Integrative Neurophysiology - Ballerini

Elisa Nerli

May 5, 2016

Contents

Chapter 1

From single neurons to brain microcircuits

1.1 Introduction

Exam: written test (true/false) and oral exam (when marks range 25-27) on Ballerini and open questions on Torre's part. Battaglini will be oral! When you register on ESSE3, send also an email to Ballerini (sissa address) with the list of names of who will give her exam (we can do only Battaglini or only Torre/ballerini or both).

1.2 Neural network biophysics

What is biophysics? Understand from the molecular point of view life processes via biology, chemistry, biochemistry, physics. Try to connect having certain cell properties and some behaviours. Neuronal activity is the way we represent perception, actions and is represented by *electrical signals*. By observing the gneration of this activity we try to understand the way neurons compute neural activity and the way they result in different output.

Try to connect synaptic properties to neuronal networks.

EEG: the first done in 1922, macroscopic activity of neurons from external recordings. Changes in the state of electrical activity correspond to hughe changes in the behaviour of the persone. Thisapproach has been used for clinical application, diagnosis. EEG for research has been deleted because of intracellular recordings: this focus interest of research for the single cell has been masking the potential of EEG. Nowadays we want to conenct the activity of a single cell to the network.

The recordings from he EEG, apart for providing tools for neurology, is

used to investigate the *pattern of activity*. These signals collected by EEG macroelectrodes are due to the neurons' electrical activity (current flow into a resistive environment). This activity is related to *large groups of synchronized neurons*, so we are looking at a sort of integration of what is going on and to a superficial activity (of the cortex).

You can put in relation the activity coming from different areas: *coherence among different areas.*

For example, α rithm before falling asleep (but still coscient). There is a *temporal structure* of the signal: this sinchronous burst of activity emerging from different areas are organized in different frequencies. This temporal organization is related to different macroscopic states. Some of this rhythm, like γ, are related to cognitive processes. The crucial point is that part of the coding of neurons (signal) is also the temporal organization, not just the amplitude.

How is this related to neuronal circuits? It depends on neuronal activity: to have a certain rhythm we must start at a certain point and stop at a certain point and this depends on a lot of factors that generates different frequencies and different output depending on neuronal state. Each neuron might have a lot amount of VOC, so depending on the membrane properties (resting potential) a neuron have different behavior. The very same structure (brain area, like thalamus) can perform different behaviours depending on the properties of the neurons at the same moment.

We have alpha rhythm, beta, theta, delta. As you go to higher amplitudes you go to slower events. *Delta* is expressed during phase 3 of sleep (non-REM): it is physiological during this hase and also during anesthesia and coma, it is related to thalamic neurons' properties. The rage of frequency is 0.5-4 Hz. The sleep is one of the way we can approach the neuroscience study of consciousness; this activity is huge! It's not a way to save energy, so we wonder why we spend so much energy to produce this activity. This may improve memory reinforcement, drive entrance of Ca in neurons (it's a big depolarization) that leads to waves of Ca. Such finally tuned activity relies on finely tuned mechanisms: we know in mammals who is driving this activity and how.

Theta rhythm is in the range of 6-7 Hz, from the limbic area (temporal area and hippocampus) and it is related to attentional state and learning. If you stimulate huppocampalo neurons in theta frequency, you can induce LTP $\longrightarrow$*theta burst* (5 Hz/250 ms).

Alpha is regular and low, in relaxed state (the twilight state before falling asleep).

These were the first rhythms colelcted by EEG, all related with sleep. All the rest of activity of EEG was called *desynchronized* during active state

and learning. There are also other activities which are high frequency, the *gamma* and *beta* rhythm, during the awake state: they are the best correlate for complex cognitive behaviour. Gamma is recorded during cosruction of conscious perception, like recognizing an object. Gamma il in the range of 30-60 Hz but could be up to 100 Hz. There is a large theory on the fact that the gamma rhythm is the way by which distant areas of the brain transiently combine into an unique pattern: for example, in visual perception (conscious) we have a unified perception, even if the brain is representing different aspect (shape, color, movement) in differen area, we percieve a coherent unique perception. How this occurs? Transiently different areas of the brain working on the same perceptional object know that they are working together (*binding theory*[1]). The binding occurs by a transient synchornization in the *gamma frequency*.

What are the molecular mechanism for this synchronization?

We have that rhythmic activity determines the *global functional states*: wake, sleep and alternation. This synchronization is done continuosly, also during development.

[1]Francesco Varela

Chapter 2

Thalamo-cortical system

Connection between different thalamic nuclei and cortex. The very same circuit might generate different activity depending on the global state of the individual. It has a role during wake phases and has different activity during sleep (the one we'll analyze).

The recordings are from EEG, which are extracellular recordings, so there are superficial voltage changes in the cortex recorded by the scalp and represent a synchornization signal from hundreds of neurons.

At a higher level of resolution there are also *local field potential LFP*, experimental: recordings in which the electrode is inserted into a tissue, it is an *extracellular signal* but generated from smaller clusters neurons. This is generated by activation at the soma, axons of dendrites of neurons which are detected by an electrode close by. LFP are easy to perform but very difficult to interpret: we can have a LFP which is a downward deflection (dendritic field potential) or an upward deflection of the potential recorded (somatic potential): If this group of neuron is firing AP, we could have on the somatic one a sudden downward deflection, called a *population spike*: this is not the Ap of a single neuron but from a population of neurons. The polarity of the signal is related to the current flow and there is a theory on the fact that neurons usually generate AP from the initial segments of the axons and receive form the dendrites, so there is a certain flow and depending on the position of the electrode related to the flow (source and sink of current) I'll detect one polarity or the other. So, *this does not mean we have a hyperpolarization or a depolarization in an excitatory or inhibitory neuron.* The direction is not directly related to what happens in the memrbane. In LFP signals may have different shape and different polarity.

We can also put an electrode in a tissue and measure the electrical activity. LFP is possible if the neurons are closely packed, but in this case with the electrode we record a multiunit or a single unit traces: we detect from

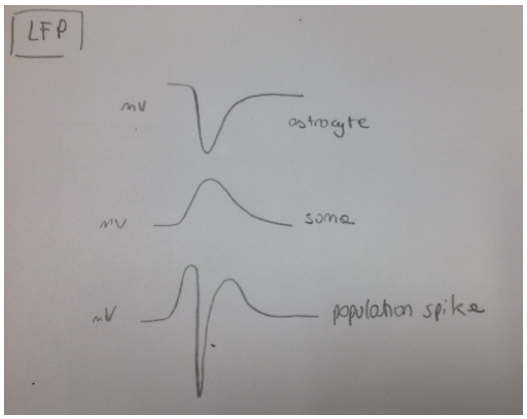

Figure 2.1: LFP recorded by soma, dendrite or population spike

extracellular space and AP, that can be the AP of a signle neuron (single unit) or of a group of neurons (multiunit). To distinguish them, we look at the amplitude: if we have a variability of the amplitude of the trace, we are in multiunit configuration. Then we have intracellular recordings (current

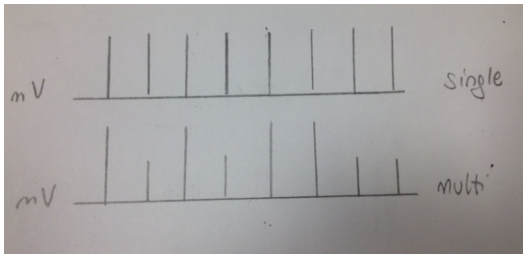

Figure 2.2: Single unit and multi unit traces

clamp and voltage clamp) from one single neuron. Voltage clamp can be done only in small neurons.

