

Computational Neuroscience

Motor control and learning

The general brain objective is to generate the movement. Movement is our only instrument for active interaction with the external world. Obvious examples range from expression of complex thoughts (writing, reading, talking, communicating...) to artistic production, sportive tasks, interpersonal relationships...

Movement is a complex task for several reasons:

- **Delays:** there are delays both in the transduction of motor command from the Central Nervous System (CNS) to the muscular system and in the conduction processes of sensorial signals to the CNS. Sensorial delays, moreover, combine with delays associated with movement itself. This delay makes the sensorial information unusable to guide at least the initial part of the movement. Skilled performances need open loop control: PREDICTION!
There are intrinsic delays. And these are even higher than the execution of the movement. When we dive, we took some time before because we need to make predictions and planning.
- **Noise:** besides delays, both sensorial inputs and motor control signals are subject to intrinsic neural noise that limits the motor system ability to simultaneously execute FAST and ACCURATE movements. To overcome this noise, the motor system has to combine actual sensorial data with a direct model: PLANNING. Planning allows, thanks to experience, the ability to induct the correct information from a noisy signal.
- **Not stationary:** the relation between motor commands and movement (dynamic) changes with time by two different points of view. First, there is an ontogenetic factor, i.e. living subjects grow up and second, our motor task actuation changes with respect to different objects and different environments. Training as well as pathologies continuously modify our actuation system and also the controlling neural mechanisms (piano players have completely different brain areas for hand control as well as tennis players have much broad brain areas for arm motion). A person suffering pain at the shoulder have a “modified” actuation system. Of course any neurological pathology does affect motor control neural circuits.
- **Non linearity:** Motor Control can't be assumed to work in a linear environment because while executing movements we always use the non linearity of this system. Two evident examples:
 - Gravity force (external non linearity). The same exact motor sequence has very different effects if executed in a microgravity environment or on the Earth. Moreover, easier to understand, the same motor command is different if executed by a lying or standing subject.
 - Joint range of motion (internal non linearity). Our joints limit the movement in a given direction and this is a straightforward example of saturation (non linearity).
- **Multidimensional:** if we consider that we have about 600 muscles in our body and we suppose to simplify that they can be contracted or relaxed, this would mean 2^{600} possible activation patterns (magnitude order of 10^{180}). BUT, if we look at the “experience” of movements, we

observe that motor tasks are actually STEREOTYPED. At the motor control level, motion is managed through synergies. We have millions of inputs and millions of outputs possible, for that reason, synergies and motor programs are necessary.

We can consider all sensorial and cognitive processes as inputs that will determine the next motor outputs. From a computational point of view, the brain is a processing system that converts inputs to outputs. **Inputs are fed back sensorial data produced by both our sensorial organs and internal signals due to cognitive processes, outputs are motor commands that act on our muscles.**

Motor control can be seen as the transformation process from sensorial inputs to motor outputs.

The motor control and motor learning problem is to regulate and adapt these sensory-motor transformations (through open- or closed-loop processes).

The action-perception loop is anyway a loop and not a unidirectional communication, so actions drive perception especially during learning.

Motor control is characterized by a learning process. Learning involves changes in behaviour that arise from the interaction with the environment and is distinct from maturation, which involves changes that occur independently from interaction.

The goal of learning in general is to improve performance. Even if some single species show no motor learning, the need for motor learning arises in species in which the organism's environment, body or task change. Specifically, when such changes are unpredictable, they cannot be pre-specified in a control system, and therefore FLEXIBILITY in the control process is required.

Motor learning is a compromise between innate capacities and learned capacities.

Innate motor behaviours need pre-specified neural connections that make these behaviours *stable* and *robust* to perturbations. This "stability" could however reduce available flexibility to learn new motor tasks because motor learning could require the interruption or change of reflexes and synergies at the CNS level.

Innate capacities are *hard-wired*, *robust* and *fast*. Instead, learned capacities are *adaptable*, *slow* and *flexible*.

In different species the "ratio" between innate and learned skills can be very different.

Simple species, like spider, don't have any motor learning. The necessity of motor learning appears in the species in which the environment, the anatomic characteristics, the objectives can change.

If changes are unexpected, then they can't be pre-codified by the control system, so they require control FLEXIBILITY.

It was shown that there's a linear positive correlation between the body mass and the brain mass. Respect to rodents, humans' synapses transfer 10 times more information.

There are linear scaling at the level of the brain: increase of brain per body mass, expansion of frontal areas, more neurons in brain and cortex. But some intrinsic features don't have this linear relationship.

Does the learning process start from scratch? No. Some facial gestures are observable in the deaf and blind from birth: we do have some INNATE behaviours.

Reflexes are not learnt. At birth a sequence of reflexes is verified to check whether the new born neurological system is properly working.

Reflexes are involuntary movements or actions. Some movements are spontaneous, occurring as part of the baby's usual activity. Others are responses to certain actions. Reflexes help identify normal brain and nerve activity. Some reflexes occur only in specific periods of development. The following are some of the normal reflexes seen in new born babies:

- *Root reflex*. This reflex begins when the corner of the baby's mouth is stroked or touched. The baby will turn his or her head and open his or her mouth to follow and "root" in the direction of the stroking. This helps the baby find the breast or bottle to begin feeding.
- *Suck reflex*. Rooting helps the baby become ready to suck. When the root of the baby's mouth is touched, the baby will begin to suck. This reflex does not begin until about the 32nd week of pregnancy and is not fully developed until about 36 weeks. Premature babies may have a weak or immature sucking ability because of this. Babies also have a hand-to-mouth reflex that goes with rooting and sucking and may suck on fingers or hands.
- *Moro reflex*. The Moro reflex is often called a startle reflex because it usually occurs when a baby is startled by a loud sound or movement. In response to the sound, the baby throws back his or her head, extends out the arms and legs, cries, then pulls the arms and legs back in. A baby's own cry can startle him or her and trigger this reflex. This reflex lasts about 5 to 6 months.
- *Tonic neck reflex*. When a baby's head is turned to one side, the arm on that side stretches out and the opposite arm bends up at the elbow. This is often called the "fencing" position. The tonic neck reflex lasts about 6 to 7 months.
- *Grasp reflex*. Stroking the palm of a baby's hand causes the baby to close his or her fingers in a grasp. The grasp reflex lasts until about 5 to 6 months of age.
- *Babinski reflex*. When the sole of the foot is firmly stroked, the big toe bends back toward the top of the foot and the other toes fan out. This is a normal reflex up to about 2 years of age.
- *Step reflex*. This reflex is also called the walking or dance reflex because a baby appears to take steps or dance when held upright with his or her feet touching a solid surface.

To understand motor learning, it has to be considered as a process that occurs in everyone life and from generation to generation as well. **The learning process, in fact, is composed by a co-adaptation of neural and anatomic structure.** For example, human manual skill has a very fine controller, but it is possible thanks to the opposable thumb. Anatomical body adaptation could occur as well. Both occur in everyone alive (e.g. tennis players' hypertrophies) and from generation to generation.

The human is the unique species living everywhere all over the Earth. They're not specialized (able to adapt to changes and not to things) and they're polyvalent.

There are 2 levels of evolution: during history and during the growth. The scale is completely different.

Also, other animal species learn motor skills as well, but the "huge quantity of learning" is unique in human species.

Perception drives the action, but at the same time, perception is not a passive task. The learning and experience become guides to the acquisition of sensorial data and they both affect perception.

Multiple stimuli present in the visual field at the same time compete for neural representation by mutually suppressing their evoked activity throughout visual cortex, providing a neural correlate for the limited processing capacity of the visual system. Competitive interactions among stimuli can be counteracted by top-down, goal-directed mechanisms such as attention, and by bottom-up, stimulus-driven mechanisms. Because these two processes cooperate in everyday life to bias processing toward behaviourally relevant or particularly salient stimuli, it has proven difficult to study interactions between top-down and bottom-up mechanisms.

A second impact on the research of motor development is the perception-action approach inspired by James and Eleanor Gibson. According to this concept, perception and action are linked together: actions need perceptual information to be planned and adaptively executed. Perceptual information needs movement to create the relevant patterns in perceptual systems.

Movement is regarded to be embedded in a continuous perception-action chain, in which perceptual information provides the basis for adaptive and prospective motor control the key to prospective control are exploratory movements. Active exploration is accounted as the link in the perception-action loop, which generates or allows for gathering information for deciding what to do next.

