The most prominent change is that of the Up states' duration, which also led to the decrease of their frequency. It seems that the lack of inhibition combined with a more depolarized state of the neuron can prolong significantly the Up states.

As a final test we combined two previous conditions: dADP activated & number of background excitatory synapses = 40 (purple trace in fig. 13). The activation of the dADP wasn't enough to generate Up states. Of all the conditions this one shows more clearly the impact of

interconnectivity. Compared to the outcome of the same manipulations in the intact network where we saw sustained activity with high firing frequency, this time the model neuron fired a few spikes without showing any sings of Up states. Another interesting finding concerns the membrane potential. Although the dADP mechanism was activated the model neuron wasn't more depolarized as in all previous cases, and it also failed to maintain the depolarizing plateau. Taken together, these results suggest that the inter-connectivity between pyramidal neurons is required for the maintenance of the depolarizing plateau of Up states when the background excitation is low and for controlling runaway excitation.

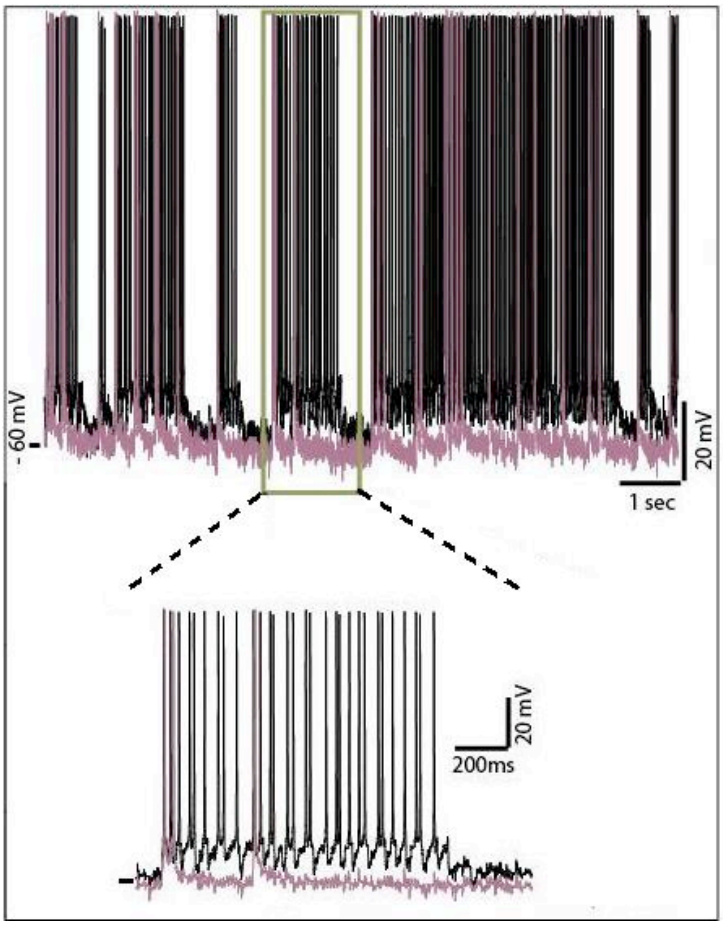


Figure 13. A representative trace were dADP is activated (4mV), and 40 background excitatory synapses and 45 inhibitory synapses are active and all pyramidal neurons are interconnected (black) overlaid with a trace with the same parameters from one of the pyramidal neurons in the microcircuit, in the absence of interconnectivity (so the number of inhibitory connections isn't important) (purple). In this condition the impact of interconnectivity is most prominent, with its absence resulting in no Up states.

8. Coefficient of variation of Up states interspike intervals

Another characteristic of Up states is a high variability in the interspike intervals (ISI) (Shu *et al.,*2003 report an average coefficient of variation (CV) = 1.74). The following table (Table 6) summarizes the interspike intervals' coefficient of variation in our model, for the control and important conditions with high firing frequency. All interspike intervals that were greater than 500ms were not used in the calculation of the CV.

The ISIs of the Up states in the control condition were highly variable (CV = 1.36). As a matter of fact the CV was larger than one in most of the cases. The smallest CV values were for the conditions where the dADP mechanism was activated and the iNMDA-to-iAMPA ratio was 1.25 (CV = 1.05) and 1.5 (CV = 0.66).

Table 6: Coefficient of Variation for different conditions

Condition	CV
control	1.36
frequency of excitatory background activity = 5 Hz	1.49
frequency of excitatory background activity = 10 Hz	1.64
frequency of excitatory background activity = 16.4 Hz	1.50
number of activated background excitatory synapses = 280, inhibitory = 180	1.60
iNMDA-to-iAMPA = 1.25	1.41
iNMDA-to-iAMPA = 1.5	1.31
dADP activated (4mV)	1.70
dADP (4mV) & iNMDA-to-iAMPA = 1.25	1.05
dADP (4mV) & iNMDA-to-iAMPA = 1.5	0.66
dADP (4mV) & number of activated background excitatory synapses = 40, inhibitory = 45	1.78
dADP (4mV) & iNMDA-to-iAMPA = 1.25 & frequency of excitatory background activity to the interneuron = 0.9	1.27

The two following tables summarize the results and p-values for all conditions tested in this work.

Table 7. A summary of all the simulated conditions and their corresponding average values (± values: standard deviation) for Up states frequency, firing frequency during Up states and Up states duration. Statistical significance is indicated for conditions compared with the control (for the Kruskal-Wallis tests between other conditions refer to Table 8). ***: p-value < 0.001, **: p-value < 0.01, *: p-value < 0.05, - : not statistically significant.

#	Simulated Conditions	Up frequency (Hz)	Firing Frequency (Hz)	Up Duration (ms)
1	control	0.17 ± 0.05	16.72 ± 3.04	688.88 ± 169.75
2	absence of interconnectivity	0.23 ± 0.06 *	26.66 ± 4.56 ***	692.21 ± 161.48 -
3	I_{CaL}	0.14 ± 0.06 -	17.84 ± 3.37 -	688.31 ± 172.18 -
4	I_{CaL} in absence of interconnectivity	0.2 ± 0.06	32.43 ± 6.24	655.07 ± 156.96
5	I_D	0.41 ± 0.07 ***	65.97 ± 5.75 ***	705.66 ± 193.66 -
6	sAHP	0.23 ± 0.06 *	27.91 ± 5.16 ***	694.7 ± 203.06 -