To investigate the ability of a network to perform a synchronized (bursting) activity, we must know how can a group of neurons synchronized the rhythm. In a minimalist approach, we can divide two mechansism by which a neuronal network could to this:

- Network driven rhythmicity: this system is synchronized due to the synaptic connection organization. (see sciancalepore) The properties of the post-synaptic events like duration are those that dictate the rhythmicity and the activity. If I block the activity of one of the 2 synapse, this blocks the rhythmicity.

- Pacemaker activity: each neuron possesses intrinsic properties which

enable a cyclic hyper/depolarization of the signle neuron. The combination of conducances will force the rhythm: biophysical properties of the memrbane

The 2 thing come together, but usually one is prevalent: we must know whether there are pacemaker neurons. Pacemaker neurons are not alway pacemaker: they need to alternate depolarization and hyperpolarization in a regenerative manner and his depend on activatio nof conductances in certain ranges of values. If I clamp one of these neurons and foce the Vm to move from one state to the other, I should swith on/off the pacemaker activity: the neuron might have different modalities of activity $\longrightarrow$pacemaker at a certan Vm, tonic firing at another Vm, silent at another one $\longrightarrow$it depends on the global state. Once found the pacemaker, I need to find the conductances responsible for this pacemaker activity!

EEG during different sleep phases: REM and non-REM phases. There are different stages of sleep with different waves of activity in the brain.

The first is the α, then stage 2-3 are characterized by huge synchronization, characterized by the *spindle waves*, group of syncro activity for the switch from conscousness to unconsciousness. Dring this activity (7-14 Hz) we have increase and decrease of activity (spindle shape), which means we have a recruitment of neurons and then they leave the synchronization. The stage 4 is the 1 Hz stage, due to a cortical slower activity. The moving from one activity to the other is cyclical during each sleep phase: it is interruped by incursion the REM sleep: in the REM, the activity of EEG is similar to the wake. The dreams we remember are those made in REM. Dream is performed by all mammals: there is a inhibition of motor output. During plasticity mechansim in development for the formation of ocular dominant column there is an impact of tis activity.

Why there is this organization? We still don't know. Surely both spindle and delta waves are generated by the thalamus nuclei. During wakeful state, thalamus is doing completely different things: it is dominated by a perceptional, sensory, motor inputs and the synapses which connects the thalamus to the cortex are extremely reliable. Neurons are relay because they re-transmitt informations. thalamus allow the brain to be completely disconnect fro mthe periphery during the sleep phase: this allow the brain to get activated by itself and for istelf, not for transferring peripheral information (apart eye movement). At the end, this activity is governed by the thalamus. The control of the cortex is less that the output that thalamus receives.

not all the thalamic nuclei are organized in that way: the visual thalamus and visual cortex give us most of the experiments.

The 2 activity of the thalamus are the spindle waves (7-14 Hz) and the delta waves (0.5-1 Hz) in the later state of sleep ⟶nice example of pacemaker activity, so neurons in thalamus can become pacemaker and switch compeletely their behaviour during wake phase.

2.1 Thalamus and circuits

Deep nuclei, near the walls of the ventriculum. It is called a *gate keeper*, there are many different nuclei; each of them has a certain specificity. 3 blocks (52 nuclei):

- Specific nuclei: both sensory and motor, they are the *relay*, or thalamocortical TC or latelar geniculate nuclei of dorsal thalamus (LGNd). They are Glutamatergic neurons, but there are also some intrinsic GABAergic neurons. The output is Glutamatergic, excitatory neurons. Each time these neurons reach certain areas of the cortex, usually S1, they activate a *feedback excitation*

- Associative nuclei of the thalamus, connected with the dfferent associative areas

- Aspecifi nuclei: they project to several areas, but one group, the *reticular nuclei* (RT), or perigeniculate neurons (PGN) or nly reticular (R). It is entirely GABAergic, so an inhibitory nuclei and is NOT projecting to the cortex but to the others thalamic nuclei! Relay neurons and cortical neurons inhibit GABAergic, which via a feedback inhibition they connect to the relay neurons. The interplay between reticular and GABA is at the base of the spindle behaviour.

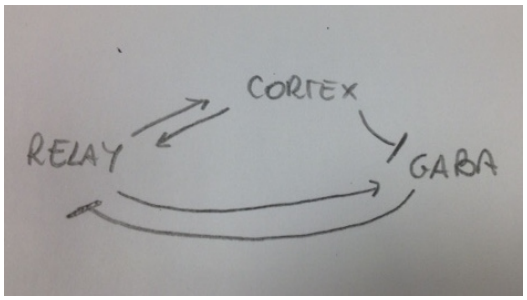

Figure 2.3

2.1.1 Differences between REM and non-REM

If you look at the traces, they are huge! In REM we have a flattening out of the electromiogram, so a strong inhibition of movement, and a total change of the EEG, which is higher in frequency similar to the wake phases, and ocular movement. These 3 major measurements are correlated with different behavior, like sensation and perception, which is absent in non-REM sleep, is present in REM but is internally generated. The movement might be episodically and uncontrolled during non-REM, while it is completely inhibited during REM sleep. The brain activity nderly a deeper state for the non-REM sleep where whe have a complete unconsciousness and in these phases we are deeply asleep, wether during REM is the contrary. During non-REM is easier for an external stimuli to wake you up, while in REM the threshold of consciousness is very low but the chance of a spontaneous wake up is higher. When you get from phase 2 to 3, the thalamus changes the activity systematically anc cyclically from spindle waves to delta. This is finely orchestrated and regulated: the same machansims which govern spindle waves when they are altered, they are responsible for epilepsy or other disfuctional states. The relation of these mechanism when no functional, lead to pathological states.

(8.03.2016)

Non-REM sleep is interrupted by REM one. We change activity from a fast syncronized activity (REM) into a slow wave activity in the nonREM activity: the latter is the one that we'll consider. We are going to look at the *spindle waves* and the *delta waves*, 2 and 3 phase of the sleep.

Electromiograph EMG: record muscular activity.

Electroencephalographic EEG and Electrooculargraphic EOG.

At the core of generation of brain activity we have the thalamic complex of nuclei and the activity generated, spindle and delta waves, are entirely generated by an interplay btw thalamic nuclei. The wake phases is continuosly transmitting signals to the motor cortex. The thalamus have several nuclei, but the interesting one is the *specific nuclei*, the relay, the LGN (lateral geniculate nucleus) and antother nuclei, the *reticulum nucleus*, GABAergic (the only one!) and not projecting to the cortex.

2.2 Dorsal thalamus

The basic circuit: relay nuclei, reticular nuclei and cortex $\longrightarrow$this interplay is repeated in the thalamus. We have TCR (thalamusc cortical relay neurons, GABAergic) which is receiving as an afference from sensory periphery

a sensory information transmitted from the thalamocortical neurons to specific areas of the cortex: in visual pathway, we have V1. This excitatory input to the cortex is also activating 2 other neurons: IN (local GABAergic interneuron), so a local inhibiting circuit that is specific of carnivores an a local refinement of the signal (feedback inhibitory loop) , and RN (reticular neurons, GABAergic), they are the inhibitory neurons of the thalamus that project to the thalamus cortical cells and is continuosly regulating the activity of relay neurons in inhibitory fashion. This inhibition is much more effecient of the IN in controlling the activity of the relay.

The basic building blocks of the circuits on rodents doesn't show IN, but only RN and relay neurons.

The first source of activation for RN is the thamaus cortical cells in themselves: from the cortex, there is an excitatory pathway which goes to re-innvervate the neurons which are delivering the signal to the very same cortex, so the cortical area, activated by the thalamic neurons, is activating the very same neurons (or not activating them). Each time I activate a relay cells, the cortex activates and excites again the relay cells. Also corticothalamic cells are delivering an input to the reticular inhibitory neurons: this is a feed-forward inhibition pathway, so I activate an inhibitory pathway that as a target the same neurons I activate directly. Rn are not receiving inputs fro mthe periphery but activate each time the relay neurons.