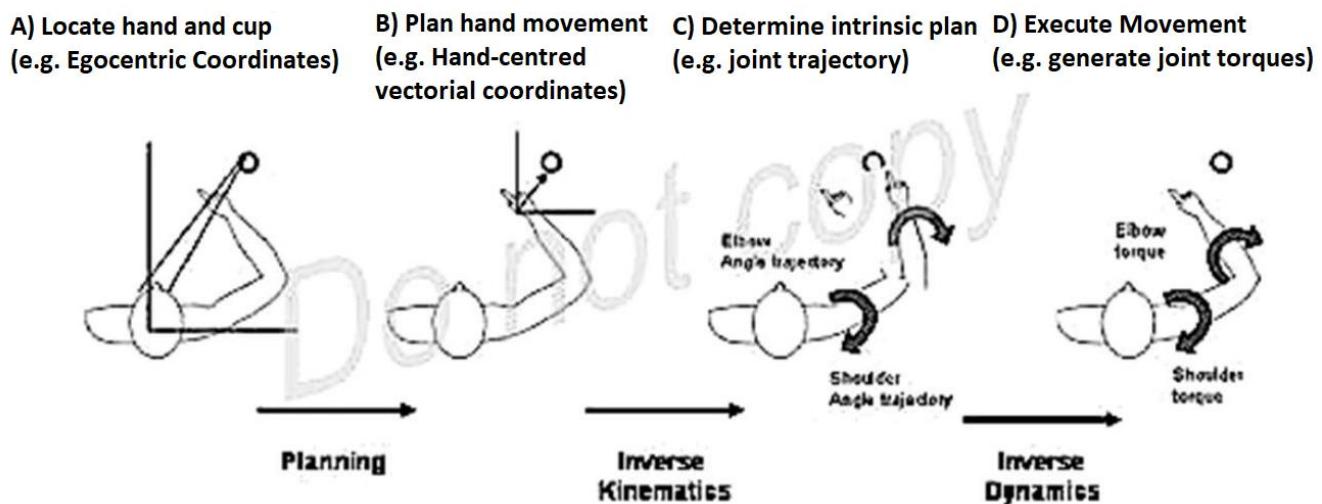
According to Piaget [1953], infants are exploring their own action system by performing certain movements over and over again and evaluating the continuous multimodal flow of sensory information. Likewise, Hofsten [1991] proposed that exploratory movements are directed towards the infant's own action system instead on the external environment, which is traditionally expected. In a nutshell, one of the predominant driving forces of changes in behaviour and development is proposed to be the exploration of the capacities of the individual.

The transformation from sensory to motor info can be subdivided into kinematic and dynamic transformations.

Kinematic transformation: conversion between the system coordinates, as for example the transformation between hand position and arm angles (e.g. a computer mouse: we have to learn the kinematic transformation between the mouse position and the cursor position on the monitor).

Dynamic transformations: it translates the coordinates in motor command, force to apply in order to obtain the desired movement (e.g. we need to learn the forces mechanism that can control the right mouse displacement on the screen and this interaction will depend on the mouse inertia and friction).

Movements happen through a transformation cascade:



Within these transformations there are multiple levels of infinite possibilities, redundancy is a key ingredient for motor control:

- Redundancy in the definition of the end effector path to reach the target;
- Redundancy in joint trajectories to accomplish the end-effector path;
- Redundancy in the muscle activation once defined the joint net torques (possible co-contraction).

Redundancy is used to assure flexibility, to adapt solution to specific situations (such as pain), to overcome unexpected perturbations... learning starts from redundancy!

We can distinguish sensory-motor transformations in two typologies: reflexes and voluntary movement.

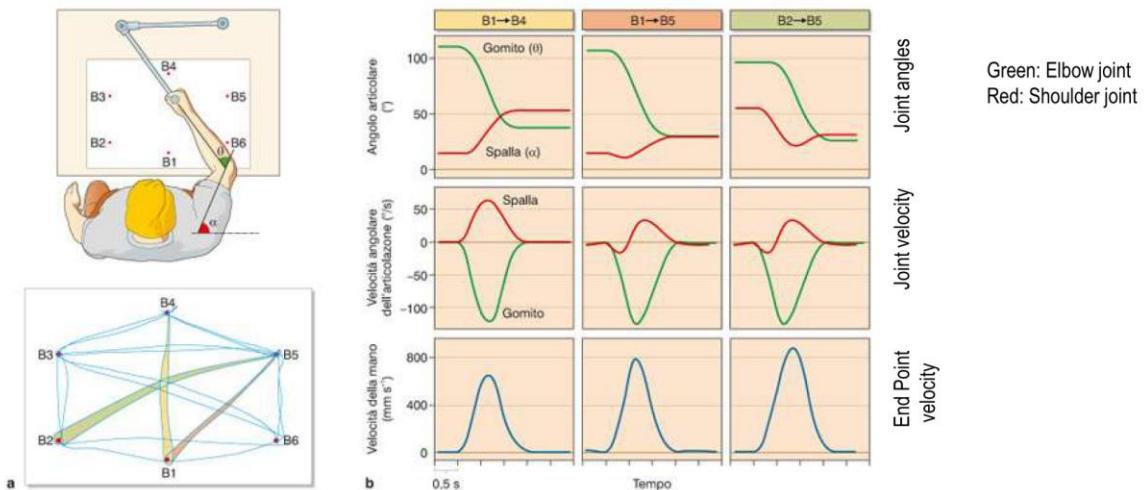
Reflexes are simple transformations occur in the same way if the same circumstances are given. They are automatic mechanisms. The input causes the direct output (e.g. tendon jerk reflex).

Voluntary movement are planned by the intention of the subject and they can be modulated by external or internal events. They have given characteristics, for example the independence from the effector (see example in the slide: we can somehow write with any part of our body. We know how to write, independently of the movement effector). Voluntary movements are organized in function of a goal-directed movement execution.

- Answer for voluntary movements to the same stimulus can be different depending on the behavioural goal, whereas reaction to reflexes is stereotyped;
- Movement efficacy grows with experience and learning;

- Voluntary movements are not simple responses to environmental stimuli but can be generated by internal instructions.

We have to circumscribe our investigation: we will primarily cover voluntary motor control.



Reaching tasks are typical volitional movements. As seen in these data, there are important similarity in the execution of reaching tasks to different targets:

- Quasi-rectilinear endpoint path
- Bell-shaped endpoint and joints velocities.

These observations suggest that, besides the infinite possible solutions, there is a tendency to solve redundancy in stereotyped solutions.

All paths are roughly straight, and all hand speed profiles have the same shape and scale in proportion to the distance covered. In contrast, the profiles for the elbow and shoulder angles for the three hand paths differ. The straight hand paths and common profiles for speed suggest that planning is done with reference to the hand because these parameters can be linearly scaled. Planning with reference to joints would require computing nonlinear combinations of joint angles.

If the brain forms a representation of a movement before its execution, does it plan the extent of the movement or does it continuously assess the distance between the hand and the target and use visual information to stop movement once the target is reached?

If the brain relied primarily on vision to stop, the initial speed of the hand might be relatively similar in movements of different extents. Instead, both the speed and the acceleration of the hand movement are scaled proportionately to the distance of the target. **This means that the extent of a movement is planned before the movement is initiated.** The representation of this plan for movement is called a motor program. The motor program specifies the spatial features of the movement and the angles through which the joints will move. These are collectively known as movement kinematics. The program must also specify the forces required to rotate the joints (torques) to produce the desired movement. This is known as movement dynamics.

The motor plan is the sequence of tasks to accomplish the target, including the amplitude, the kinematics and the dynamics of the movement. Velocity and acceleration change with the distance of the target.

Laws of voluntary movements

I law: voluntary movements show invariant features

The CNS plans in abstract way the final output of the movement independently from the mechanisms which will be carried out to accomplish it. Performance does depend on the effector; thus the natural solution chooses the effector which gets the best performance. ("the more the use, the better the performance")

In the early 1950s the psychologist Donald Hebb observed that individual motor actions share important characteristics even when performed in different ways. For example, our handwriting appears about the same regardless of the size of the letters or of the limb or body segment used to produce them. Hebb called this "motor equivalence". Motor equivalence suggests that a purposeful movement is represented in the brain in some abstract form rather than as a series of joint motions or muscle contractions.

The continuous motion of drawing a figure eight consists of regular increases and decreases in the angular motion of the hand. These changes in angular motion occur at regular intervals during which the hand describes approximately equal angles, a feature termed isogony. The duration of each hand movement is the same regardless of the length of the hand path, a feature termed isochrony. Studies of more complex movements, such as those made during random continuous scribbling, show a similar segmentation. Such studies also reveal a consistent relationship between the speed of hand motion and the degree of curvature of the hand path: Velocity varies as a continuous function of the curvature raised to the $2/3$ power. This two-thirds power law governs virtually all movements and expresses an obligatory slowing of the hand during movement segments that are more curved and a speeding up during segments that are straight.

II law: reaction time increases with the information to be processed

Reaction time increases nonlinearly with the number of response alternatives available to the subject and also with the number of inputs.

External Stimulus → identification of the stimulus → selection of the answer → programming of the answer execution → Motor Output

III Law: Speed-accuracy tradeoff – Fitt's law

The accuracy of a movement varies in direct proportion to the speed of movement. Subjects held a stylus and had to hit a straight line lying perpendicular to the direction in which they moved the stylus. Subjects could not see their hand and thus were unable to correct their movement.

The variability in the motion of the subjects' arm movements is shown here as the standard deviation of the extent of movement plotted against average speed (for three different movement times). The

variability in movement increases in proportion to the speed and therefore to the force producing the movement. (From Schmidt et al. 1979.)