7	frequency of excitatory background activity = 5 Hz	0.46 ± 0.08 ***	21.43 ± 3.71 ***	742.64 ± 236.51 -
8	frequency of excitatory background activity = 10 Hz	0.53 ± 0.07 ***	33.48 ± 6.22 ***	1520.5 ± 996.85 ***
9	frequency of excitatory background activity = 16.4Hz	0.46 ± 0.06 ***	43.59 ± 7.66 ***	1897.55 ± 1194.61 ***
10	frequency of excitatory background activity = 2Hz	0.01 ± 0.02 ***	14.64 ± 2.63 -	580.75 ± 66.43 -
11	frequency of excitatory background activity = 10 Hz & number of activated background excitatory synapses = 40, inhibitory = 45	0.63 ± 0.12	11.74 ± 2.23	1012.66 ± 576.46
12	number of activated background excitatory synapses = 40, inhibitory = 45	0.07 ± 0.05 **	7.28 ± 1.30 ***	662.27 ± 158.02 -
13	number of background excitatory synapses 40, in absence of interconnectivity	No Up states	-	-
14	number of activated background excitatory synapses = 280, inhibitory = 180	0.28 ± 0.05 ***	25.29 ± 4.62 ***	703.54 ± 195.55 -
15	Reduced frequency of membrane fluctuations by 20%	0.17 ± 0.06 -	16.43 ± 2.67 -	699.01 ± 174.79 -
16	iNMDA-to-iAMPA = 1.25	0.29 ± 0.05 ***	18.27 ± 3.27 **	728.52 ± 194.76 -
17	iNMDA-to-iAMPA = 1.5	0.49 ± 0.08 ***	20.94 ± 4.16 ***	1202.24 ± 1020.38 ***
18	dADP activated (4mV)	0.53 ± 0.05 ***	27.11 ± 7.58 ***	1302.29 ± 781.36 ***
19	dADP (4mV) & iNMDA-to-iAMPA = 1.25	0.18 ± 0.09	36.67 ± 12.59	5182.92 ± 6820.08
20	dADP (4mV) & iNMDA-to-iAMPA = 1.5	0.04 ± 0.01	51.96 ± 14.27	24492.68 ± 10769.49
21	dADP (4mV) & iNMDA-to-iAMPA = 1.25 & frequency of excitatory background activity to the interneuron = 0.9	0.22 ± 0.08	35.15 ± 11.00	4186.31 ± 4797.61
22	dADP (4mV) in absence of interconnectivity	0.16 ± 0.07	26.27 ± 11.04	6039.91 ± 5793.65
23	dADP (4mV) & number of activated background excitatory synapses = 40, inhibitory = 45	0.14 ± 0.05	19.13 ± 12.64	5601.16 ± 7292.91
24	dADP (4mV) & number of background excitatory synapses = 40, in absence of interconnectivity	No Up states	-	-
25	20% increase in the amplitude of membrane fluctuations	0.17 ± 0.05 -	16.42 ± 2.6 -	699.01 ± 174.79 -
26	Blocking of MNDARs and compensate excitability by increasing activity of	No Up states	-	-

	AMPAR			
27	Blocking of MNDARs and compensate excitability by increasing activity of AMPAR in absence of interconnectivity	No Up states	-	-

Table 8. Statistical significance of the differences observed between conditions tested against each other (but not with the control condtion) with a Kruskal-Wallis test. ***: p-value < 0.001, **: p-value < 0.01, *: p-value < 0.05, - : not statistically significant.

Conditions tested		**Up frequency**	**Firing frequency**	**Up duration**
ICaL in absence of interconnectivity	ICaL	-	***	-
	absence of interconnectivity	-	***	-
frequency of excitatory background activity = 10 Hz	frequency of excitatory background activity = 10 Hz & number of activated background excitatory synapses = 40, inhibitory = 45	-	***	***
dADP (4mV) in absence of interconnectivity	dADP(4mV)	***	-	***
	absence of interconnectivity	*	-	***
dADP(4mV) & number of activated background excitatory synapses = 40, inhibitory = 45	dADP(4mV)	***	***	*
	number of activated synapses excitatory = 40, inhibitory = 45	**	***	***
dADP (4mV) & iNMDA-to-iAMPA = 1.25	dADP (4mV)	***	***	***
	iNMDA-to-iAMPA = 1.25	**	***	***
	dADP (4mV) & iNMDA-to-iAMPA = 1.25 & frequency of excitatory background activity to the interneuron = 0.9	-	-	-
dADP (4mV) & iNMDA-to-iAMPA = 1.5	dADP (4mV)	***	***	***
	iNMDA-to-iAMPA = 1.5	***	***	***

4. Discussion

In this study we used a biophysical microcircuit model of PFC layer V neurons to investigate the impact of various parameters in the generation and properties of Up and Down states, as well as to find a possible link with the generation of sustained firing activity observed during working memory tasks. With the exception of the model created by Compte *et al.* (2003), models appearing so far on this subject mainly used a large number of non-linear integrate and fire neurons (Parga & Abbott, 2007; Destexhe, 2009; Ngo *et al.,* 2010). We used a small network, therefore we did not examine the dynamics of the wave propagation. Instead we chose to create a biologically plausible model based on anatomical and electrophysiological data by integrating many biophysical properties (such as anatomical connections, intrinsic and synaptic mechanisms) and incorporate stochastic events (eg. the membrane noise, the opening and closing of intrinsic channels, the latencies of the connections).

The mechanisms that have been proposed to be involved with the emergence and characteristics of Up and Down states are the intrinsic makeup of layer V pyramidal neurons, the properties of the local network and synaptic background activity. Because the exact role of each mechanism isn't clear we incorporated all three mechanisms in our model. Although there is evidence supporting the emergence of Up states in a small number of cells (Cossart *et al.,* 2003), it was not certain that our model would replicate that. However it exhibited spontaneous generation of Up and Down states, with characteristics that closely resembled the ones reported by McCormick *et al.* (2003) from their *in vitro* recordings in PFC slices. The difference of the membrane potential between the Down and Up states in our model was ~15 mV – other reported values include 10 mV (Destexhe *et al.,* 2003), 12 mV (Cossart *et al.,* 2003), 7.6±2.1 mV (McCormick *et al.,* 2003), ~12 mV (Sanchez-Vivez *et al.,* 2000) and ~20mV (Seamans *et al.,* 2003). The next step was to modify specific components of these mechanisms and see their effect on Up and Down states.

The frequency of Up states was influenced by the: i) frequency of the excitatory background activity, ii) strength of the excitatory background activity, iii) iNDMA-to-iAMPA ratio, iv) I_D (D-type potassium current), v) sAHP and vi) activation of the dADP mechanism (delayed afterdepolarization). *The firing frequency during Up states was effected by the*: i) strength of the excitatory background activity, ii) frequency of excitatory background activity, iii) iNDMA-to-iAMPA ratio, iv) I_D (epileptiform activity), v) sAHP, vi) dADP, vii) reverberation. *The duration of the Up states increased by*: i) increasing the frequency of the excitatory background activity, ii) increasing the iNMDA-to-iAMPA ratio, iii) activating the dADP, iv) reducing the background excitation and activating the dADP. *The depolarizing plateau of Up states was abolished when*: i) we deactivated the NMDA receptors and ii) when we removed the network's reverberation and reduced the background excitation. Of all the parameters we investigated, the frequency of the

background synaptic excitation was the only one capable of altering the time of Up and Down states' emergence. Therefore network, intrinsic and synaptic mechanisms all contribute to shape the characteristics of Up and Down states. Our model also replicates the high variability of interspike intervals (ISIs) during the Up states (CV=1.74 according to Shu *et al.,* 2003). In the control condition the ISIs coefficient of variation was 1.36, indicating that the firing activity depends on more than one parameters, in accordance with our conclusions from the manipulations we performed.