C are the cortical thalamic terminals, excitatory. The terminals from RN are usually ocated in a different position: there is a local circuit on the dendrites, regulating the inputs incoming, but this is only local; the inhibitory role of these IN is not inhibiting the somatic activity of these cells! It inhibits the *distal dendrites*. We'll never see the cell hyperpolarized by these IN. Only RN can hyperpolarize these cells!

So, summarizing: there is a circuit btw thalamus and cortex. 3 major elements: relay neurons (thalamus-cortical neurons and LGN neurons) that project axons to the cerebral cortex, excitatory; then IN located within the relay nuclei; then extrinsic GABAergic neurons, RN, they are in the thalamus but not into the relay nuclei, they send axons to the area of dorsal thalamus. RN innervate the thalamus in a *divergent manner*: they can simultaneously inhibit a bunch of neurons!

2.2.1 Relay nuclei

Sensory fibers frm periphery which forms the medial lemniscus, the somatosensory pathway along the spinal cord ending to the gracile and cuneatum nuclei in the brainstem and reach the thalamus, then the optic tract, the lateral lemniscus (autidoty tract). These are very well described and they are

the first input; the secon is the cortico-thalamic fibers, from the cortex to the thalamus, exciting the thalamus. Then we have reticulo-thalamic axons from reticulo-thalamic neurons which are inhibitory. Each time the signal goes to the cortes, it goes activating also the reticular cells and when it come back from the cortex, it still activates the reticular cells as well, which in turns get to the relay neurons. if we imagine this, there is acontinuous balance btw excitation and inhibition and, depending on how much this activity is activating the inhibiory one, we'll have at the end a very precise and sophisticated tuning of the relay neurons of the thalamus, which are continuously regulated based on their own activity.

The system is regulated continuosly by sensory fibers. What is the outcome during wakefulness? A continuous stimulation. If i look at relay neurons during wake, is a tonic firing, there is not a construction of synchronization. The trigger is the continuous arrival of sensory inputs.

In sleep phases, all these inputs are silenced: this structure is not anymore governed by sensory inputs but switches to a complete dufferent regulation activity. The first change is that al lthese neurons are more negative in thei Vm, they get hyperpolarized due to this silencing of activity. One they remove all these inputs, what is governing the activity of the thalamus is the interplay btw these nuclei: this actvity is transferred to the cortex, so there will be a dialogue btw thalamus and cortex. This is crucial in the way this activity will emerge and there are 2 signs to switch the activity from tonic to bursting: *removal of sensory inputs* and *neurons get hyperpolarized* and this will unmask different membrane properties. During wake phases neurons are at a certain Vm (ex -55 mV), during sleep phases they get hyperpolarized: there might be some voltage dependent channels that are active at more active at more hyperpolarized Vm. The same neuron is changing properties and becoming a bursting neuron! It can turn into a pacemaker neuron in some ranges of Vm.

put figure circuit

In the generation of the spindle and delta waves from the thalamus, the cortex is implicated in transmission of the signal but does not participate to the generation of the rhythm: this is due to mechanisms from relay and reticular neurons. We can focus on properties of relay and reticular and describe the system even not considering the cortical.

I usually record the spindle waves from the cortex, all these phenomena are recorded from the cortex: if I remove the cortex, I can still record the activity from the thalamus, because the mechanism responsible for this activity are in the interplay btw Rn and relay neurons.

We have a *reciprocal excitation*, the excitation that wen relay neurons are activated, this activates also the neighbouring one and this will activate

also the cortical neurons, btw thalamus and cortex, then *reciprocal inhibition* because they are inhibitory neurons within the all system, so each time a neuron get activated is activating an inhibitory neurons, which is back inhibiting the very same neuron, and *parallel excitation and inhibition*. When cortical neurons innervates the thalamus, they activate relay with an excitation and reticular neurons that are inhibited, so the same inputs activated two pathways.

2.2.2 Reticular nuclei

The first synaptic connection demostrated were the one btw PGN (reticular neurons for lateral geniculate nucleus LGNd, also called perigeniculate neurons) and the relay neuron. There is a monosynaptic connection GABAergic btw them: I need to have 2 electrodes in each of these neurons and Im expecting that if I activate the inhibitory neuron I will record in the relay an inhibitory postsynaptic potential. This is done with *sharp intracellular recordings*. In current clamp, there is injection of depolarizing step of current in the inhibitory neuron $\longrightarrow$series of AP $\longrightarrow$I expect in the relay and IPSP (inhibitory postsynaptic potential). in voltage clamp it will be an outward current, corresponding to a reduction of positive charges, because we follow the positive charges (otward for hyperpolarization, innward for depolarization). I'm pushing the Vm to a value more distant from the threshold to generate AP. I'll need a stronger input to activate those neurons. When we describe acurrent, we have to describe the direction: if movement of negative changer, we have the contrary than when positive charges moves. A hyperpolarization is the Vm more negative: if I look at this, is Cl ions entering, but if I look at the current (positive ions!) it will be an outward current. Each time we have a inward current, we have a depolarization mediated by positive charges. This was the first demostration of an inhibitory synaptic connection btw reticular neurons and relay neurons. So, relay or thalamocortical (TC) or LGNd (visual thalamus) or VB (somatosensory thalamus).

AP in relay neuron $\longrightarrow$EPSP[1] into PGN: this is an excitatory connection, a transient depolarization which can reach or not the threshold. We can know if we have the activation of one synapse or more by measuring the delay btw AP and EPSP: if it is 10 ms, there is only one synapse involved. If we have more than 10 ms delay, we have a multisynapse path. We add delay with adding synapses.

A huge burst of activity of an inhibitory neuron $\longrightarrow$inhibition of the LGNd cells. When we stop this inhibition, the activity starts again we go

[1]Excitatory connection, activating a Glutamatergic synapse.

back to EPSP. The way the system is conencted is able to perform a *repetitive cycling behaviour*.

Reticular neurons have long dendrites and axons and are fusiform cells, while relay have a radial, huge amount of dendrites which are similar in terms of properties. They are different also in terms of way to generate AP: if I record from one neuron and I don't know wheter is it relay or reticular, I can understand if it is relay or reticular depending on the *profile of activation*: reticular neurons, when injected with a depolarizing step of current, generate a delayed burst of AP $\longrightarrow$it has properties that allow a delayed AP. Relay neurons instead generate AP in a faster way and generates a wave of depolarization and it is a shorter depolarization. So, these neurons are also biochemically different: dark staining is the presence of GABA, so reticular cells, while the relay neruons are not provided of GABA.

2.2.3 IN

The role of these neurons is at the level of dendrites: the dendritic arboriztion of a neuron is what provides the neuron a variable computational power. the ability to integrate. The way dendrites are represented are with resistance and capacitance: we relay to morphology of dendrites with ability to transfer charges through long distances. See length constant and time constant. Whatherver is going on at the top of the dendrites is not instantaneously transferred to the soma: it depends from where the inhibition is located and how effective it is.

Usually, relay neurons have a quite regular arborization of dendrites. They are radially oriented: these neurons also give rise to axons, thick for a CNS axon, and we have intrinsic inhibitory IN around 25% of the neurons. These IN are smaller and give rise to synapses on dendrites, because they are inefficient in controllin the degree of hyper/depolarization of the entire cell. The presence of these small GABAergic neurons is very insignificant in terms of controlling major changes in the polarization of Vm at the cell soma. The fact that we are studying the thalamic rhythm in neurons which do not possess these neurons does not affect our hypothesis.

The difference is that reticular GABAergic neurons make inhibitory synapses more to the proximal dendrites, very close to cell soma, therefore if I look at localization of inhibition coming from local IN and from reticular neurons, both inhibitions are important but, to hyperpolarize the relay, the IN are not effective due to their localization in dendrites (more distal), only the reticular neurons are effective.

In this distribution, reticular neurons only have the power to control what the cell is doing.