The higher the velocity, the lower the accuracy. Multiple factors concur to the worsening of accuracy for fast motions:

- If the available time is short, feedback loops does not enter into play
- Signal dependent noise: higher speed → more recruited motor units (EMGs) → higher signal-dependent noise → lower accuracy
- Higher speed → more recruited motor units (EMGs) -> more variance

Minimum variance is an important criterion of optimization for movement learning.

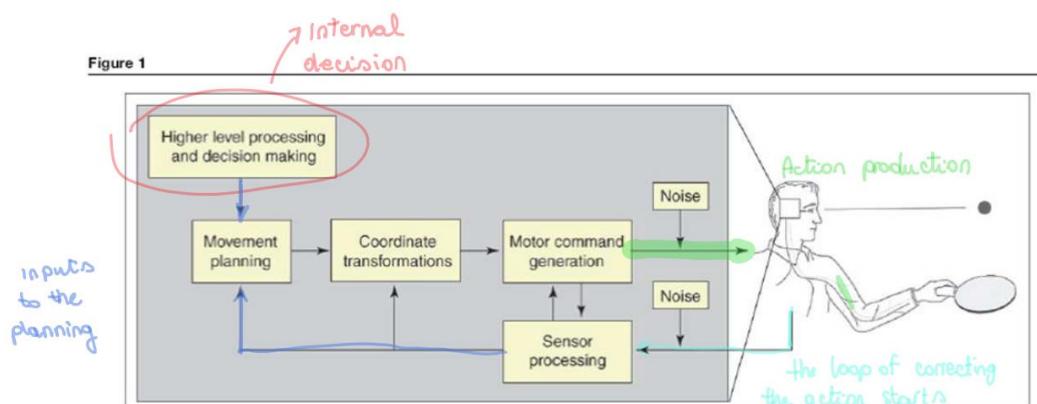
In the 1890s the psychologist Robert Woodworth showed that fast movements are less accurate than slow ones. This is in part because fast movements leave less time for feedback corrections. In fact, the fastest movements are shorter than the reaction time itself. But lack of time for correction does not explain fully why fast movements are less accurate and more variable than slow ones; faster movements made without visual feedback are also more variable in both extent and speed.

Several factors contribute to the increase in variability with speed. One of these is the recruitment of additional motor neurons to produce rapid increases in force, since the excitability of motor neurons is subject to random variations. A constant incremental increase in force is produced by progressively smaller numbers of motor neurons. Therefore, as force increases, fluctuations in the number of motor neurons lead to proportionately greater fluctuations in force and thus velocity. This proportional relationship is maintained over most of the range of contractile force and corresponds to the proportional increase in variability with the speed of movement and the distance of the target.

IV law: Movement efficacy grows with experience and learning

The more you repeat a task, the more efficient you will be in performing it. Variability also arises because subjects may be uncertain about the forces and loads that are needed to oppose movement. This uncertainty decreases with practice, however, so that both the accuracy and the speed of movement increase.

MOTOR CONTROL DIAGRAM



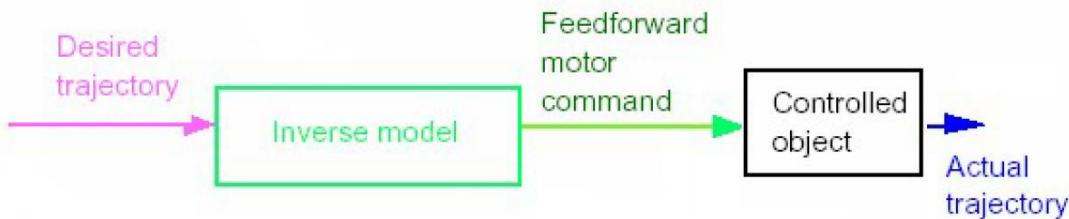
This scheme is assuming a double control system both feedforward and feedback--- can we prove experimentally the existence of a feedforward control?

The **feedforward control** is an anticipatory control which put together sensorial information and previous experience.

The **feedback control** is an actual control. It is dependent on the actual sensory information and make comparison with a reference signal. It is characterized by a gain.

To model the brain functions in motor control we use **internal models**. These are representations of the sensorimotor and motor sensory transformation present within our brain. In order to fulfil an efficient motor control, they mimic, inside our brain, the functions of the system to be controlled.

FEEDFORWARD CONTROL: INVERSE MODEL



Inverse model: estimates the motor commands required to achieve the desired sensory feedback (anticausal direction). It converts the desired task into a motor command.

It is fast, so it cancels delays, and it's not able to correct the movement on errors occurring because of its inaccuracy or because of unexpected disturbances.

Open loop control is essential for most natural movements, where the eventual timing of closed loop control would be available too late to effectively guide the movement. Inverse model is built with learning and experience.

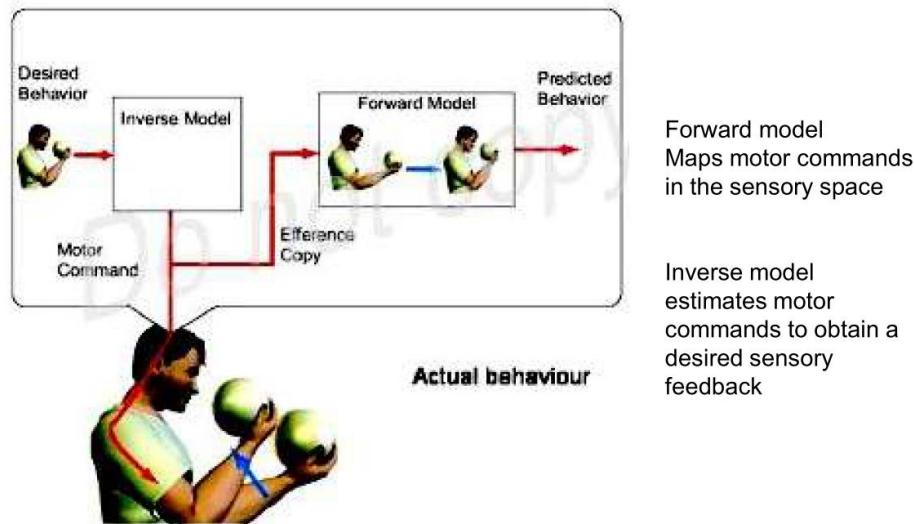
Note: the eventual error can't correct the current movement execution, but it can be used to correct the inverse model itself (adaptive models).

An example is the **Vestibulo-Ocular reflex (VOR)**, which is not actually a reflex, but a voluntary task. This task keeps the scene fixed while I rotate my head.

To execute a feedforward control of a given movement and later on to integrate a feedback control based on sensorial information due to actual executed movement, we introduce a second internal model (**forward model**) that has the task of predicting the effect of outgoing motor commands. The forward model mimics external world aspects and motor system aspects to predict a causal relationship between actions and their consequences.

It has as input, the efference copy, and as output, the predicted behaviour, the predicted sensory feedback caused by our own action. This way, it allows to

- Estimate the state (without delays and noise);
- Reduce the sensory feedback due to our own action.



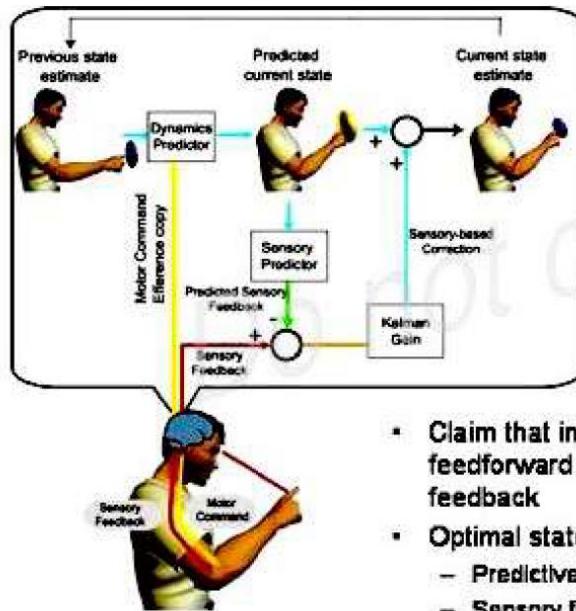
Efference copy + forward model allows us to distinguish between our own actions and external events.

The experimental proof of the existence of the forward model is the “Two eyes for an eye”. Usually when two kids fight, each one says that the other kid kick him harder. With this experiment, it has been shown that both kids tell the truth and that the escalation of perception is a consequence of natural neural elaboration.

A possible reason is that this process comes from a predictive step where consequences of our own movements are anticipated and used to attenuate the perception of the related sensations.

Why can't you tickle yourself? The consequences of our own actions are perceived differently from the same input due to external actions, because we have a different sensorial processing of what comes from our own action.