Further support for our model arises from the fact that the smallest number of activated background excitation synapses with which we would observe even a small number of depolarizing plateaus lasting more than 500ms was 40. This is consistent with the results of Gasparini *et al.* (2004) according to which the pyramidal neuron needs only a set of less than 50 active synaptic contacts to trigger a regenerative dendritic response. This implies that thousand of synaptic inputs need not be active for the generation of a plateau potential that would propagate to the soma and change a Down state into an Up state. On the contrary, the activation from afferents carrying relevant informational content, especially if they are spatially and temporally clustered (Poirazi & Mel, 2001), is sufficient to generate an Up state, supporting the theory that individual dendritic branches may serve as independent computational units (Segev & Rall, 1998; Poirazi *et al.,* 2003a,b; London & Häusser, 2005; Branco & Häusser, 2011; Sheffield *et al.* 2011).

When we deactivated the current mediated by NMDA receptors (while compensating the loss of excitability by increasing correspondingly the AMPA current), the depolarizing plateau of Up states was completely abolished, although the pyramidal neurons continued to fire at a lower frequency. Therefore the NMDA plateau is crucial in maintaining an Up state and when we also take into account its effect on the firing frequency we can assume that NMDA receptors contribute to the rate control of the firing activity and thus cortical information processing as proposed by Antic *et al.* (2010) and Compte (2006).

Seeing how activity during Up states has similar characteristics with the awake state, where the pyramidal neurons are constantly depolarized and fire with an average frequency of 10 Hz (Destexhe *et al.,* 2003; Boustani *et al.*, 2007), we can hypothesize that the sustained firing observed during working memory tasks uses the same mechanisms. Stimulation of neuromodulatory inputs providing cholingergic or dopaminergic input to cortex have been shown to produce sustained activity much more prolonged than those occurring spontaneously, sometimes lasting tens of seconds (Steriade *et al.*, 1993; Lewis & O'Donnell, 2000). Because dopamine is released during working memory tasks, we tried to simulate its neuromodulatory effect by increasing the iNDMA-to-iAMPA ratio (Seamans *et al.*, 2001), activating the dADP mechanism (Sidiropoulou *et al.*, 2009)

and finally by combining both conditions. In all cases our model was able to generate sustained activity, and particularly when the iNDMA-to-iAMPA ratio was 1.5 and the dADP mechanism was activated (resulting in a 4mV depolarization of the soma) the sustained activity lasted for more than 25 seconds. Therefore our original assumption that Up states can turn into sustained firing given the appropriate conditions is supported by these findings, revealing a common basis for the two phenomena.

We also deactivated various intrinsic currents (I_D, sAHP, I_{CaL}). Counterintuitively, deactivation of the I_{CaL} had no statistically significant impact. However deactivation of the I_D resulted in an epileptiform activity, supporting the argument that I_D may shape the neuronal membrane potential (Wilson, 1992; Nisenbaum *et al*., 1994; Wilson *et al.*, 1996).

The most interesting and counterintuitive result of our study was the generation of Up and Down states in the absence of a reverbatory network. The significance of the balance between excitation and inhibition has been pointed out in many studies, mainly because Up states were always associated with a synaptic bombardment of EPSPs and IPSPs. However, in absence of reverberation and all other parameters as in the control condition, we observed Up and Down states, with a higher firing frequency (26.66±4.56Hz as opposed to 16.72±3.04), presumably due to the lack of inhibition. Thus provided a strong background excitation, the neurons are able to show Up and Down states. On the contrary, when we reduced the strength of the background excitation (40 activated synapses instead of 180) the depolarizing plateau generated after a spike was abolished and this was the case even if the dADP mechanism was activated. On the contrary when the network was intact and the dADP was activated, a reduced background excitation lead to sustained activity. Overall, we propose that the reverberation is necessary for the generation of the Up state, particularly when the background excitation is low. Additionally what can also be seen from these results is that when the balance of excitation and inhibition is perturbed Up states can turn into sustained activity.

McCormick *et al.* (2003), reported low firing during the Down state. This suggests that these neurons receive sufficient input to generate a few spikes, however they fail to generate Up states. We can assume that the adjacent neurons receive similar input, therefore they potentially could also fire an action potential. However they don't, possibly because they are still in a refractory period, unable to generate spikes. As a consequence, during the Down state, the neuron doesn't receive enough excitation form the recurrent connections to generate an Up state. This explanation agrees with our conclusion, that when the background excitation is low, the recurrent connections are necessary to generate an Up state.

A next step would be to investigate the role of inhibition by removing the inhibitory connections to the pyramidal neurons while leaving their interconnections intact. The most probable outcome would be either runaway excitation, suggesting that inhibition is required for stable Up and Down states or a more moderately altered firing frequency, which would imply that the inhibition is necessary for the fine-tuning of the pyramidal neurons firing. Another issue we can address is to provide current injections during the Up and the Down state and see if we can replicate the results of McCormick *et al.* (2003).

In summary, this work presents the first model microcircuit of 5 PFC layer V neurons that is able to support the emergence of Up and Down states whose characteristics resemble those of the in vitro recordings. Our findings include several experimentally testable predictions that shed new light on the mechanisms underlying the Up and Down states phenomenon and its strong link with sustained firing observed during working memory tasks.

5. References

Amaral, D.G. (2000) The anatomical organization of the central nervous system. In ER, K. (ed) *Principles of neural science*. McGraw-Hill, pp. 317-336.

Antic, S.D., Zhou, W.L., Moore, A.R., Short, S.M. & Ikonomu, K.D. (2010) The decade of the dendritic NMDA spike. *Journal of neuroscience research*, **88**, 2991-3001.

Bacon, S.J., Headlam, A.J., Gabbott, P.L. & Smith, A.D. (1996) Amygdala input to medial prefrontal cortex (mPFC) in the rat: a light and electron microscope study. *Brain research*, **720**, 211-219.

Barbas, H. (2000) Connections underlying the synthesis of cognition, memory, and emotion in primate prefrontal cortices. *Brain research bulletin*, **52**, 319-330.

Barbas, H. & Zikopoulos, B. (2007) The prefrontal cortex and flexible behavior. *Neuroscientist*, **13**, 532-545.

Boustani, S., Pospischil, M., Rudolph-Lilith, M. & Destexhe, A. (2007) Activated cortical states: experiments, analyses and models. *Journal of physiology, Paris*, **101**, 99-109.

Branchereau, P., Van Bockstaele, E.J., Chan, J. & Pickel, V.M. (1996) Pyramidal neurons in rat prefrontal cortex show a complex synaptic response to single electrical stimulation of the locus coeruleus region: evidence for antidromic activation and GABAergic inhibition using in vivo intracellular recording and electron microscopy. *Synapse (New York, NY)*, **22**, 313-331.

Branco, T. & Haeusser, M. (2011) Synaptic Integration Gradients in Single Cortical Pyramidal Cell Dendrites. *Neuron*, **69**, 885-892.

Carmichael, S.T. & Price, J.L. (1995) Sensory and premotor connections of the orbital and medial prefrontal cortex of macaque monkeys. *The Journal of comparative neurology*, **363**, 642-664.

Cash, S. & Yuste, R. (1999) Linear summation of excitatory inputs by CA1 pyramidal neurons. *Neuron*, **22**, 383-394.

Cerqueira, J.J., Almeida, O.F.X. & Sousa, N. (2008) The stressed prefrontal cortex. Left? Right! *Brain Behav Immun*, **22**, 630-638.

Chauvette, S., Volgushev, M. & Timofeev, I. (2010) Origin of Active States in Local Neocortical Networks during Slow Sleep Oscillation. *Cerebral cortex (New York, NY : 1991)*, **20**, 2660-2674.