There is a very strong connection btw thalamus and cortex and vice versa. We can simplify this system with 3 major blocks, efficient in being activated, controlled and tuned during wakefulness but becoming the generator of activity during sleep phases. They are those interactions of intrinsic emmbrane properties, and the interaction of the system is the one generating rhythmic oscilaltions during the sleep oscillation. We know the mechansism that lead to a certain temporal structure of this activity. This is important because these oscillations are recurrent during sleep phases, but they may appear in pathological conditions not during sleep. There is a large amount of work which give insight for the properties of cells of the circuits related to the ability to perform synchronized oscillations. Thes eproperties of a signel neuron membrane are crucial for the colelctive behaviour. If I generate transgenic animals, I'll change the shape of sleep phase of that animal.

Absence seizures are characterized by SW during wakefulness.

The 2 activities generated by this system are spindle waves and delta waves.

2.3 Spindle waves

Occurring at the beginning of the sleep phase. This activity is characterized by a certain appearance and frequency: they are bursts of activity like synchronous waves. They are btw 7-14 Hz. Each spindle is characterized by increasing-decreasing of the signal; the duration of each event is arounf 2-4 sec, while the intrafrequency os 7-14 Hz and the tibe btw each event can be 2-10 seconds. Why this frequency of 7.14 Hz? Why an increase and decrease od the events? Why it is silenced ad a certain point (2-4 seconds)? Why we have an interval of 2-10 seconds?

All these points are known fro mthe molecular point of view. The neurons implicated are Rn and relay: the cortical one are implicated, but not essential because the frequency is governed by relation btw RN and thalamo-cortical. There are several factors relevat: presence of particular proterties of RN and of synapses btw Rn and relay.

In vivo recordings form a cortical area, so it is a LFP, and simultaneously from a relay neuron of LGNd in an anhestesized cat: the first evidence of something that is going on in the thalamus. We see spindle form the cortical area (cortical LFP): in the thalamus we have a mutliunit recording (from more than a neuron) and the activity detected is the same of the cortex.

put figure 6

The first work is from Steriade et al, Journal of Neuroscience vol 13 (1993). Since spindle waves is a circular event, sooner or later we'll have the beginning of the system, so let's record it from the reticular neuron RN. Dur-

ing the spindle wave, in the RN we see that they perform a bursting activity characterized by the appearance of bursts of AP which have a frequency of 7-14 Hz, generated by the presence of *Ca spikes* that subscribe on a slow wave of depolarization. If I look at reticular neurons during spindle, these neurons start generating bursts of AP at 7–14 Hz and are subscribed on a small wave of depolarization. This activity will be generated by the presence of Ca spike and consist of *burst of AP clustered on top of Ca spikes*, which are slow depolarization with a shape similar to AP. This activity is whithin the frequency of 7–14 Hz and is generated on a small depolarization due to Ca spikes. When we look at more details, we see that each group of AP is generated by a slower depolarization which is a Ca spike that rhythmically occur: this is due to activation of T-type Ca channels, which transiently activates and deactivates..

figure 7

The relevant factor is that each time I have these bursts of AP in reticular neurons, this will affect the relay cells in terms of inhibition, so: SW $\longrightarrow$reticualr neurons start oscilalting (pacemakers) and performing on top of Ca waves some AP rhythmically, with a frequency of 7–14 Hz. Each time these neurons bursts at this frequency, the relay cells will receive a barrage of inhibitory potentials whithin 7–14 Hz. If the SW is occurring at the level of reticular neuron, if I'm on thalamocortical neurons, I'll see a barrage of IPSP arriving, and since the innervation from GABAergic neurons to the relay is divergent, it means that these neurons will synchronize large amount of relay neruons in performing a series of IPSP.

This is related to the fact that this transient inhibition of TC neuron will induce a post-inhibitory rebound, an activation. This rebound phenomenon is the *core of the spindle wave*. In this way, I synchronized the activity at the frequency of the barrage of the IPSP: the inhibition is occurring at a certain frequency. RN start firing to a certain frequency, but I need also TC to have the barrage of IPSPS and then I need a rebound excitation of these neurons $\longrightarrow$rebound is intrinsic property.

figure9

What is governing the frequency is not only the frequency of activation or RN, but also the *duration of IPSP*, because the rebound will occur at tha end of IPSP, so IPSP duration impose the timing of the system. This is an efficient system: if I want to synchronize al lthe TC in acertain ragne of activity, I'll need a stong depolarization. To inhibit symultaneously a large group of neurons is easy, because activating these GABAergic connection is efficient, each time I open Cl Ill have hypperpolarization. If the neuron posseses a property to provoke a rebound, I have a strong probability to synchronize this system if I hyperpolarize everybody to the same stage and let go and when they come back from hyperpolarization, they have a higher probability to generate AP, and they will be synchronized. The inhibition

plays a crucial role to synchronize neurons at a certain frequency. Naturally, this is because these neurons possess the ability to generate a rebount which is related to a voltage dependent channel, so to the Vm each neuron is sitting. This describes only a certain window of possibility of the spindle to occur. The conductance involved into the rebound is the I_T voltage gated Ca channels: they need to be activated after hyperpolarization near Vm.

Interplay btw Rn and TC is crucial to generate spindle waves: this is why it is called *network driven rhythmicity*, becuase synaptic conenction is crucial and goversn the frequency characterizing this system, altough we have pacemakers.

(15.03.2016)

So, spindle waves are AP on the top of Ca spikes. They lead to activation of relay neurons. The frequency of IPSP is 7-14 Hz because it depends on oscillation of relay neuron. There is also a post-inhibitory rebound in the relay cells. The probability to have these neurons firing at that sequence synchronized is higher due to the divergent inhibitory inputs.

The most important features of spindle waves are the inhibitory synaptic conenction and the presence of intrinsic memrabne proteins that leads to post-inhibitory reboun potential. Retilar an thalamocortical sight of spindle waves is different. This spindle waves generation can be studied in thalamic acute slices: taken from furret (furetto), they contains both LGNd and PGN (nuclei and reticular part) and is able to generate spindles, so circuitry and minimal mechanisms to generate spindlses are contained there. The acute slices such as cultured neurons or organotypic slices are artifacts: if a mechanism is described there, this is a possibility, but this is not surely occurring in vivo. Acute slices is a wonderful tool for basic neuroscience: 300-400 μm tissue alive for 8-10 h. There are still changes in the tissue: even if in CREB solution, the layer reconstruct new synapses whhich are not there in vivo. This system, even so semplifyed, generate spindles. When you record in the single slice, there is a similar activity to the in vivo one: during in vivo preparation we have the nfluence of te entire brain. The activity that you have at the relay neuron is based on the presence of a post-inhibitory rebound Ca spike (abbreviated PIR), which usually identify the transiet hyperpolarization of a neuron and the cming back to Vm: the probability to display an additional depolarization is increased. The real core of the generation of spindles from relay neuron is this rebound.

figure10

Rebound depolarization requires T-type Ca channels, while *anode break* is a transient change in the threshold in a neuron which might improve the ability of neurons to generate AP when coming back from hyperpolarization. If you inject a depolarizing step of current we induce serier of AP, while if we inject an hyperpolarizing step, when coming back from hyperpol there is

the appearance of and AP and of a depolarization after the AP. This is a post-inhibitory rebound. In other condition there is the appearance of only an AP: this is the anode break. MOr ein detail, inject a hp step of current in patch clamp or intracellular recordings, as soon as we stop HP we have the appearance of AP. We don't have Ca spikes, but a simple AP. This is called anode-break excitation: before, at the same Vm, we don't have AP, so the resting Vm is far from the threshold. hen I'm coming back to the same value from a transient period and I have AP, it means that the threshold has changed.