When there is a difference between the predicted movement and the actual executed one, there is a growing difference between prediction and sensorial feedback and therefore perceived sensation is not attenuated (or less attenuated). These results support the hypothesis that tactile perception attenuation is self-produced, it is due to tactile prediction correlated to stimuli. It is the correlation (and not the movement itself) between movement and produced sensation (self-produced) that determines the attenuation of the perceived sensation.



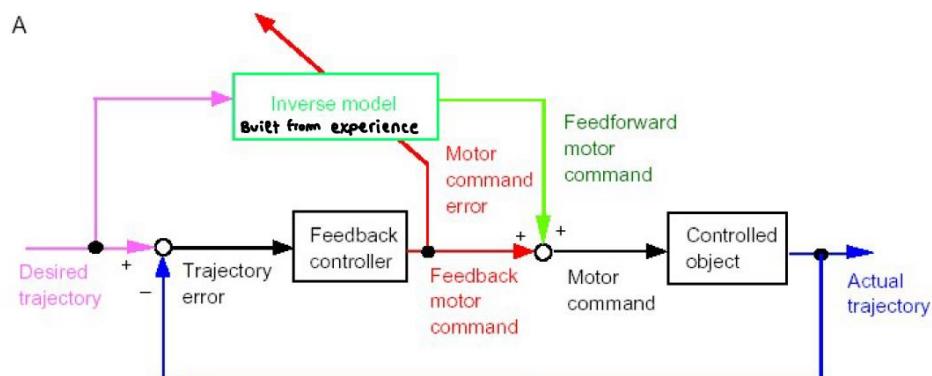
Re-afference and Kalman filter

- Claim that initial movement is feedforward and then final part is feedback
- Optimal state estimation is a mixture
 - Predictive estimation (FF)
 - Sensory feedback (FB)

The Kalman filter module, with a sort of “gain”, modulates the reciprocal role of forward model and effective perception, feedback contribution. If something happens that is different from our prediction, we perform correction.

If you don't know how to play tennis, you rely more on feedback. If you know how to play, you rely more on planning. This is what Kalman filter do.

How to train the inverse model?



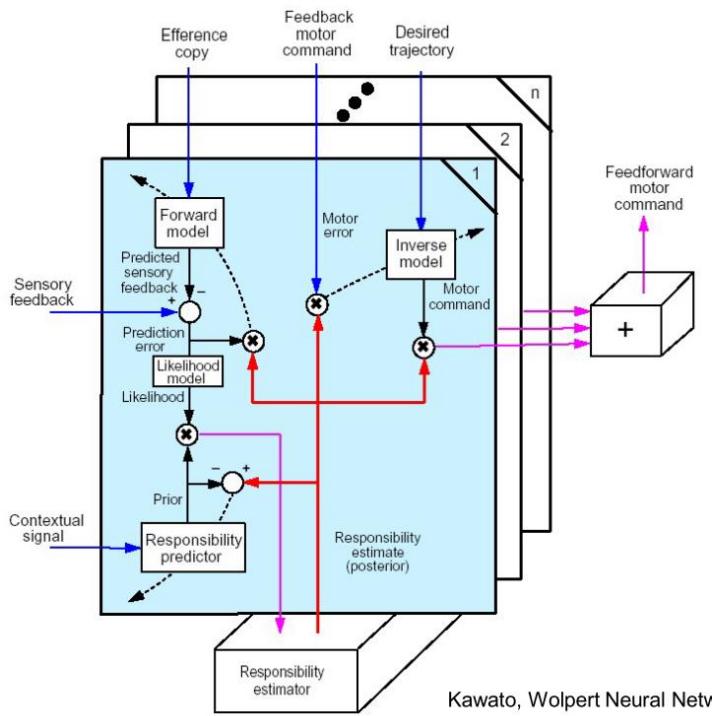
The problem of training an inverse model is strictly linked to the unavailability of a physical error on model output, thus an error on motor commands is unavailable. In fact, we do have an error signal measured by sensorial data (e.g. if we move an arm, we can have visual error feedback), but we do not have any information about the error on motor commands that generated the movement. Or again, when we speak, we perceive the error in terms of acoustic stimulus, but not as muscle error.

Kawato et al. (1996) proposed the existence of a cerebellar feedback controller. The feedback controller transforms trajectory errors (e.g. sensorial coordinates) in motor command feedbacks, that are used to

update inverse models. Training signals represent sensorial error signals converted in the motor command coordinates.

Skilled motor behaviour relies on accurate predictive models of both our own body and the tools we interact with. As the dynamics of our body change during development and as we experience tools which have their own intrinsic dynamics, we need to continuously acquire new models and update existing models. Thus, forward models are not fixed entities but must be learnt and updated through experience. Forward models can be trained and updated using prediction errors, that is by comparing the predicted and actual outcome of a motor command. Well-established computational learning rules can be used to translate these errors in prediction into changes in synaptic weights which will improve future predictions of the forward model.

Multiple paired forward-inverse model



The figure shows a paired forward-inverse model. There are N modules that are represented as different sheets. Here are shown details for the first module and the interaction between different modules are possible thanks to the responsibility estimator.

Each module is composed by three parts that interact. The first two parts, the forward model and the responsibility predictor, are used to determine module responsibility. This “responsibility signal” (fuchsia line) reflects the degree to which the single module gets the actual context and therefore to which degree it should participate to motor command. The objective is that multiple models learn to subdivide experience so that at least a forward model could predict the consequences of a

given action in a given context. The similarity that a particular forward model could get is determined through its prediction error. The smaller the error, the better the sensory feedback and the efference copy of this model are suited for the present context and the greater the module’s responsibility. In any case the forward model can be used only once the movement started and action results are known. To define a module’s responsibility before the movement (initial guess), a responsibility predictor is needed. Responsibilities are used to control forward model learning, where the error received by each module is proportional to the responsibility weight. This way a competitive learning takes place that gives more responsibility to different modules depending on action and context where it is executed.

The third component in each module is the controller that has to be learned and it has to correspond to each forward model. The controller is the inverse model that generates the motor commands, given a desired trajectory. The inverse model has to learn the control signal of the context where its paired

forward model gives a good prediction. Error signals for inverse module learning are weighted with the same responsibility degree and therefore the better the prediction of the paired forward model was, the more the paired inverse model gain part of the error signal and therefore it is trained to better answer to the context.

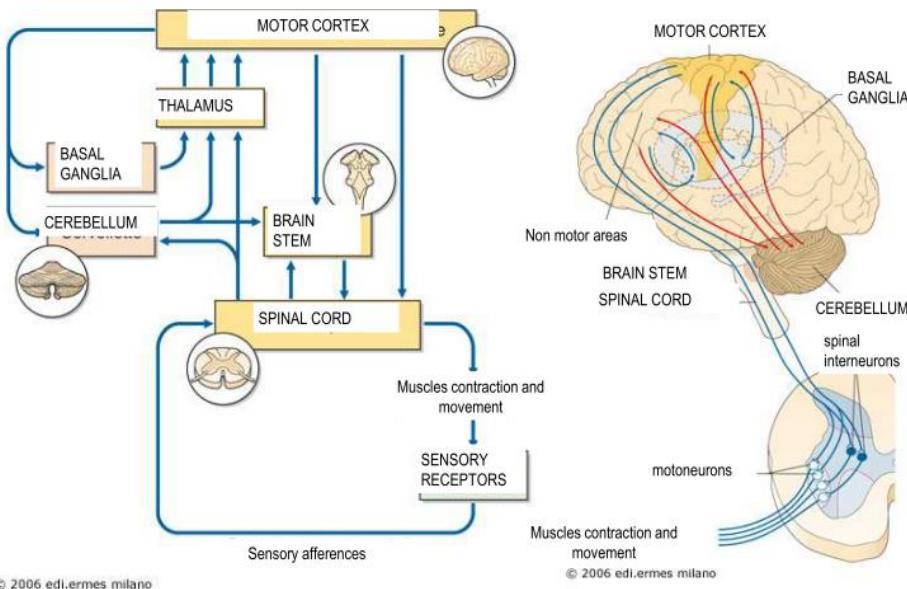
Responsibilities signal weight the motor command, output of each module, and form the general feedforward motor command.

Do these models actually exist? On the one hand, we applied control theory to understand human motor control behaviour and we draw from this approach of investigation the existence of internal models. On the other hand, we need to go back to neurophysiology to prove that the organization of human brain indeed includes these models.

As a matter of fact, the demonstration is not so straight forward, the human brain is still mysterious to neuroscientists, and a lot of research in neuroscience is currently ongoing on these topics (for example US BRAIN INITIATIVE and EU flagship HUMAN BRAIN PROJECT).

We're now presenting the major areas of the brain involved into motor control and for each of them we summarize the features.

Neural bases of Motor control



Motor systems are organized hierarchically and parallelly. Motor areas of the cerebral cortex can influence spine directly or through the Brain Stem (Encephalic trunk). Sensorial afferences are conveyed to motor cortex through Thalamus, directly or after a pre-processing of the Basal Ganglia and/or the Cerebellum. We can highlight 5 loops:

1. Spine loop (arc reflex)
2. Spinal-cortex loop
3. Cerebro (cortex)-cerebellum loop
4. Cerebellum-spine loop
5. Cortex- basal ganglia loop

Primary motor cortex (M1): area from which it is possible to evoke movements with the minimum stimulation intensity.