Compte, A. (2006) Computational and in vitro studies of persistent activity: edging towards cellular and synaptic mechanisms of working memory. *Neuroscience*, **139**, 135-151.

Compte, A., Brunel, N., Goldman-Rakic, P.S. & Wang, X.J. (2000) Synaptic mechanisms and network dynamics underlying spatial working memory in a cortical network model. *Cerebral cortex (New York, NY : 1991)*, **10**, 910-923.

Compte, A., Sanchez-Vives, M.V., McCormick, D.A. & Wang, X.J. (2003) Cellular and network mechanisms of slow oscillatory activity (<1 Hz) and wave propagations in a cortical network model. *Journal of neurophysiology*, **89**, 2707-2725.

Contreras, D., Timofeev, I. & Steriade, M. (1996) Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. *The Journal of physiology*, **494 (Pt 1)**, 251-264.

Cossart, R., Aronov, D. & Yuste, R. (2003) Attractor dynamics of network UP states in the neocortex. *Nature*, **423**, 283-288.

Destexhe, A. (2009) Self-sustained asynchronous irregular states and Up-Down states in thalamic, cortical and thalamocortical networks of nonlinear integrate-and-fire neurons. *Journal of computational neuroscience*, **27**, 493-506.

Destexhe, A. & Paré, D. (1999) Impact of network activity on the integrative properties of neocortical pyramidal neurons in vivo. *Journal of neurophysiology*, **81**, 1531-1547.

Destexhe, A., Rudolph, M. & Paré, D. (2003) The high-conductance state of neocortical neurons in vivo. *Nature reviews Neuroscience*, **4**, 739-751.

Dodt, H.U., Frick, A., Kampe, K. & Zieglgänsberger, W. (1998) NMDA and AMPA receptors on neocortical neurons are differentially distributed. *The European journal of neuroscience*, **10**, 3351-3357.

Doiron, B., Noonan, L., Lemon, N. & Turner, R.W. (2003) Persistent Na+ current modifies burst discharge by regulating conditional backpropagation of dendritic spikes. *Journal of neurophysiology*, **89**, 324-337.

Dombrowski, S.M., Hilgetag, C.C. & Barbas, H. (2001) Quantitative architecture distinguishes prefrontal cortical systems in the rhesus monkey. *Cerebral cortex (New York, NY : 1991)*, **11**, 975-988.

Durstewitz, D., Seamans, J.K. & Sejnowski, T.J. (2000) Neurocomputational models of working memory. *Nature neuroscience*, **3 Suppl**, 1184-1191.

Egorov, A.V., Hamam, B.N., Fransén, E., Hasselmo, M.E. & Alonso, A.A. (2002) Graded persistent activity in entorhinal cortex neurons. *Nature*, **420**, 173-178.

Erreger, K., Dravid, S.M., Banke, T.G., Wyllie, D.J. & Traynelis, S.F. (2005) Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. *The Journal of physiology*, **563**, 345-358.

Gabbott, P.L., Warner, T.A. & Busby, S.J. (2006) Amygdala input monosynaptically innervates parvalbumin immunoreactive local circuit neurons in rat medial prefrontal cortex. *Neuroscience*, **139**, 1039-1048.

Gasparini, S., Migliore, M. & Magee, J.C. (2004) On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **24**, 11046-11056.

Goldman-Rakic, P.S. (1995) Cellular basis of working memory. *Neuron*, **14**, 477-485.

Golomb, D. & Ermentrout, G.B. (2001) Bistability in pulse propagation in networks of excitatory and inhibitory populations. *Physical review letters*, **86**, 4179-4182.

Gorelova, N.A. & Yang, C.R. (2000) Dopamine D1/D5 receptor activation modulates a persistent sodium current in rat prefrontal cortical neurons in vitro. *Journal of neurophysiology*, **84**, 75-87.

Hines, M.L. & Carnevale, N.T. (1997) The NEURON simulation environment. *Neural Computation*, **9**, 1179-1209.

Hines, M.L., Morse, T., Migliore, M., Carnevale, N.T. & Shepherd, G.M. (2004) ModelDB: A Database to Support Computational Neuroscience. *Journal of computational neuroscience*, **17**, 7-11.

Holcman, D. & Tsodyks, M. (2006) The emergence of Up and Down states in cortical networks. *PLoS computational biology*, **2**, e23.

Jackson, M.E., Homayoun, H. & Moghaddam, B. (2004) NMDA receptor hypofunction produces concomitant firing rate potentiation and burst activity reduction in the prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 8467-8472.

Jay, T.M. & Witter, M.P. (1991) Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *The Journal of comparative neurology*, **313**, 574-586.

Kroener, S., Chandler, L.J., Phillips, P.E. & Seamans, J.K. (2009) Dopamine modulates persistent synaptic activity and enhances the signal-to-noise ratio in the prefrontal cortex. *PloS one*, **4**, e6507.

Larkum, M.E., Zhu, J.J. & Sakmann, B. (1999) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature*, **398**, 338-341.

Lewis, B.L. & O'Donnell, P. (2000) Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D(1) dopamine receptors. *Cerebral cortex (New York, NY : 1991)*, **10**, 1168-1175.

Lisman, J.E., Fellous, J.M. & Wang, X.J. (1998) A role for NMDA-receptor channels in working memory. *Nature neuroscience*, **1**, 273-275.

London, M. & Häusser, M. (2005) Dendritic computation. *Annual review of neuroscience*, **28**, 503-532.

Losonczy, A. & Magee, J.C. (2006) Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron*, **50**, 291-307.

Magee, J.C. & Johnston, D. (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science (New York, NY)*, **275**, 209-213.

McCormick, D.A., Shu, Y., Hasenstaub, A., Sanchez-Vives, M., Badoual, M. & Bal, T. (2003) Persistent cortical activity: mechanisms of generation and effects on neuronal excitability. *Cerebral cortex (New York, NY : 1991)*, **13**, 1219-1231.

Miller, E. & Wallis, J. (2008) The prefrontal cortex and executive functions. In Squire, L.R. (ed) *Fundamental Neuroscience*. Elsevier, pp. 1119-1223.

Milojkovic, B.A., Radojicic, M.S., Goldman-Rakic, P.S. & Antic, S.D. (2004) Burst generation in rat pyramidal neurones by regenerative potentials elicited in a restricted part of the basilar dendritic tree. *The Journal of physiology*, **558**, 193-211.

Milojkovic, B.A., Zhou, W.L. & Antic, S.D. (2007) Voltage and calcium transients in basal dendrites of the rat prefrontal cortex. *The Journal of physiology*, **585**, 447-468.

Ngo, H.V., Koehler, J., Mayer, J., Claussen, J.C. & Schuster, H.G. (2010) Triggering up states in all-to-all coupled neurons. *Epl*, **89**, 68002.

Nisenbaum, E.S., Xu, Z.C. & Wilson, C.J. (1994) Contribution of a slowly inactivating potassium current to the transition to firing of neostriatal spiny projection neurons. *Journal of neurophysiology*, **71**, 1174-1189.

Ongür, D. & Price, J.L. (2000) The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral cortex (New York, NY : 1991)*, **10**, 206-219.