The threshold depends from balance btw Na and K conductances: favouring K, the threshold is more positive. During the hyperpolarization phasse, we remove the inavtivation of Na channels and reduce the driving force for K (leak channels). This transiently change the threshold: this is for 10 ms and is due to the generation of an AP. Why the PIR require the activation of Ca channels? T-type have a particular kinetic and generate a Ca spike with a shape of an AP, altough this lasts longer. On top of Ca spike, we have AP $\longrightarrow$rebound. the real PIR is blocked any time I remove Ca from outside or when I apply blockers of T-channels like Nichel at low concentration.

How was it shown in thalamic slices? 2 electrodes in the slice where we have LNGd (relay) and PGN (reticular, GABAergic). OR is optical radiation, because there are fibers coming from the optic nerve and sometimes these nerves are stimulated to induce the appearance of spindle (artifact!). We have A, A1 and C, layers of cells in the thalamus: in humans they are 6, in rodents 3 etc and they are segregated group of neurons connected with ipsi or controlateral retina. The fibers which reach each thalamus comes from both ispi and controlateral retina (optic chiasm) and these fibers carry informations from the same area of visual field but from the 2 retinae. In the thalamus, there is not a combination fo these 2 inputs, these inputs are segregated in layers, so we have 2 controlateral and 1 ipsilateral layer. The first neurons which combine these 2 inputs are in the visual cortex. This segregation is important. In these slices, recording activity with an extracellular eletrod and one intracellular, we notice the appearance of spindle waves: if I look at each event with different time scale, the extracellular has the appearance of a spindle and the intracellular recording is a barrage of IPSP with PIR. We might have IPSP not followed by PIR, other followed by a Ca spikes which do not have on top the AP, other followed by PIR with AP on top: this is sthocastic.

Ca spike is the rebound and the duration is shaped by the IPSP: the frequency at which the system is working depends on the synaptic connection with reticular neuron and the duration of the events regulates the frequency of the spindle. IPSP are important to induce the rebound and in shaping the

frequency of the system.

One advantage of the presence of PIR is that if I wanto to investigate the mechansisms of rebound, I can induce a Ca spike artificially injecting an hyperpolarizg with duration and frequency of IPSP; I can end up with a PIR.

What are the mechanisms due to the rebound? Usually, these neurons in the wake are acting in different manner, do not perform post-inhibitory rebound because they are more depolarized. The mechansism are due to low threshold currents activated at very negative potential, close to resting Vm, that inactivate.

2.3.1 T-type Ca channels

T are very low conductance, very low channels. They have low activation; depending on the neurons, we'll find different negative values. (Delcour and Tsien, 1993). In the gating scheme, to remove the inactivation they need to step at negative Vm (transient hyperpolarization). That's why they are so keen to perform rhythmic activity etc. One classifcation is T, L, P, Q, N, R.

N-channels are involved in the neurotransmitter release. T channels have particular gating properties $\longrightarrow$rhythmic activity. We have a close state (actually they are more!) at the hyperpolarized, the small activation open the channel $\longrightarrow$influx f Ca $\longrightarrow$depolarization $\longrightarrow$inactivation $\longrightarrow$hyperpolarization that removes the inactivation $\longrightarrow$close state. Window currents: a range of Vm values in which channels are open but not completely inactivated $\longrightarrow$steady-state depolarizing current an inward current. There are several models proposed for T-type

There are several models for T-type gating: the close state or inactivated state are the most probable at negative potential. Then via small depolarization we have intermediate closed states C1, C2, C3 etc. and then we get to open state. From the open state, the channels, in a transiently inactivated state and the equilibrium will drive the channels to get fastly to the activated state. This means that we have the fast rise of inactivation and this gives the shape as an action potential. The Ca spike will last hundreds of milliseconds, so longer than an AP. There are several inactivated states (depending o the model). These channels has antoher peculiarity: activate at negative potential, fastly inactivate, need to deactivate do go back to open and, in addition, this channel can be facilitated in a voltage-dependent manner, meaning that the probability of opening is higher. This is due to the fact that previous strong depolarizations are able to increase the T-type Ca current, which might be important for mechanisms more related to plasticity. We have a *voltage dependent facilitation* of T-type channels. This can be due

to different effectors or be a pure voltage facilitation. It might be a direct voltage effect on the Ca channels itself or via inhibition of Tyr Pi.

There is an additional mechansism: we have a neuron and a Vm color coded. When tese neurons fire AP at tight frequency, they can propagate it into the dendrites (Ca spike)! These are *back propagating AP*, usually decremental with distance and they get slower and slower. If high frequency, this decremental depolarization being slower can *summate* i nthe dendrites and this may lead to the activation of T-type channels in the dendrites.

The other way to classify the channels is via pharmacology.

Ca spike in thalamic slice is dependent on T-channel expression. When I tonically depolarize the cell, I'll remove the possibility to generate a PIR $\longrightarrow$stop the spindle waves. This was done to incvestigate the GABA-ergic connections. Based on the Nernst equation, if I move the Vm, depending how far from reverse potential of an ion, I can identify which ions are involved in synaptic transmission.

During the spindle waves induced in OR by a strong stimulus, when we have IPSP barrage and Ca spikes and AP, if we move to more positive or negative AP, we change the amplitude of IPSP. Hyperpolarizing current are larger at more positive value, but the rebound is not anymore there because the relay neurons are too much depolarized. Same thing if they are hyperpolarized: IPSP are smaller but we don't get anymore to the threshold to activate T-type channels, Vm is too negative! These experiments are called *DC shift*: moving Vm. There is a clear window of possibilities to obtain the spindles and this depends of Vm of the relay neurons, and this is few mv!. *IPSP are appearing as Cl-mediated events*: this was the first prove (0 at -85 mV).

If in the pipette there is high KCl solution: it takes 10-15 minutes to dialize the cytoplasm and we change the IPSP barrage into excitatory depolarizing events. The way the system works: we have a loop connection btw relay and reticular neurns, therefor if I record from relay neruons(intracell) and from reticular (extracell), if I inject an hyperpolarizing step in relay I will have a rebound $\longrightarrow$activation of reticular neurons bursts: IPSP $\longrightarrow$way of recruiting cells in the spindle. There can be some failures of the connections, a sthocastic effect. This recruitment is occurring also in vivo, probably: the cortex is back-looping to the thalamus.

So, relay neurons during every spindle waves are bombarder by IPSP barrages, due to the activation of reticular cells, and these IPSP were demostrated to be due to the activation of GABAergic synapses and usually what happens is that IPSP are followed in relay cells by rebound Ca potential, due to the T-type Ca channels, I_T currents. This might explain the waxing, even if we don't still perfectlyunderstand the waning.

Why the reticular neurons start doing this activity? They are *pacemakers*: spindle waves are an example of network-driven rhythmicity. They are pacemakers only in certain condictions: they can have also a tonic firing. 7-14 Hz frequency is due to the rpesence of pacemakers, PIR and GAGAergic synapses.

Reticular neurons are generating a pacemacker activity that was observed before the rest! PGN on furret, acute slices: isolating PGN from the thalamus, if these neurons are at the right Vm they will behave as pacemaker, so produce oscillaion due to intrinsic properties. They have 2 modalities of activity: *tonic activty* at 30-60 Hz during wakeful stae, or *oscillatory activity* btw 7-14 Hz. These oscillation are due to properties that emerge only at certain Vm values. Tonic activity or burtsing manner $\longrightarrow$completely different informations!

The oscillatory activity will depend from the interplay btw I_T and Ca-dependent K conductance $I_{K_{Ca}}$ or I_{AHP}. Therefore, this iterplay appears only at certain ranges of Vm values, which appears *during the first stages of sleep.* During sleep phases we have progressivly hyperpolarization of the membrane and this progressively hyperpolarization is the one driving these different functional states, which are of single neurons of the nuclei and became of the all brain. We start having Ca spikes, then again strong hyperpolarization due to $I_{K_{Ca}}$ and another spike: each one is build up on a more depolarized state and the depolarization drives us back to a tonic discharge and the oscillation is turned off. This build up of depolarization is crucial to regulate the duration of the spindle in reticular neuron.