Motor maps (somatotopic organization) were built through electrical stimulation. Each group of neurons were associated to a particular motor district based on “the first neurons that produce activation in the given motor district”, e.g. neurons that have the lowest threshold for that motor district activation.

While a less intense stimulus can evoke a single muscle contraction, a single muscle can always be activated through stimulation of different cortical districts. This demonstrates that neurons from different cortical districts project on the same muscle. Moreover, most stimuli activate more muscles, whereas we can only rarely observe single muscle activation. In fact, cortico-spinal terminations of a single axon diverge, and they distribute over different motor neurons that innervate different muscles.

Somatotopic motor cortex organization is not fixed, but it can be modified with learning and after brain lesions. E.g. tennis player has a bigger arm map and a piano player has bigger finger map.

In a classic experiment, Evarts (1960s) showed that during wrist flexion, the firing frequency of M1 neurons change in relation to the force that the animal had to exercise rather than the amplitude of the movement. The activity of M1 neurons code direction and intensity of the force needed to produce a movement rather than joint angle variation.

Tanji and Evarts found another unexpected property of M1 neurons. The activity of Basal M1 neurons changes while the animal is expecting the signal to start the movement in the selected direction (motor task execution with delay) called SET RELATED activity. This property demonstrates that the intention to do a movement modifies the firing of some M1 neurons some hundreds of milliseconds before movement execution.

But, the activity of a cortico-motor cell and the activity of target muscle are NOT directly correlated.

The multiplicity of motoneuron connection allows that activity is flexible and it can well adapt to the motor task it has to execute. In fact, they demonstrated that depending on the movement objective (e.g. precision grip vs force grip), primates have a different cortical activation pattern.

The largest part of movements involve many joints and they require synergistic, sequential and accurate muscle activations in time. Which movement aspects are coded in M1? Do M1 neurons code spatiotemporal characteristics? Or do they code global movements aspect such as direction, amplitude and angle variation?

Georgopoulos' experiment (1982). A pool of neurons from an M1 area vigorously fire before a movement starts and during its execution with a quite broad directional sensitivity.

Hp: movement direction is coded by a pool of neurons and not by a single neuron. Georgopoulos built a population vector. The single neuron contribution was represented through a vector whose amplitude indicates the activity level that is presented in the selected movement direction. Single neuron vector direction was selected as the one where the neuron has the highest firing rate. This direction was kept constant during movement direction variation, while its amplitude was modulated with firing rate (with respect to maximal one). Then for each movement direction, he summed the contribution of all neurons from the population, and he constructed the population vector. The population vector directions were surprisingly corresponding to movement directions.

While changing force movement direction, the firing rate changes as well. This way it has been demonstrated that activity of M1 neurons code lower level parameters such as force that muscles have to develop, and high-level parameters such as those related to hand trajectory during reaching movements. CM codes for the force required to maintain a trajectory.

The cortex includes 6 billion neurons, it is thick 1/8 inch and it has a very uniform structure base of 6 layers of neurons.

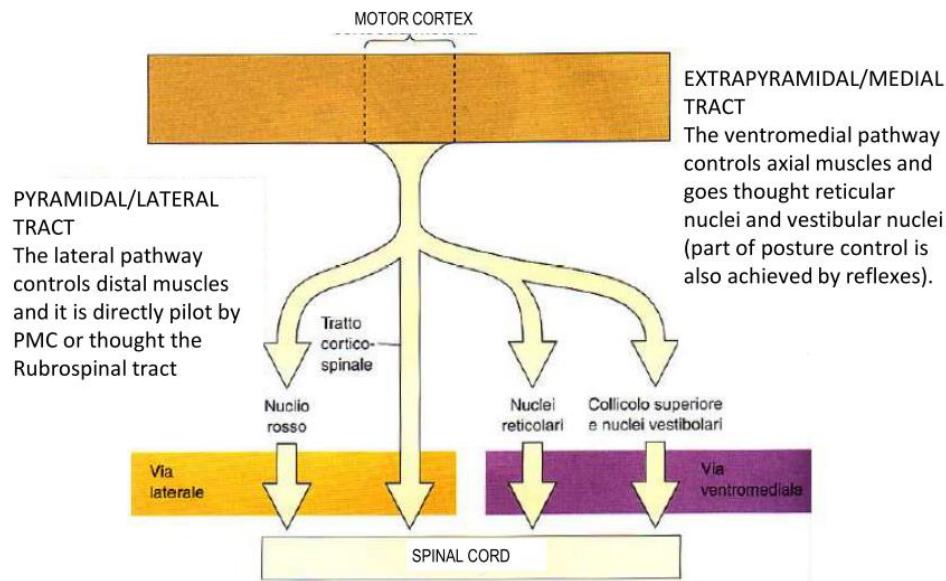
The uniform organization would suggest that there might be a common computational principle operating across cortex... which is it? We still don't know! Research in neuroscience is aiming at proving answer to this issue.

Primary Motor Cortex has a double fold control:

- A low-level control of single muscles (homunculus)
- A high-level control of multiple muscles depending on High content motor parameters

Exercise and training modify both functions.

SPATIAL ORGANIZATION OF SPINAL CORD NEURONS



Flexor-extensor rule: motor neurons that innervate flexor muscles are located posteriorly to motor neurons that innervate extensor muscles.

Proximal-distal rule: motor neurons that innervate distal muscles (e.g., hand muscles) are located lateral to motor neurons that innervate proximal muscles (e.g., trunk muscles).

Descending motor pathways are organized into two major groups:

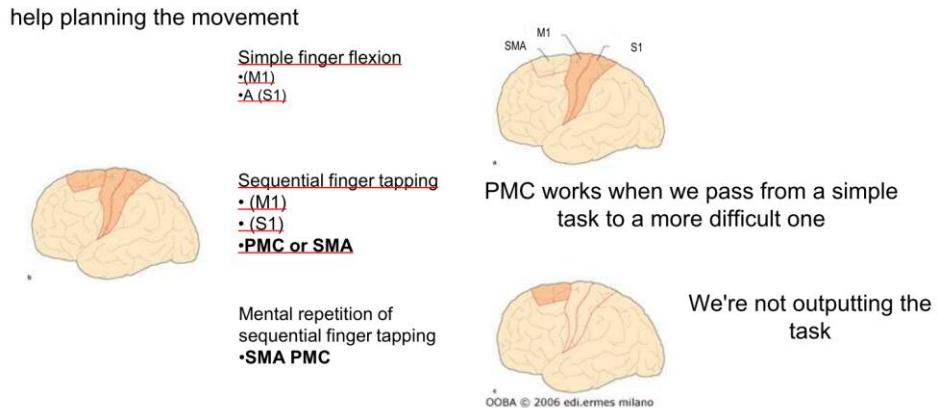
- **Lateral pathways (pyramidal) control both proximal and distal muscles** and are responsible for most voluntary movements of **limbs**, arms and legs. They include the lateral corticospinal tract and rubrospinal tract.
- **Medial pathways (Extrapyramidal) control axial muscles** and are responsible for posture, balance, and coarse control of axial and proximal muscles. They include the vestibulospinal tracts (both lateral and medial), reticulospinal tracts (both pontine and medullary), tectospinal tract, anterior corticospinal tract.

The **spinal cord** has an important role in cyclic motor task. Rhythmic tasks are a combination of voluntary tasks and reflexes. The trigger is, usually, volitional while the continuation is based on spinal reflexes.

Rhythmic motor tasks include rhythmic repetition of gestures and alternate coordination of two sides or of different body parts. They are controlled by spinal circuits and brain stem, under the supervision

of cortex. A tonic action of the motor cortex is translated into oscillatory activity, central pattern generators. Their existence at spine level is recognized but their functioning is still not fully understood

Supplementary motor area and Premotor cortex: they are activated in case of complex motor tasks and to program sequences.



Motor cortex receives the desired trajectory from Premotor cortex and frontal lobe, receives information about sensory data from the Sensory Cortex and the parietal lobe and receives inputs from the cerebellum (through the deep nuclei and the thalamus) and projects to the spinal cord and to the cerebellum (through the Pons) and the nuclei. Thalamus is the point where most of the sensory feedback go before going to other places.

Neural bases of Motor Control – Focus on the Cerebellum

The **cerebellum** is a small structure, located in the occipital part. It receives more inputs than creates outputs (inputs = 40*outputs).

Inputs are info on the objective of motor actions, info on the motor commands, sensorial feedback signals associated to the planning and execution of movements.