Orozco-Cabal, L., Pollandt, S., Liu, J., Vergara, L., Shinnick-Gallagher, P. & Gallagher, J.P. (2006) A novel rat medial prefrontal cortical slice preparation to investigate synaptic transmission from amygdala to layer V prelimbic pyramidal neurons. *Journal of neuroscience methods*, **151**, 148-158.

Papoutsi, A., K., S. & Poirazi, P. (Year) Mechanisms underlying persistent activity in a model PFC microcircuit. BMC Neuroscience. City.

Parga, N. & Abbott, L.F. (2007) Network model of spontaneous activity exhibiting synchronous transitions between up and down States. *Frontiers in neuroscience*, **1**, 57-66.

Petrides, M. (2005) Lateral prefrontal cortex: architectonic and functional organization. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*, **360**, 781-795.

Poirazi, P., Brannon, T. & Mel, B.W. (2003a) Arithmetic of subthreshold synaptic summation in a model CA1 pyramidal cell. *Neuron*, **37**, 977-987.

Poirazi, P., Brannon, T. & Mel, B.W. (2003b) Pyramidal neuron as two-layer neural network. *Neuron*, **37**, 989-999.

Poirazi, P. & Mel, B.W. (2001) Impact of active dendrites and structural plasticity on the memory capacity of neural tissue. *Neuron*, **29**, 779-796.

Polsky, A., Mel, B. & Schiller, J. (2009) Encoding and decoding bursts by NMDA spikes in basal dendrites of layer 5 pyramidal neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **29**, 11891-11903.

Polsky, A., Mel, B.W. & Schiller, J. (2004) Computational subunits in thin dendrites of pyramidal cells. *Nature neuroscience*, **7**, 621-627.

Rall, W. (1959) Branching dendritic trees and motoneuron membrane resistivity. *Experimental neurology*, **1**, 491-527.

Sanchez-Vives, M.V. & McCormick, D.A. (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nature neuroscience*, **3**, 1027-1034.

Schiller, J., Major, G., Koester, H.J. & Schiller, Y. (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. *Nature*, **404**, 285-289.

Seamans, J.K., Durstewitz, D., Christie, B.R., Stevens, C.F. & Sejnowski, T.J. (2001) Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 301-306.

Seamans, J.K., Nogueira, L. & Lavin, A. (2003) Synaptic basis of persistent activity in prefrontal cortex in vivo and in organotypic cultures. *Cerebral cortex (New York, NY : 1991)*, **13**, 1242-1250.

Segev, I. & London, M. (2000) Untangling dendrites with quantitative models. *Science (New York, NY)*, **290**, 744-750.

Segev, I. & Rall, W. (1998) Excitable dendrites and spines: earlier theoretical insights elucidate recent direct observations. *Trends in neurosciences*, **21**, 453-460.

Sheffield, M.E.J., Best, T.K., Mensh, B.D., Kath, W.L. & Spruston, N. (2011) Slow integration leads to persistent action potential firing in distal axons of coupled interneurons. *Nature neuroscience*, **14**, 200-U285.

Shu, Y., Hasenstaub, A. & McCormick, D.A. (2003) Turning on and off recurrent balanced cortical activity. *Nature*, **423**, 288-293.

Sidiropoulou, K., Lu, F.M., Fowler, M.A., Xiao, R., Phillips, C., Ozkan, E.D., Zhu, M.X., White, F.J. & Cooper, D.C. (2009) Dopamine modulates an mGluR5-mediated depolarization underlying prefrontal persistent activity. *Nature neuroscience*, **12**, 190-199.

Steriade, M., Nuñez, A. & Amzica, F. (1993) A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **13**, 3252-3265.

Stuart, G.J. & Sakmann, B. (1994) Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature*, **367**, 69-72.

Thierry, A.M., Gioanni, Y., Dégénétais, E. & Glowinski, J. (2000) Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. *Hippocampus*, **10**, 411-419.

Timofeev, I., Grenier, F. & Steriade, M. (2001) Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 1924-1929.

Tobias, T.J. (1975) Afferents to prefrontal cortex from the thalamic mediodorsal nucleus in the rhesus monkey. *Brain research*, **83**, 191-212.

Tseng, K.Y. & O'Donnell, P. (2005) Post-pubertal emergence of prefrontal cortical up states induced by D1-NMDA co-activation. *Cerebral cortex (New York, NY : 1991)*, **15**, 49-57.

Uylings, H.B., Groenewegen, H.J. & Kolb, B. (2003) Do rats have a prefrontal cortex? *Behavioural brain research*, **146**, 3-17.

Van Eden, C.G., Lamme, V.A. & Uylings, H.B. (1992) Heterotopic Cortical Afferents to the Medial Prefrontal Cortex in the Rat. A Combined Retrograde and Anterograde Tracer Study. *The European journal of neuroscience*, **4**, 77-97.

Wang, H., Stradtman, G.G., Wang, X.J. & Gao, W.J. (2008) A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 16791-16796.

Wang, X.J. (1999) Synaptic basis of cortical persistent activity: the importance of NMDA receptors to working memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **19**, 9587-9603.

Wilson, C.J. (1992) Dendritic morphology, inward rectification, and the functional properties of neostriatal neurons. *Single neuron computation*.

Wilson, C.J. & Kawaguchi, Y. (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **16**, 2397-2410.

Wong, R.K. & Prince, D.A. (1978) Participation of calcium spikes during intrinsic burst firing in hippocampal neurons. *Brain research*, **159**, 385-390.

Yang, C.R. & Seamans, J.K. (1996) Dopamine D1 receptor actions in layers V-VI rat prefrontal cortex neurons in vitro: modulation of dendritic-somatic signal integration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **16**, 1922-1935.

Yang, C.R., Seamans, J.K. & Gorelova, N. (1996) Electrophysiological and morphological properties of layers V-VI principal pyramidal cells in rat prefrontal cortex in vitro. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **16**, 1904-1921.

Zhao, M.G., Toyoda, H., Lee, Y.S., Wu, L.J., Ko, S.W., Zhang, X.H., Jia, Y., Shum, F., Xu, H., Li, B.M., Kaang, B.K. & Zhuo, M. (2005) Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. *Neuron*, **47**, 859-872.

Zikopoulos, B. & Barbas, H. (2007) Parallel driving and modulatory pathways link the prefrontal cortex and thalamus. *PloS one*, **2**, e848.