If you transiently depolarize the membrane, we have a tonic firing. We have the *ramp experiment*: we have acontinuous stepping the cell through hyperpol/depolar state and we see the neuron switching from oscillation and tonic firing. In a pacemaker, oscillatory asctivity is strictly related to Vm. Changes s Vm are not dramatic, only few mV. The other property of the pacemaker is that oscillations are independent from the synaptic connections: experimentally, we can induce a series of oscillation and then tonic activity. These oscillations, if not related to synaptic network connection, should be there even blocking the synaptic activity. I block it with tetrodotoxin $\longrightarrow$able to oscillate $\longrightarrow$this is a pacemaker: it can perform in the absence of synaptic connection.

After hyperpolarization is reated to Ca activated K channels: increasing intracellular EGTA to chelate Ca and to prevent the accumulaiton of Ca inside the cell, which activated K current, the oscillation disappears, we have a long burst. If we put Cesium inside the pipette: if inside the cell, it blocks all K conductances, while if in extracellular solution it blocks only I_h $\longrightarrow$no oscillations, only a big Ca spike. Apamin is a venom and blocks SK channels

(small Ca activated K channels), involved i ngeneration of AHP. In this case, the interplay is done by I_T and by $I_{K_{Ca}}$: the first lead do tepolarization and to a series of AP, so a huge influx of Ca $\longrightarrow$Ca accumulates and activates SK channel $\longrightarrow$hyperpolarizaiton.

I_{AHP} allows for certain frequency of firing and it is important for synchronized activity as well. It can be BK and SK: the differences are dimentions (big and small), pharmacology and voltage dependence. Both are indirectly voltage-dependent, because they are activated by Ca and the influx of Ca is voltage depenent. They can be activated either by increasing intracellular Ca or by depolarizing Vm (BK) while SK are not provided of voltage dependence.

On Y axis: % of time open of the channe, so activation; then in one of the x axis Ca concentration, so we expects in BK that by increasing Ca concentratio I'll have more and more activation. On the other x-axis we have voltage dependence: at -50, we need a high amount of intracellular Ca to activate 50% the channel. If we are at +10 mV, we reach the same 50% of activation with less Ca (5 μm) and this is peculiar to BK, SK needs only Ca concentration increase.

(22.03.2016)

Considering the role of I_T channels in generating rebound, the back propagating waves of depolarization can summate in the dendrites $\longrightarrow$activation of Ca channels $\longrightarrow$Ca spike.

Puttin 2 pipettes at different position in the neurons and follow generation of series of AP, we see that the back-propagating one are decremental, so they don't reach 0 mV. If this activity of back propagation activates T-channels, they will reach a much higher depolarization $\longrightarrow$incoming inputs from afferent synapses might be activated coincidently with this depolarization $\longrightarrow$spike dependent plasticity.

There are neurons very efficient in back-propagating, like cortical one, or nuerons which cannot do it at all.[2] GABAergic neurons: oscillation btw 7-14 hz and generate activity at the ground of the barrage of IPSP. The activity of reticular neurons is oscillatory and appears at certain levels of depolarization of Vm. In that case, these neurons start oscillating and when are hyperpolarized enough they unmask pacemaker activity $\longrightarrow$cycling de/hyperpolarization od the membrane. The reason of frequency set at 7-14 Hz relies on pacemaker properties of these reticular neurons and rebound activity or relay cells.

I_T and I_{AHP} are crucial: if negative step of current $\longrightarrow$hyperpolarization $\longrightarrow$go back to Vm $\longrightarrow$activation of I_T $\longrightarrow$depolarization $\longrightarrow$activation of Ca

[2]Not related to the thalamic system.

channels $\longrightarrow$Ca spike + AP on the Ca spike. Influx in Ca, increase of intracellular Ca concentration that leads to the activation of $I_{K_{Ca}}$ $\longrightarrow$activation of SK channels apamine sensitive. If we sum up inactivation of T channels, of Na channels and activation of K channels and activation of Ca dependent K channels, we have a fast hyperpolarization that will inactivate I_T and this will lead to a second oscillation. There is a series of oscillations and then a slight depolarization of membrane potential and a tail of tonic activity. The system works with series of oscillations and we end-up with depolarization and tail of AP. To switch off pacemakers, we have to move Vm to different values: we exit the range of values which allows the pacemaker activity to emerge. To stop spindle wave activity $\longrightarrow$depolarization $\longrightarrow$tonic firing.

Why do we have depolarization? there are Ican conductances which are activated: Ca dependent aspecific cationic conductances. Each time we have bursting activity, we have It that generates Ca spikes and the Ca spike generate Na-mediated AP, so the depolarization $\longrightarrow$increase activation of voltage dependent Ca channel and I accumulate more and ore Ca in the cell which activate K conductance. The ability to buffer Ca decrease with time $\longrightarrow$acculumation of Ca and activation of Ican conductances. this lead to slow conductances $\longrightarrow$slow depolarization which stays there until the Ca is buffered, so the conductances close back.

So, we know why they have 7-14 Hz frequency, the duration is 2-4 s and there is an increasing activity and a decreasing activity. We have the *interbust* that is 10-20 s, a sort of refractary period due to properties of the relay cells. We can think that the best way to switch off the system is to depolarize the reticular neurons or the relay ones, because depolarizing relay neurons the barrage of IPSP is not able to remove T channels inactivation.

The activity of spindle waves has been investigated because the perfect example of network synchronization and rhythmic activity: it is due to interaction of reticular and relay neurons. Usually, reticular cells generates burst activity (IPSP barrage) and the activity is silenced probabilly by Ican activity, that is a sort of emory of that cell because it depends on accumulation of Ca. Therefore, reticular thalamic cells can work in 3 modalities:

- At depolarizing potentials, the activity is a *tonic firing*;
- In the first stage of sleep, the fisrt activity which appears are *spindle waves*, so at that Vm (more negative) reticular cells start performing their pacemaker activity, so large part of the cortex is forced to work synchronously.
- At stage 3 of sleep, we have the appearance of a slower activity, *delta waves*, when Vm is more hyperpolarized (0.5 Hz, very slow with large

oscillations)

2.4 Delta waves

Hallmark that we enter a different stage of sleeping. This activty is not generated by pacemaker, because 0.5 Hz, but it is due to relay neurons which dominate the scene during delta waves and become themselves pacemaker but at a different frequency. The realy neurons, once more hyperpolarized, are disengaged by the spindles and start oscillating at a frequency intrinsicly produced, that is slow and impone to the system slow and long oscillations.

As for spindle, the first observation experimentally was coming from E-G coupled recordings of LGNd. These are in vivo recordings: EEg acyivity is mirrored by activity of LGNd acivity and LGNd recoridngs (intracellular, in vivo with a sharp electrode in thalamus) appears as oscillatoring burst, so maybe these neurons are pacemakers generating delta waves. They need synaptic connection to the cortex and feedback. This activity disappears in wake or REM phase of the sleep, so these are neurons that can generate tonic firing of pacemaker oscillations depending on the Vm value.

Stage 4 is the slower activity, generated by cortex, where stage 2-3 are generated by thalamus (spindle and delta waves). This is a very organized actiity, meaning that the system is not resting at all! This delta waves activity is a perfect example of pacemaker activity: we have not an interplay, but this is generated by single relay neurons, so these neurons in certain window of Vm posses the intrinsinc property to generate depolarization and hyperpolarization. In this activity we have I_T for Ca spike, then I_h activated by membrane hyperpolarization. In this stage of slee, Vm is more hyperpolarized, so we have removal of I_T inactivation but we need something to bring Vm to the potential of inactivation of I_T, so we need depolarization. This is the *pacemaker potential*, the ability to depolarize in response to an hyperpolarization.