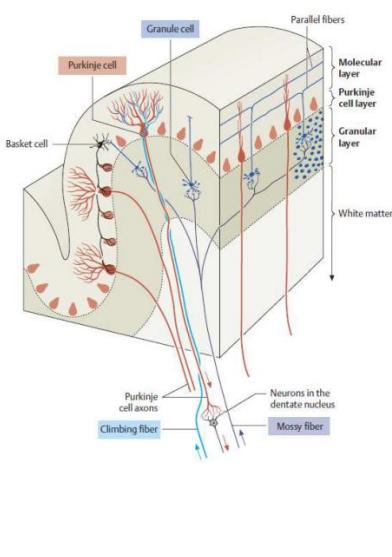
The output projections of the cerebellum are focused mainly on the premotor and motor systems of the cerebral cortex and brain stem, systems that control spinal interneurons, and motor neurons directly. It has the property of modulation of the input/output connections (adaptation and motor learning: synaptic plasticity).

The activity of neurons of three major spinal pathways involved in locomotion control (reticulo, vestibulo and rubro-spinal) are compared in case of a physiological functioning of the cerebellum and after cerebellum removal. The overall activity is extremely modified by the removal of the cerebellum, even if the capability to walk is still preserved by the patient.

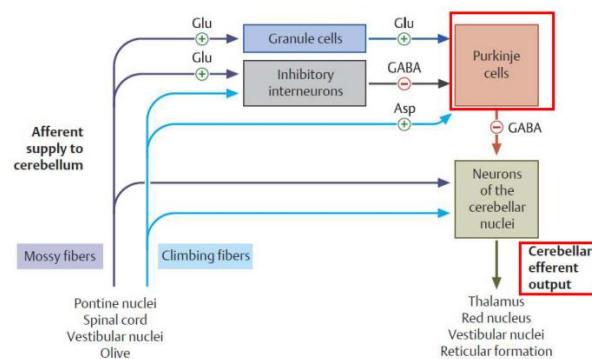
Cerebellum has a very specific structure. Differently from the cortex, we know more about interconnection of cerebellum. It's the part of the brain more studied in computational models.

The two main inputs are represented by **mossy fibers** (mf) originating in various brain stem and spinal cord nuclei, and by **climbing fibers** (cf) originating from the IO (Inferior Olive).

The structure of the cerebellum is formed by the **granular layer** (containing GrC, granular cells, bodies and GoC, Golgi cells) and the **molecular layer** (containing PC, Purkinje cells, SC, Stellate cells, and BC, Basket cells) and the **parallel fibers** (pf- axons of GrC).



Purkinje are processing some common inputs and some specific inputs.



In the granular layer, inhibition is provided by GoC, in the molecular layer by SC and BC. Finally, PCs inhibit DCN. The IO, which is also activated by brain stem and spinal cord nuclei, controls PC activity

through a single powerful synapse. Thus, the whole system can be seen as a complex mechanism controlling the DCN output.

Mossy fibers are originated from:

- nuclei in the spinal cord and brain stem carrying sensory information from the periphery
- the cerebral cortex (cortical MFs) carrying motor commands (efference copy)

They have excitatory synapses on the dendrites of granule cells (state generator; not-recurrent; sparse coding: high divergence rate of connections), which activate all the other cortical elements.

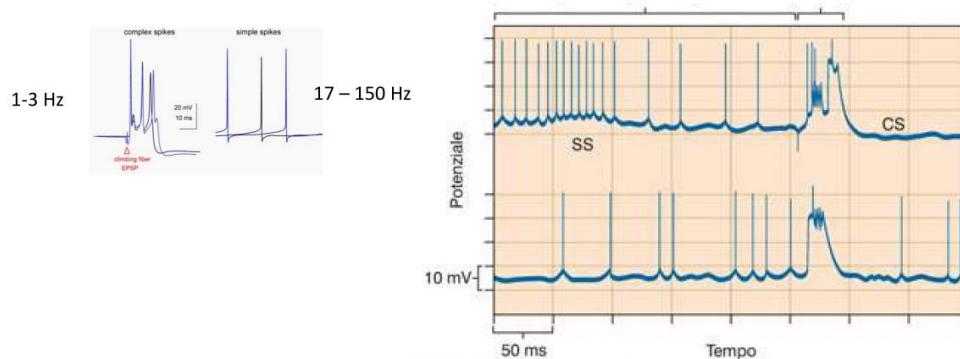
Granule cells: excite large numbers of Purkinje inducing a constant simple spike (SS). The frequency of the SS could codify the intensity and the duration of the peripheral or the behaviours generated by the CNS. They have a centre-surround coding.

Thanks to parallel fibers, a large number of Purkinje cells have the SAME input.

Climbing fibers (IO): have excitatory synapses on the Purkinje cells (generating complex spike, CS). Each Purkinje neuron receives only one climbing fiber. They generate low frequency CS; The CS could codify the temporal features of the peripheral events and/or act as starting signals for behavioural actions.

The climbing fibers wrap the Purkinje cell so that when it generates the CS, the Purkinje cell is dominated by the electrical event induced by the climbing cell. CF originate from the inferior olfactory nucleus and convey somatosensory, visual, or cerebral cortical information.

Cerebellum output:



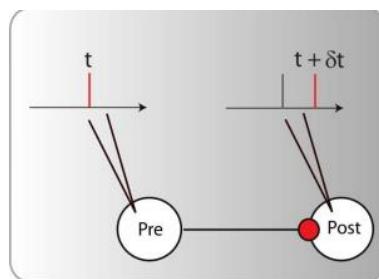
SS: simple spikes come from mossy fibers inputs; CS: occurred when climbing fiber is spiking.

The combination of the mossy fiber active and the climbing fiber active will provide a change in the synaptic connection (Purkinje cell and mossy fibers), so SS will change, and this will produce learning. Each combination will produce a different output.

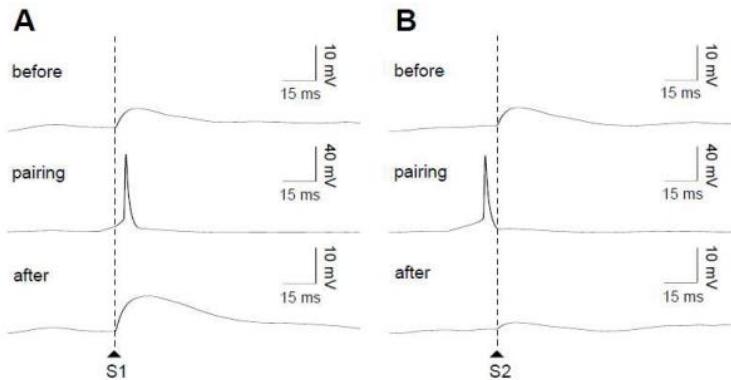
Purkinje cells receive a lot of parallel fiber and one climbing fiber.

HEBBIAN PLASTICITY: Spike Timing Dependent Plasticity (STDP)

Hebb hypothesis 1949
 Experimental proofs:
 Bi 1998, Markram 1997,
 Gerstner 1996



Experimental protocol of Spike Timing Dependent Plasticity in vitro. Pre and Post synaptic neurons are patched and forced to fire with a time difference, while the modification of the synaptic strength is monitored



The best experimental setup for exploring **plasticity** in a controlled manner is the in vitro setup. By using pairs of neurons clearly isolated and connected (using either brain slices or cultured neurons), one can patch the pre- and the post-synaptic neuron and observe the synaptic modifications between them according to their discharge.

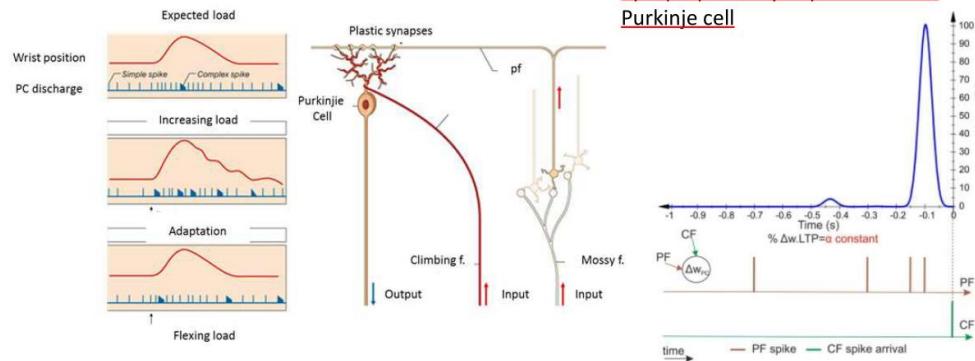
There is indeed several evidence in many brain areas that the efficiency of a synaptic connection between two neurons may be regulated by the precise timing of the joint activity of the neurons. This postulate, originally made by Hebbian, has been demonstrated in a lot of in vitro experimental studies in the form of the STDP rule. Hebb did not, however, postulate the existence of synaptic weakening.

STDP is an associative rule. As one can see in Figure, when pre-post pairings are made repeatedly at a fixed frequency of 1 Hz, with a particular time difference δt between pre and post spikes, synaptic modifications are observed whose magnitude depends on δt . The weight of the synapse is measured as the amplitude (or initial slope) of the postsynaptic potential. For positive values of δt , when pre-synaptic spike occurs before the post, the synapse is potentiated. Oppositely, if δt is negative, the synapse is depressed. Both mechanisms occur in relatively short time windows of ≈ 20 ms, and a double exponential fit made on the data is the classical shape everybody has in mind when talking about STDP. That 20 ms time scale is the time window for triggering a change, but the actual change happens much more slowly. The STDP phenomenon as seen in vitro is appealing from a theoretical point of view.