6. Appendix

Code written in Matlab to:

A) Determine Up states duration, firing frequency and Up states frequency and export results to excel. We run this code for each condition separately.

```
%load soma
 for r=1:10
    k = ['load soma1' int2str(r) '.dat'];
    eval(k)
 end

 %duration of UP states and spike number

thr = -60; %mV threshold for UP
for i=1:10
    s = eval(sprintf('soma1%d', i));
    UP = [];
    temp = [];
    h = 1;

    [a, b] = butter(5, 10/10000); %create filter
    filt = filtfilt(a, b, s);
%     figure;
%     plot(filt);

        for j=1:100

            t1 = find(filt(:,1)>thr, 1, 'first'); % finds time of first spike
            filt = filt(t1:length(filt),:); % remove entries prior to t1

            if j==1, T1=t1;
            else T1=T2 + t1;
            end

            t2 = find(filt(:,1)<thr, 1, 'first'); %finds time of last spike in this
            putative UP
            if isempty(t2), t2 = 299999; T2 = 299999; filt = [];
                else filt = filt(t2:length(filt),:); %remove entries prior to B
                T2 = t2 + T1;
            end

            sp = 0;  % sp: number of spikes
            for l=T1:T2  %find number of spikes in all putative up states
                if (s(l)<0 && s(l+1)>0)
                    sp = sp+1;
                end
            end

            if isempty(t1), break, end

            dt = (T2-T1)/10; %in miliseconds
            T_1 = T1/10000; % in seconds
            T_2 = T2/10000; % in seconds

            %save dt, corresponding  spikes and times in [up]
            if j==1, up=[dt sp T_1 T_2 i];
            else up=[up ; dt sp T_1 T_2 i];
```

```
            end

            e = 1;
            for f=1:length(up(:,1))
                if up(f,1)>500 %discard values <500 ms
                   temp(e,:) = up(f,:);
                   e = e+1;
                end
            end

        %save UP states' duration, spikes, beginning and end in [UP]
          UP = strcat('UP', int2str(i));
          assignin('base', UP, temp);

        if isempty(filt), break, end

        end
end


UPALL = [UP1; UP2; UP3; UP4; UP5; UP6; UP7; UP8; UP9; UP10];

% STATISTICS %

A = size(UPALL);
a1 = A(1,1); %number of UP states in 300sec
up_f = a1/300; % Up frequency

 for i = 1:10
    ss = eval(sprintf('UP%d',i));
    [siz1 siz2] = size(ss);
    up_fi = siz1/30; % Up frequency in each run

    UP_fi = strcat('UP_fi', int2str(i));
    assignin('base', UP_fi, up_fi);
 end

UP_f = [UP_fi1; UP_fi2; UP_fi3; UP_fi4; UP_fi5; UP_fi6; UP_fi7; UP_fi8; UP_fi9;
UP_fi10];
up_f_std = std(UP_f); % standard deviation of Up frequency

B = mean(UPALL);
up_dur = B(1,1); % average UP duration
C = std(UPALL);
up_std = C(1,1); %standard deviation of UP duration

ff = UPALL(:,2)*1000 ./ UPALL(:,1); %firing frequeny of each Up
ff_m = mean(ff); % average firing frequency
ff_std = std(ff); % standard deviation of firing frequency


stat = [up_f, up_f_std, ff_m, ff_std, up_dur, up_std];

final = horzcat(UPALL, ff);

xlswrite('try.xls', final, 'fc_0.4', 'A2');
xlswrite('try.xls', stat, 'fc_0.4', 'J2');
xlswrite('try.xls', UP_f, 'fc_0.4', 'H2');
```