The relay cells can switch from oscillatory mood (with AP on top of Ca spike) and we have tonic spike if we inject depolarizig current, then again oscillatory mode. If you record the activity, you might have co-expression of different rhythms. This seems to be tuned by the Vm value.

Pacemacker activity due to interplay btw I_T na I_h. In the depolarizing phase, I_T channels de-inactivate, so they can be re-opened, but they need to be at the threshold and here we are far from the threshold. We need the activation of I_h at this hyperpolarized Vm: it is a slow conductance which does not inactivate. Influx of + ions, basically Na $\longrightarrow$depolarization of Vm which is called *pacemacker potential*, which reaches the threshold for T-channels

to activate, so we have a huge Ca spike with on top AP. When Ca spike declines, this is due to I_T channels inactivation together with deactivation of I_h, that close at positive Vm and opens at positive Vm: this brings the Vm back to the resting level, that is negative $\longrightarrow$remove T channels inactivation etc. 0.5 Hz is due to the duration of Ca spike (400 ms).[3] What is crucial is I_h, a strange conductance. When Vm is more depolarized, we have the *tonic firing*, the system does not oscillate anymore. Btw these 2 conditions, we have the range of oscillation of relay neurons $\longrightarrow$spindle waves.

2.4.1 I_h

I_h is a mixed cationic current: it is Na and K current, K is not that important in driving the charge of current, the important one is Na, an inward positive current. We can find different values of activaition, any cells can regulate and tune the activation curve of this cation current. It is a slow oepning and slow closing, but not inactivation: it does *deactivate*. This depolarization is called *pacemaker potentials*, it is slow depolarization and leads to activation of I_T etc.

We are interested in the properties of these conductances I_h: McCormick was the first to find out that Ih contributes to generation of rhythmic oscillations. I_h, h stays for hyperpolarization, has different names: I_f like funny current, I_Q like queer (strange) and I_{AR} like anomalous rectifyer. If we are voltage-calmping and inject negativ step of currents to hyperpolarize Vm, we'll notice that at the beginning we have almost no current (steps used to measure *input resistance of a cell* as a passive properties). When going more negative we have a huge inward current appearing that takes a while to develop, like 2 second, a slow current. If we want to measure the current at the steady state and the one that we have at the beginning, the difference is I_h, because it is something which develop slowly and leads to an inward current, so the first strange thing is the *slow activation upon hyperpolarization* of the membrane. We may notice also that not only it increasing the activation at more and more negative Vm, but also the latency of of appearance the activity is shorter, so we fasten the activation at more negative potential, but there is a high difference from the istantaneous current and the steady-state one.

The steady state current does not activate immediately: during hyperpolarization you activate more and more current, we have a higher current, so we have a delay. This is a long step, so measuring the difference btw the current at the beginning of the hyperpolarization, we have a small value I_S,

[3] We go more negative than the spindle waves Vm

but if we measure the values of the current at the end, the steady state I_{SS}, and we make the difference btw these 2, we see that I_S is a leak current, while I_{SS} is voltage depending, so it is I_h. The delay is voltage-dependent as well. Another hyperpolarization activated conductance, the I_{IR}, inward rectifyer K current (K enters $\longrightarrow$depolarization).

What maked I_h conductance completely different from the others? Slow activation as a inward current following hyperpolarization. The magnitude of the current and the activatio rate are increase increased hyperpolarization. Injectng more negative potential steps, we have firstly a leak current which keep the Vm towards negative values because it goes agains Nernst potential for K. This curent is linear, no rectification and it is resistant to application of extracellular Cesium, because Ce inside the cell block all the K ocnductances, while outside the cells it will block only I_h $\longrightarrow$istanteneous current is resistant to Ce, while the steady state currents will disappear.

The activation of I_h has a delay, it is slow and it can accumulate this current. It reaches a steady state, where the current does not increase anymore: this delay inactivation is reduce d upon hyperpolarization so gating system is voltage dependent. Therefore, this delay is reflecting some intrinsic channel mechansism of activation, which are voltage dependent. I_h does not inactivate but it does deactivate, so Vm at which it opens or closed depends o nthe voltage dependence of the channel. This is a strong voltage dependence: for small difference in Vm, we have a high difference of the amount of channels which are open.

This conductance works as pacemaker, so comparing activation of I_T, it is quite fast, while activation of I_h is very slow. Then there is another role of these conductances: it is not only involved in oscilaltory pacemaker activity but also in tuning regulating the value of resting Vm: at rest, the Vm will be more and more negative to be close to resting potential for K (-90 mV). The reason why this is not the case is that towardds this path they crosses the activation of I_h, because at very negative potentials I_h id depolarizing a bit the membrane. So we have the leak conductances I_K and I_h responsible of maintaining Vm. In a normal system, putting a blocker of I_h Vm would be more negative (5-6 mV more negative).

Another peculiarity of I_h is that it loose the *independece principles*: usually, in a mixed cation current, the 2 ions behave indepdentently one from the other, whether is I_h they are not independent: if I increase extracellular K concentration, I will increase the amplitude of the current, because I'm increasing the affinity of the pore for Na $\longrightarrow$more Na entering $\longrightarrow$more Na current.

I_h also opposes to strong hyperpolarization and can cause a sort of rebound. The last peculiarity is the activation curve, so the range of Vm in

which I might expect to have I_h open, can be regulated by second messenger systems, by the metabolic state of the neurons, in particular by cAMP: gating of I_h is shifted to certain Vm values depending on the direc bining of cAMP to the channel. The *second messenger status of the cell* will regulate when I_h mediated depolarization has to contribute to the system: the slow depolarization which appear is not fixed, it can changes and it is tuned by the metabolic activity of the cell, and we need to have a partial activation of the current to havea decrease or an increa of cAMP. If the current is not activated, cAMP has no effect. An elevation of cAMP $\longrightarrow I_h$ easier to open.

We can modulate I_h via experimental tool: use an analogous of cAMP which is not degraded, or stimulate AC or inhibit phosphodiesterase etc. This modulation can be pharmacologically tined. Inhibiting AC, we have a smaller activaiton of the current. To have the regulation: open channel + change cAMP concentration, these 2 things must be coupled. cAMP binds to the channel and changes the gating properties. The activation curve after phosphorylation will be shifted at -65 mV instead of -80 mV. All this has to occur during the activation of the channel!

(31.03.2016)

I_h in lateral geniculate nueurons involved in the generation of sleep activity as spindle waves and δ-waves (pacemaker clls due to interplay between I_T and I_h). R expresses I_h $\longrightarrow$appearance of this cuurrent related to hyperpolarization.

Demonstration of I_h into talamocortical neurons: I_h is important foe the refractory period. In voltage clamp (intracellular patch clamp technique) I record the current and I inject negative steps of V so that I hyperpolarize the membrane and I notice the inward current due to the influx of Na (mixed cation current, but it is due to influx of Na). There is an acceleration of the current the more I go to negative potentials. I_h are slowly activated.

In current clamp mode I look at the voltage I will inject negative steps of current and record V. An inward current correspond to a depolarization of the membrane: if I_h is present when I put negative current, I should observe positive V (depolarization). This is called *voltage seak*, a current activated by hyperpolarization). Researchers found out that when we give the hyperpolarizing step, we have influx of positive charges and so a depolarization (inward current in voltage clamp) or we have the appearance of a seak in the V when I'm recording in current clamp: two signature of I_h.