If a pre-synaptic spike occurs just before a post-synaptic one, the strength of the synapse between the two neurons tends to be increased. Conversely, if the pre-synaptic spike comes just after a post-synaptic one, the synaptic strength tends to be decreased.

This rule establishes a link with Hebb's postulate and could allow neurons to learn causal chains of information: if pre-synaptic information is important in the discharge of the post-synaptic neuron, then synapse is strengthened, otherwise it is weakened.

3 neurons



There's a change just before the CF spike. This action is different than before: if connection (or parallel) predict the spike the connection is strengthened.

parallel fiber

Changes in the strengths of parallel fiber–Purkinje cell synapses could store stimulus-response associations by linking inputs with appropriate motor outputs, following a Hebbian learning approach but with supervision of Cf discharge.

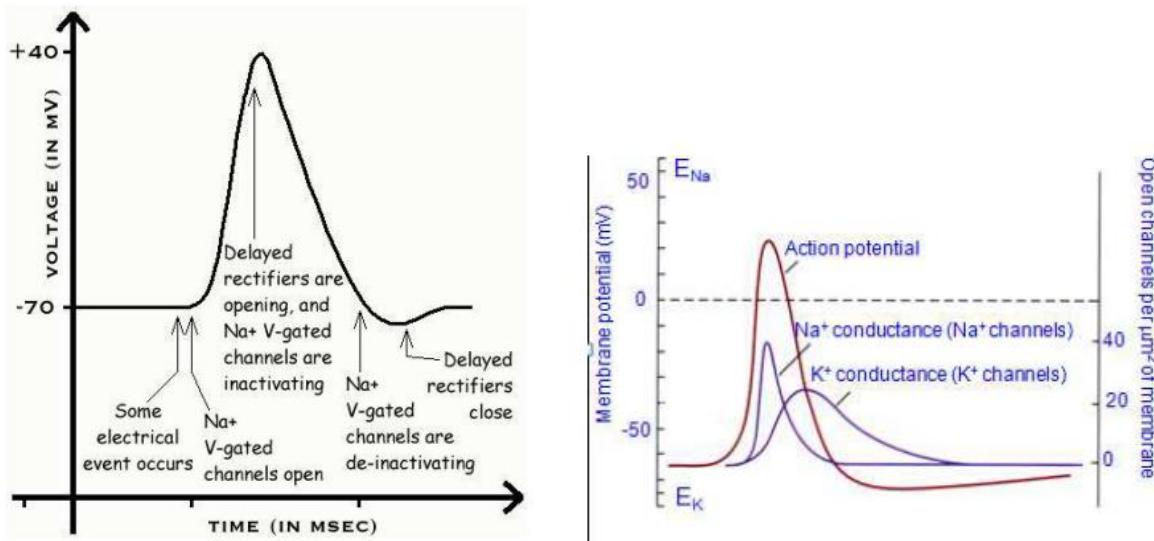
Structure and organization of the brain suggest computational analogies:

- INFORMATION STORAGE: Physical and chemical structure of neurons and synapses
- INFORMATION TRANSMISSION: electrical and chemical signaling
- PRIMARY COMPUTING ELEMENT: the neuron
- COMPUTATIONAL BASIS: still unknown!!!

Modelling and simulation

The cerebellum is specialized in supervised learning, the cerebral cortex in unsupervised learning and the basal ganglia in reinforcement learning.

The Hodgkin-Huxley model



The membrane of neurons contains voltage-gated ion-channels. These channels let through only one particular type of ion, typically Na or K, with a high selectivity. For instance, the channel for Na is called a Na-channel. Due to an exquisite mechanism that relies on conformational changes, the open probability of the channel depends on the voltage across the membrane.

When an action potential initiates, the following events happen: 1) close to the threshold voltage a few Na channels start to open. 2) Because the sodium concentration is higher outside the cell (its reversal potential is +40mV), the sodium starts to flow in, depolarising the cell. 3) This positive feedback loop will open even more Na channels and the spike is initiated. 4) However, rapidly after the spike starts, sodium channels close again and now K channels open. 5) The K ions starts to flow out the cell, hyperpolarising the cell, roughly bringing it back to the resting potential.

Consider a single compartment, or a small membrane patch (Compartmental model)

In order to calculate the membrane potential, we collect all currents. In addition to the leak current and capacitive current (which flow in rest neurons), we now (@ spike) have to include Na and K currents. Let's first consider the sodium current. The current (per area) through the sodium channels is:

$$I_{Na}(V, t) = g_{Na}(V, t)[V(t) - V_{Na}^{rev}]$$

The current is proportional to the difference between the membrane potential and the Na reversal potential. The current flow will try to make the membrane potential equal to the Na reversal potential.

The total conductance through the channels is given by the conductance of the single Na channel multiplied by the number of open channels, expressed by the product of the density of channels and the open probability. The Na channel's open probability ($P_{open}(V,t)$) turns out to factorise as the product of three switches (m) and one h .

$$g_{Na}(V, t) = g_{Na}^0 \rho_{Na} P_{open}(V, t)$$

$$P_{open}(V, t) = m^3(V, t)h(V, t)$$

g_{Na} is the open conductance of a single Na channel;

ρ_{Na} is the density of Na channels per area;

P_{open} is the Na channel's open probability.

Microscopically, the gates are like little binary switches that switch on and off depending on the membrane voltage. The Na channel has 3 switches labelled m and one labelled h . In order for the sodium channel to conduct all three m and the h have to be switched on. The gating variables describe the probability that the gate is in the 'on' or 'off' state. Note that the gating variables depend both on time and voltage; their values range between 0 and 1.

The gating variables evolve as in the next equations:

$$\begin{aligned}\frac{dm(V,t)}{dt} &= \alpha_m(V)(1 - m) - \beta_m(V)m \\ \frac{dh(V,t)}{dt} &= \alpha_h(V)(1 - h) - \beta_h(V)h\end{aligned}$$

The interesting part for the voltage gated channel is that the rate constants depend on the voltage across the membrane. Therefore, as the voltage changes, the equilibrium shifts, and the gating variables will try to establish a new equilibrium. Importantly, the m opens with increasing voltage, but h closes with increasing voltage. **The m is called an activation variable and h is an inactivating gating variable.** The inactivation causes the termination of the Na current. Because the inactivation is much slower than the activation, spikes can grow before they are killed.

n : gating variable is for K channels.

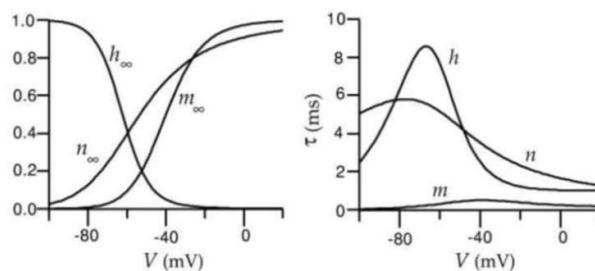
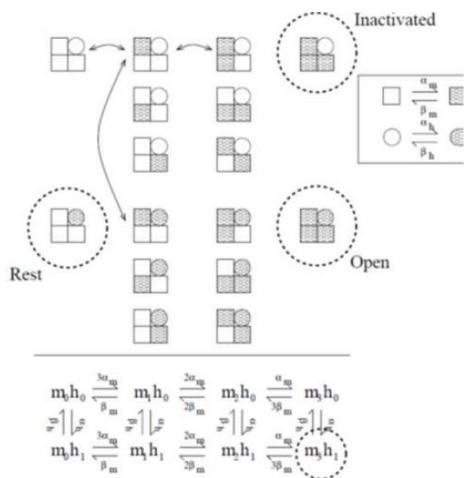


Figure 3.2: Left: The equilibrium values of the gating variables. Note that they depend on the voltage. Also note that the inactivation variable h , switches off with increasing voltage, whereas m switches on. Right: The time-constants by which the equilibrium is reached. Note that m is by far the fastest variable. From(Dayan and Abbott, 2002).

Markovian model



We can write down a Markov state diagram for a single Na channel. There are 4 gates in total (3 m's, and 1 h), which each can be independently in the up or down state. So, in total there are $2^4 = 16$ states, where one of the 16 states is the open state, Fig top. However, in the diagram it makes no difference which of the m gates is activated, and so it can be reduced to contain 8 distinct states, Fig. bottom.

In the original HH model, all gating variable are continuous real quantities, not switches. In contrast, in the stochastic version, one has a discrete number of channels each with activation variables (h, m, n) that flip between on and off states, this in turns leads to a flickering of the conductances. The rate constants give the probability that they flip. In the limit of a large number of channels, the stochastic description matches of course the original one.