B) Create three excel files (up_freq.xls, firing.xls, duration.xls) and group Up states frequency, firing frequency and Up states duration respectively, for pairs of conditions on which we would perform the Kruskal-Wallis test.

```
%File name: load_xls.m
%load data from all runs.
```

```
control = xlsread('try.xls', 'control');
five_wo = xlsread('try.xls', '0.5_wo');
fourty_w = xlsread('try.xls', '40_w');
fourty_wo = xlsread('try.xls', '40_wo');
ADP_eight_w = xlsread('try.xls', 'ADP_8_w2');
ADP_eight_wo = xlsread('try.xls', 'ADP_8_wo');
ADP_fcin = xlsread('try.xls', 'ADP_15_fcin');
ADP_fifteen_w = xlsread('try.xls', 'ADP_15_w');
ADP_thirtyfive_w = xlsread('try.xls', 'ADP_35_w');
ADP_fourty_w = xlsread('try.xls', 'ADP_40_w');
ADP_fourty_wo = xlsread('try.xls', 'ADP_40_wo');
DA_fifteen = xlsread('try.xls', 'DA_15');
DA_thirtyfive = xlsread('try.xls', 'DA_35');
fc_six = xlsread('try.xls', 'fc_0.6');
fc_eight = xlsread('try.xls', 'fc_0.8');
IKs = xlsread('try.xls', 'IKs');
KCa = xlsread('try.xls', 'KCa');
Ltype = xlsread('try.xls', 'L-type');
Ltype_wo = xlsread('try.xls', 'L-type_wo');
noise = xlsread('try.xls', 'noise');
stim = xlsread('try.xls', 'stim');
syn = xlsread('try.xls', 'syn_280');
fc_eight_fourty = xlsread('try.xls', 'fc_0.8_40');
fc_four = xlsread('try.xls', 'fc_0.4');
fc_one = xlsread('try.xls', 'fc_1.0');
```

```
%file name: up_frequency.m
%UP frequency

%load_xls.m

warning off MATLAB:xlswrite:AddSheet

xlswrite('upfreq.xls', control(:,8), '1', 'A1');
xlswrite('upfreq.xls', five_wo(:,8), '1', 'B1');

xlswrite('upfreq.xls', control(:,8), '2', 'A1');
xlswrite('upfreq.xls', KCa(:,8), '2', 'B1');

xlswrite('upfreq.xls', control(:,8), '3', 'A1');
xlswrite('upfreq.xls', IKs(:,8), '3', 'B1');

xlswrite('upfreq.xls', control(:,8), '4', 'A1');
xlswrite('upfreq.xls', Ltype(:,8), '4', 'B1');

xlswrite('upfreq.xls', Ltype(:,8), '5', 'A1');
xlswrite('upfreq.xls', Ltype_wo(:,8), '5', 'B1');

xlswrite('upfreq.xls', five_wo(:,8), '6', 'A1');
xlswrite('upfreq.xls', Ltype_wo(:,8), '6', 'B1');

xlswrite('upfreq.xls', control(:,8), '7', 'A1');
xlswrite('upfreq.xls', stim(:,8), '7', 'B1');

xlswrite('upfreq.xls', control(:,8), '8', 'A1');
xlswrite('upfreq.xls', syn(:,8), '8', 'B1');

xlswrite('upfreq.xls', control(:,8), '9', 'A1');
xlswrite('upfreq.xls', fc_four(:,8), '9', 'B1');

xlswrite('upfreq.xls', control(:,8), '10', 'A1');
xlswrite('upfreq.xls', fc_six(:,8), '10', 'B1');

xlswrite('upfreq.xls', control(:,8), '11', 'A1');
xlswrite('upfreq.xls', fc_eight(:,8), '11', 'B1');
```

```
xlswrite('upfreq.xls', control(:,8), '12', 'A1');
xlswrite('upfreq.xls', fc_one(:,8), '12', 'B1');

xlswrite('upfreq.xls', fc_eight(:,8), '13', 'A1');
xlswrite('upfreq.xls', fc_eight_fourty(:,8), '13', 'B1');

xlswrite('upfreq.xls', control(:,8), '14', 'A1');
xlswrite('upfreq.xls', noise(:,8), '14', 'B1');

xlswrite('upfreq.xls', control(:,8), '15', 'A1');
xlswrite('upfreq.xls', fourty_w(:,8), '15', 'B1');

xlswrite('upfreq.xls', fourty_w(:,8), '16', 'A1');
xlswrite('upfreq.xls', fourty_wo(:,8), '16', 'B1');

xlswrite('upfreq.xls', five_wo(:,8), '17', 'A1');
xlswrite('upfreq.xls', fourty_wo(:,8), '17', 'B1');

xlswrite('upfreq.xls', control(:,8), '18', 'A1');
xlswrite('upfreq.xls', ADP_eight_w(:,8), '18', 'B1');

xlswrite('upfreq.xls', ADP_eight_w(:,8), '19', 'A1');
xlswrite('upfreq.xls', ADP_eight_wo(:,8), '19', 'B1');

xlswrite('upfreq.xls', ADP_eight_w(:,8), '20', 'A1');
xlswrite('upfreq.xls', ADP_fourty_w(:,8), '20', 'B1');

xlswrite('upfreq.xls', ADP_fourty_w(:,8), '21', 'A1');
xlswrite('upfreq.xls', ADP_fourty_wo(:,8), '21', 'B1');

xlswrite('upfreq.xls', ADP_fourty_w(:,8), '22', 'A1');
xlswrite('upfreq.xls', fourty_w(:,8), '22', 'B1');

xlswrite('upfreq.xls', ADP_fourty_wo(:,8), '23', 'A1');
xlswrite('upfreq.xls', ADP_eight_wo(:,8), '23', 'B1');

xlswrite('upfreq.xls', ADP_fourty_wo(:,8), '24', 'A1');
xlswrite('upfreq.xls', fourty_wo(:,8), '24', 'B1');

xlswrite('upfreq.xls', five_wo(:,8), '25', 'A1');
xlswrite('upfreq.xls', ADP_eight_wo(:,8), '25', 'B1');

xlswrite('upfreq.xls', control(:,8), '26', 'A1');
xlswrite('upfreq.xls', DA_fifteen(:,8), '26', 'B1');

xlswrite('upfreq.xls', control(:,8), '27', 'A1');
xlswrite('upfreq.xls', DA_thirtyfive(:,8), '27', 'B1');

xlswrite('upfreq.xls', ADP_fifteen_w(:,8), '28', 'A1');
xlswrite('upfreq.xls', ADP_eight_w(:,8), '28', 'B1');

xlswrite('upfreq.xls', ADP_fifteen_w(:,8), '29', 'A1');
xlswrite('upfreq.xls', DA_fifteen(:,8), '29', 'B1');

xlswrite('upfreq.xls', ADP_fifteen_w(:,8), '30', 'A1');
xlswrite('upfreq.xls', ADP_fcin(:,8), '30', 'B1');

xlswrite('upfreq.xls', ADP_thirtyfive_w(:,8), '31', 'A1');
xlswrite('upfreq.xls', ADP_eight_w(:,8), '31', 'B1');

xlswrite('upfreq.xls', ADP_thirtyfive_w(:,8), '32', 'A1');
xlswrite('upfreq.xls', DA_thirtyfive(:,8), '32', 'B1');
```

```
%file name: firing_freq.m
%Firing frequency
```

```
%load_xls.m

warning off MATLAB:xlswrite:AddSheet

xlswrite('firing.xls', control(:,6), '1', 'A1');
xlswrite('firing.xls', five_wo(:,6), '1', 'B1');

xlswrite('firing.xls', control(:,6), '2', 'A1');
xlswrite('firing.xls', KCa(:,6), '2', 'B1');

xlswrite('firing.xls', control(:,6), '3', 'A1');
xlswrite('firing.xls', IKs(:,6), '3', 'B1');

xlswrite('firing.xls', control(:,6), '4', 'A1');
xlswrite('firing.xls', Ltype(:,6), '4', 'B1');

xlswrite('firing.xls', Ltype(:,6), '5', 'A1');
xlswrite('firing.xls', Ltype_wo(:,6), '5', 'B1');

xlswrite('firing.xls', five_wo(:,6), '6', 'A1');
xlswrite('firing.xls', Ltype_wo(:,6), '6', 'B1');

xlswrite('firing.xls', control(:,6), '7', 'A1');
xlswrite('firing.xls', stim(:,6), '7', 'B1');

xlswrite('firing.xls', control(:,6), '8', 'A1');
xlswrite('firing.xls', syn(:,6), '8', 'B1');

xlswrite('firing.xls', control(:,6), '9', 'A1');
xlswrite('firing.xls', fc_four(:,6), '9', 'B1');

xlswrite('firing.xls', control(:,6), '10', 'A1');
xlswrite('firing.xls', fc_six(:,6), '10', 'B1');

xlswrite('firing.xls', control(:,6), '11', 'A1');
xlswrite('firing.xls', fc_eight(:,6), '11', 'B1');

xlswrite('firing.xls', control(:,6), '12', 'A1');
xlswrite('firing.xls', fc_one(:,6), '12', 'B1');

xlswrite('firing.xls', fc_eight(:,6), '13', 'A1');
xlswrite('firing.xls', fc_eight_fourty(:,6), '13', 'B1');

xlswrite('firing.xls', control(:,6), '14', 'A1');
xlswrite('firing.xls', noise(:,6), '14', 'B1');

xlswrite('firing.xls', control(:,6), '15', 'A1');
xlswrite('firing.xls', fourty_w(:,6), '15', 'B1');

xlswrite('firing.xls', fourty_w(:,6), '16', 'A1');
xlswrite('firing.xls', fourty_wo(:,6), '16', 'B1');

xlswrite('firing.xls', five_wo(:,6), '17', 'A1');
xlswrite('firing.xls', fourty_wo(:,6), '17', 'B1');

xlswrite('firing.xls', control(:,6), '18', 'A1');
xlswrite('firing.xls', ADP_eight_w(:,6), '18', 'B1');

xlswrite('firing.xls', ADP_eight_w(:,6), '19', 'A1');
xlswrite('firing.xls', ADP_eight_wo(:,6), '19', 'B1');

xlswrite('firing.xls', ADP_eight_w(:,6), '20', 'A1');
xlswrite('firing.xls', ADP_fourty_w(:,6), '20', 'B1');

xlswrite('firing.xls', ADP_fourty_w(:,6), '21', 'A1');
xlswrite('firing.xls', ADP_fourty_wo(:,6), '21', 'B1');

xlswrite('firing.xls', ADP_fourty_w(:,6), '22', 'A1');
```

```
xlswrite('firing.xls', fourty_w(:,6), '22', 'B1');

xlswrite('firing.xls', ADP_fourty_wo(:,6), '23', 'A1');
xlswrite('firing.xls', ADP_eight_wo(:,6), '23', 'B1');

xlswrite('firing.xls', ADP_fourty_wo(:,6), '24', 'A1');
xlswrite('firing.xls', fourty_wo(:,6), '24', 'B1');

xlswrite('firing.xls', five_wo(:,6), '25', 'A1');
xlswrite('firing.xls', ADP_eight_wo(:,6), '25', 'B1');

xlswrite('firing.xls', control(:,6), '26', 'A1');
xlswrite('firing.xls', DA_fifteen(:,6), '26', 'B1');

xlswrite('firing.xls', control(:,6), '27', 'A1');
xlswrite('firing.xls', DA_thirtyfive(:,6), '27', 'B1');

xlswrite('firing.xls', ADP_fifteen_w(:,6), '28', 'A1');
xlswrite('firing.xls', ADP_eight_w(:,6), '28', 'B1');

xlswrite('firing.xls', ADP_fifteen_w(:,6), '29', 'A1');
xlswrite('firing.xls', DA_fifteen(:,6), '29', 'B1');

xlswrite('firing.xls', ADP_fifteen_w(:,6), '30', 'A1');
xlswrite('firing.xls', ADP_fcin(:,6), '30', 'B1');

xlswrite('firing.xls', ADP_thirtyfive_w(:,6), '31', 'A1');
xlswrite('firing.xls', ADP_eight_w(:,6), '31', 'B1');

xlswrite('firing.xls', ADP_thirtyfive_w(:,6), '32', 'A1');
xlswrite('firing.xls', DA_thirtyfive(:,6), '32', 'B1');
```

```
%file name: duration.m
%UP duration

%load_xls.m

warning off MATLAB:xlswrite:AddSheet

xlswrite('duration.xls', control(:,1), '1', 'A1');
xlswrite('duration.xls', five_wo(:,1), '1', 'B1');

xlswrite('duration.xls', control(:,1), '2', 'A1');
xlswrite('duration.xls', KCa(:,1), '2', 'B1');

xlswrite('duration.xls', control(:,1), '3', 'A1');
xlswrite('duration.xls', IKs(:,1), '3', 'B1');

xlswrite('duration.xls', control(:,1), '4', 'A1');
xlswrite('duration.xls', Ltype(:,1), '4', 'B1');

xlswrite('duration.xls', Ltype(:,1), '5', 'A1');
xlswrite('duration.xls', Ltype_wo(:,1), '5', 'B1');

xlswrite('duration.xls', five_wo(:,1), '6', 'A1');
xlswrite('duration.xls', Ltype_wo(:,1), '6', 'B1');

xlswrite('duration.xls', control(:,1), '7', 'A1');
xlswrite('duration.xls', stim(:,1), '7', 'B1');

xlswrite('duration.xls', control(:,1), '8', 'A1');
xlswrite('duration.xls', syn(:,1), '8', 'B1');

xlswrite('duration.xls', control(:,1), '9', 'A1');
xlswrite('duration.xls', fc_four(:,1), '9', 'B1');
```

```
xlswrite('duration.xls', control(:,1), '10', 'A1');
xlswrite('duration.xls', fc_six(:,1), '10', 'B1');

xlswrite('duration.xls', control(:,1), '11', 'A1');
xlswrite('duration.xls', fc_eight(:,1), '11', 'B1');

xlswrite('duration.xls', control(:,1), '12', 'A1');
xlswrite('duration.xls', fc_one(:,1), '12', 'B1');

xlswrite('duration.xls', fc_eight(:,1), '13', 'A1');
xlswrite('duration.xls', fc_eight_fourty(:,1), '13', 'B1');

xlswrite('duration.xls', control(:,1), '14', 'A1');
xlswrite('duration.xls', noise(:,1), '14', 'B1');

xlswrite('duration.xls', control(:,1), '15', 'A1');
xlswrite('duration.xls', fourty_w(:,1), '15', 'B1');

xlswrite('duration.xls', fourty_w(:,1), '16', 'A1');
xlswrite('duration.xls', fourty_wo(:,1), '16', 'B1');

xlswrite('duration.xls', five_wo(:,1), '17', 'A1');
xlswrite('duration.xls', fourty_wo(:,1), '17', 'B1');

xlswrite('duration.xls', control(:,1), '18', 'A1');
xlswrite('duration.xls', ADP_eight_w(:,1), '18', 'B1');

xlswrite('duration.xls', ADP_eight_w(:,1), '19', 'A1');
xlswrite('duration.xls', ADP_eight_wo(:,1), '19', 'B1');

xlswrite('duration.xls', ADP_eight_w(:,1), '20', 'A1');
xlswrite('duration.xls', ADP_fourty_w(:,1), '20', 'B1');

xlswrite('duration.xls', ADP_fourty_w(:,1), '21', 'A1');
xlswrite('duration.xls', ADP_fourty_wo(:,1), '21', 'B1');

xlswrite('duration.xls', ADP_fourty_w(:,1), '22', 'A1');
xlswrite('duration.xls', fourty_w(:,1), '22', 'B1');

xlswrite('duration.xls', ADP_fourty_wo(:,1), '23', 'A1');
xlswrite('duration.xls', ADP_eight_wo(:,1), '23', 'B1');

xlswrite('duration.xls', ADP_fourty_wo(:,1), '24', 'A1');
xlswrite('duration.xls', fourty_wo(:,1), '24', 'B1');

xlswrite('duration.xls', five_wo(:,1), '25', 'A1');
xlswrite('duration.xls', ADP_eight_wo(:,1), '25', 'B1');

xlswrite('duration.xls', control(:,1), '26', 'A1');
xlswrite('duration.xls', DA_fifteen(:,1), '26', 'B1');

xlswrite('duration.xls', control(:,1), '27', 'A1');
xlswrite('duration.xls', DA_thirtyfive(:,1), '27', 'B1');

xlswrite('duration.xls', ADP_fifteen_w(:,1), '28', 'A1');
xlswrite('duration.xls', ADP_eight_w(:,1), '28', 'B1');

xlswrite('duration.xls', ADP_fifteen_w(:,1), '29', 'A1');
xlswrite('duration.xls', DA_fifteen(:,1), '29', 'B1');

xlswrite('duration.xls', ADP_fifteen_w(:,1), '30', 'A1');
xlswrite('duration.xls', ADP_fcin(:,1), '30', 'B1');

xlswrite('duration.xls', ADP_thirtyfive_w(:,1), '31', 'A1');
xlswrite('duration.xls', ADP_eight_w(:,1), '31', 'B1');

xlswrite('duration.xls', ADP_thirtyfive_w(:,1), '32', 'A1');
```

```
xlswrite('duration.xls', DA_thirtyfive(:,1), '32', 'B1');
```