With voltage clamp we can plot Vm at which we put the step with the amount of current normalixed to the max, we have info about the half max of activation and the slope is the dependence of this system by the charge (V). Further evidence: from 2.5 mM of K going to 7.5 we have an increase in current. The istantaneous current is a leak conductance, linear, shifted

when we can change the extracellular K concentration, because leak channels are lead by K. 2.5 mM is the physiological concentration of K, we have the rectfication that corresponds to the steady state, further increased to 7.5 mM of K. So, we have an increase in amplitude. If I reduce extracellular Na, I should reduce the current because we have a lower driving force and this is the case. The instantaneous current doesn't change. The current is modulated by K, it is build up by Na, high selective nlockers for this channel of if we want to block we have to replace K with Cs^{2+}. So, we put Cs in the pipette and t will block K conductances, but if we apply Cs at the extracellular level we will block I_h! In presence of Cs I should reduce the current and this was the case. The instantaneous current doesn't change again.

I_h contribute to properties of the thalamic relay cells, first to resting membrane potential Vm. This ability to avoid to get too negative can be tuned because activation curve of I_h can be changed depending on the metabolism of the cell. In addition, I_h mediates oscillatory activity of δ-waves with I_T in a certain range of Vm: this oscillatory activity is also present in presence of TTX due to intrinsic properties.

I_h is also important for the spindle activity (related to the interplay between relaty and reticular nucleus), interplay between I_T and $I_{K_{Ca}}$ that generates IPSP in the reticular and this cause a rebound hyperpolarization due to I_T. I_{CAN} due to the accumulation of Ca that causes depolarization in the spindle. This I_{CAN} and depolarization can cause a block of the system. I_{CAN} is not a long lasting current, so it cannot explain the refractory period (10 to 20 s). The idea is that this has to be related to changes in the thalamocortical nuclei's intrinsic properties. Changes that are long lasting and a sort of memory of the last activity. During the spindle, something has to occur: if we have something that after the spindle is causing a depolarization, so and ADP, this will be a way to block the spindle, because I'm moving the Vm and so I don't have conditions to have a rebound. So, during the refractory period, we have a depolarization that we do not have during the spindle. Only I_h are the candidate that give depolarization at hyperpolarizing potentials.

How can we have the activation of I_h if they activate at more negative potentials? This is due to something that changes the activation curve for I_h so a change in the metabolic phase. During the spindle, we have a series of IPSP, each time we have hyperpolarization of Vm we have a partial activation of I_h, then we have the Ca spike with AP, the inhibitory rebound due to Ca. If we repeat this, together this time with the I_h current and we will end up wth activation of more and more current that end up with a depolarization that endures for 10-20 s. This was demonstrated in an experiment.

Experiment If we record intracellular spindle from the thalamocortical neurons, we can observe this ADP after each spindle but we have to stay intracellularly (extracellularly not detected). The refractary period will depend on the metabolic state of the cell and is governed by the spindle itself. Upregulation of I_h due to change in the activation curve and it is at the ore ofthe spindle refractory period. Patch clamp recordings on thalamocortical slices relay neurons: they make fake spindles, each time I put an hyperpolarizing potential I'll get a rebound Ca spike. Rebound can be spontaneous due to IPSP or induced by negative current. We see series of IPSP mimicked by injection of negative current that give a fake spindle with Ca spike at the frequency of the spindle, and we observe also the apearance of ADP. This ADP is due to inward current, but to demonstrate it I need to change in V clamp made to analyze the current.

If I block with Ni^+ the entrance of Ca, so the Ca spike and so one of the mechanisms which is inolved in the upregulation of I_h, this wil lreach the steady-state earlier, so we need also Ca in addition to IPSP. If we inject a Ca chelator as BAPTA, in this case we have Ca spike, but it doesn't end up into an increase of Ca concentration because we buffer it. there fore the rebound is there but not Ca and blocks the ADP. the ADP depends also on the IPSP and Ca spikes. After single step, we do not affect I_h but we need more than one step.

To prove te same thing, we increase the intracellular concentration of Ca: if it is enough, even one step is sufficient to get upregulation of I_h. This was done using cage compound that are activated by light in which Ca is limited and in UV we have release of Ca (quantum dot comp). If we block I_h with ZD and if we flash with light without the cage compound with Ca I'll not obtain the same effect. So, this is the mechanism that regulate the refractory period, but these were all performanced in vitro.

Spindles are correlated to a form of epilepsy, where we have apprearance of spindles even during the wake and not only sleep. Does the amplitude of IPSP have an impat on the appearance of the rebound? If IPSP are longer, the frequency of post inhibitory rebound will be different. It is called *no threshold spike*. The rebound changes depending on the IPSP and this was investigated eith the dinamic clamp which is a continuous injection into the cell of V or current so it is not a step. If we prolong IPSP we will have acceleration in the post-inhibitory rebound, so we have different activity of the network. Even the post inhibitory rebound (PIR) can be shaped. Weknowthat IPSP are due to opening of GABA A recetors that we can modulate with BDZ, so even the PIR is not passive but depolarization of the system can be tuned.

2.4.2 Manipulation of I_T

(So the rebound) If we remove I_T, we wil laffect spindle because we do not have the spindle. Strain of mice missing the functional subunit of I_T in the thalamus, so the channel doesn't work and they not have the spike. They have reduction of the spindle and δ-waves, but we do not have problems with the non-REM waves slow frequency (1 Hz) and REM is also untouched.Showed that thalamocortical neurons and appearance of I_T are important for the spindle and δ-waves, but not the other, and also the critical role in the genesis of oscillation will lead to the loss of oscillation and changes into ttransit from one phase of the sleep to the other. I_T is also called LTS. They investigated the sleep activity with EEG activity.

Wt-transgenic plot the E of the system toward the frequency we have a plot. Peak: high voltage, so the system is synchronized at that frequency. 1-4 Hz (δ) peak, the second peak at 9 Hz (spindle). The dashed line is the transgenic that shows total absence of peaks in these frequencies, so they are not anymore synchronized.

Going back to sleep, REM is characterized by the appearance of EEG phase which is reminescent of wake phase but in a complete ipotonic state (no muscle movement except for eye movement). In the REM, the frequencies distributed are similar in the two animals, in non-REM we have loss of the frequency of spindle and δ, but the animals are sleeping. So, what change their behaviour? Are they really important? The rats sleep during day, the total amount of sleep in the transgenic mice is reduced due to a reduction in the non-REM sleep, but not the REM not affected. What is affected is the long part of the sleep (non-REM).

Why sleep phases are reduced? BEcause we do not have change into the total time of sleep. The sleep of these animals is characterized by the appearance of brief awake moment. When we wake, we are not ipotonic anymore, so there is a big jump from a state of consciousness to the other very quick and it is important because for any animal sleeping is dangerous so they are exposed to predators. So the wake for them is important and they are ready to run. In the transgenic mice the brief wakenings are not so brief but longer, not different into the incidence of this "brief period" but they are more frequenc. Spindle impact and protect our sleep and change between sleep and awake, which is crucial for the animal. Spindle are claimed to be important in memory trace; maybe also with the QI. So maybe the change between non-REM - REM - wake is governed by spindles and so I_T because block of I_T is sufficient to block efficiently the generation of the spindle.

The spindle are implicated in different brain functions, sleep quality, difficulty in walking people due to the fact that the spindle are working very

wel las a filter; also the reinforcement of memory, protecton from oxidative species during night. In plasticity is not involved into LTP but also omeostatic plasticity which balanced a network, if we have a potentiation at one synapse we have depression to another and we need a balance. This omeostatic plasticity is a way in which a neuronal retwork change, but remaining anyway equal.

The spindle are different depending on where we are recording. non-REM/REM rapporto changes during like, the non-REM is higher and higher. The generation of spindle is more complex in humans and we can divide in slow and fast.

2.4.3 Spindle and LTP

In vivo we have larger volume of the cortes and associative learning with coincidence of spindle. If we affect sleep phase, we will affect the memory and attention. Also consolidation seems to be due to the filter activity of the spindle waves. REM contributes to maturation of visual cortex and dominance, so it is important for developmental phases. Why do we sleep? What is the advantage for the brain? The motor cortex is still active during REM phase even if we do not have the tonic movement of muscles.