C) Perform the Kruskal-Wallis test on pairs of conditions and write the results to an excel file

```
% load_xls.m
% duration.m
% up_frequency.m
% firing_freq.m


dur=[];
fir=[];
upf=[];

m = 32; %number of pairs of conditions

for i = 1:m

    %create matrixes from each pair of conditions
    a = xlsread('duration.xls', i);
    dur = strcat('dur', int2str(i));
    assignin('base', dur, a);

    b = xlsread('upfreq.xls', i);
    upf = strcat('upf', int2str(i));
    assignin('base', upf, b);

    c = xlsread('firing.xls', i);
    fir = strcat('fir', int2str(i));
    assignin('base', fir, c);

    %perform kruskal-wallis test on each pair
    A = eval(sprintf('dur%d', i));
    B = eval(sprintf('upf%d', i));
    C = eval(sprintf('fir%d', i));

    kd = kruskalwallis(A);
    ku = kruskalwallis(B);
    kf = kruskalwallis(C);

    %save p values in krusk
    if i == 1, krusk = [kd, ku, kf];
    else krusk = [krusk; kd, ku,kf];
    end

end

xlswrite('try.xls', krusk, 'p_values', 'A1');
```

D) Create histogram of the control condition to visualize Up and Down states.

```
for r=1:10
    k = ['load soma1' int2str(r) '.dat'];
    eval(k)
 end

 D = (-80:1:56);
 d = transpose(D);
 A = [soma11; soma12; soma13; soma14; soma15; soma16; soma17; soma18; soma19;
soma110];


 figure;
 hist(A, d);
```