The Hodgkin-Huxley model is complicated, and no analytical solutions are known. It is a four-dimensional, coupled equation (the dimension are V, h, m, n). To solve it one has to numerically integrate the equations. The simplest, but not the most efficient, way would be: initialise values for voltage (and the gating variables) at time 0. Next, we calculate the voltage a little bit later. Calculate the rate constants at the current voltage. From this calculate the change in the gating variables. From this follows the Na and K conductance. Now the new value of the potential can be calculated. Repeat this for the next time-step.

Although the Na and K channels of the HH model are the prime channels for causing the spike, many other channel types are present. The cell can use these channels to modulate its input-output relation, regulate activity its activity level, and make the firing pattern history dependent (by adaptation).

$$c_m \frac{dV(t)}{dt} = -g_{leak}[V(t) - V_{leak}] - g_{Na}(V, t)[V(t) - V_{Na}^{rev}] - g_K(V, t)[V(t) - V_K^{rev}] + I_{ext}$$

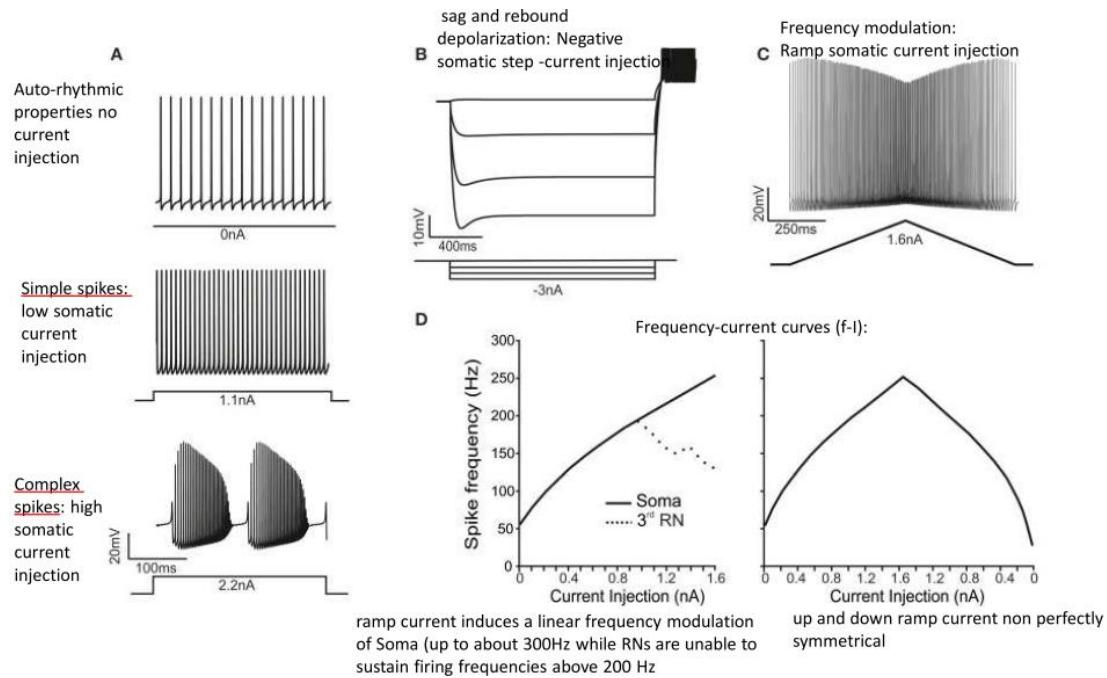
The above description is for a single compartment. If one wants to describe propagation of the spike in the axon, one has to couple the compartments like we did in the cable equation. The equation holds for each compartment in the axon. Guarantee continuity in the compartments.

$$\frac{dV}{dt} = -\frac{1}{C_m} * \left\{ \sum [g_i * (V - V_i)] + i_{inj} \right\}$$

With this model we are able to model dendrites, soma, AIS, myelin, Ranvier nodes, collateral and see, for every compartment which ions are involved.

Gating equations can be written either in Hodgkin-Huxley (HH) style or Markovian style.

To see if the model is right, we need to provide inputs to the real cell and record some feature, then give those input to the model and see the output.



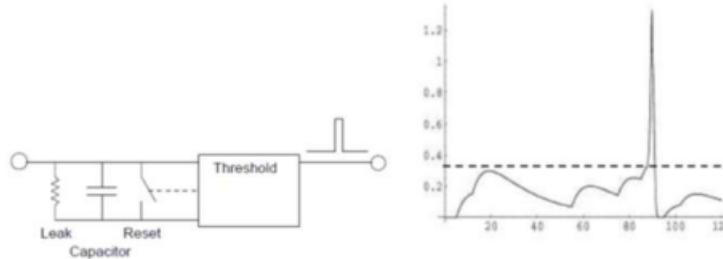
To test the model, we developed two experimentally-based protocols aimed at reproducing the main PC properties in turn. Both protocols were run after each conductance change. The first protocol was designed to test the ability of PCs to generate spontaneous firing and evaluated the PC model discharge in the absence of current injection. The second protocol was designed to evaluate the PC model electroresponsiveness upon somatic current injection.

Electroresponsive properties of the PC model. **(A)** The traces show spikes in the soma during spontaneous firing and in response to moderate (1.1 nA) and high (2.2 nA) step-current injections in the soma, demonstrating the transition from simple spikes to complex bursting. **(B)** A series of negative step-current injections in the soma determines voltage responses showing the typical sag and rebound depolarization generated by the H-current. **(C)** A ramp-current injection (from 0 to 1.6 nA and back) causes a frequency-modulated response in the PC model. **(D)** In response to step-current injection from 0 to 1.6 nA (0.1 nA steps), the PC model generates proportionately higher spike frequencies. Conversely, the RNs are unable to sustain firing frequencies above 200 Hz (dotted line). In response to ramp-current injection increasing from 0 to 1.6 nA, the f-I curve closely resembles that obtained using step-currents. However, on the way back, the f-I curve is asymmetrical.

From neuron to microcircuits

We pass from cells detailed compartmental models to single point neuron to simulate the functional properties of microcircuits.

The most common reduced model for spiking is the **integrate-and fire model**. Because of its easy and efficient implementation integrate-and-fire neurons are used frequently in modelling studies. The integrate and fire model describes the sub-threshold behaviour with a simple passive circuit:



$$C^* dV_m(t)/dt = -1/R_m * [V_m(t) - V_{rest}] + I_{ext}(t)$$

In addition, a spike threshold is supplied:

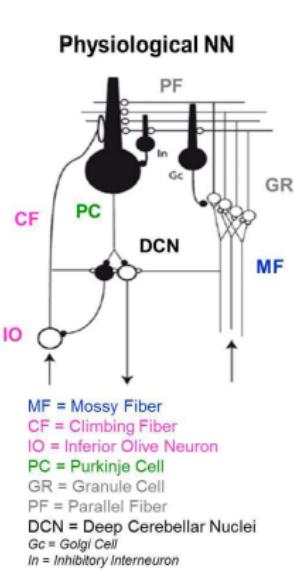
if $V > V_{thr}$ then Spike and $V = V_{reset}$

This is the so called leaky integrate and fire model. The simplification without the R_m -term is called the leak-less I&F model.

From neurophysiology to neural networks

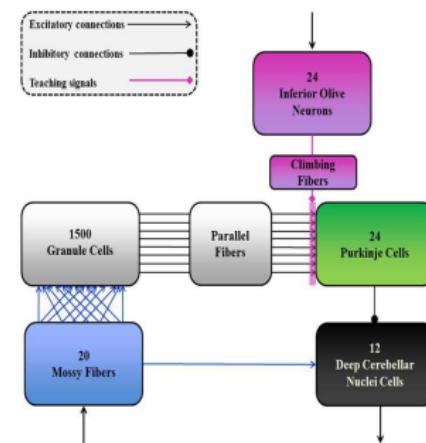
Spiking Neural Networks

The neuron activation functions are not analog values representing indirectly the spike frequency, but they emit directly spikes (0-1), based on thresholding functions (Integrate&Fire neurons).



CEREBELLUM

Modeled Spiking NN



It's mimicking as much as possible the complete model, but the parameter are compensated and tuned to act like it (bio-inspired neuron: the parameters and rate of connectivity is derived from experiments or neurophysiology).

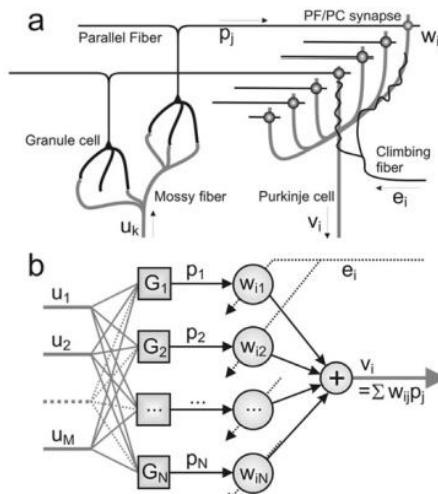
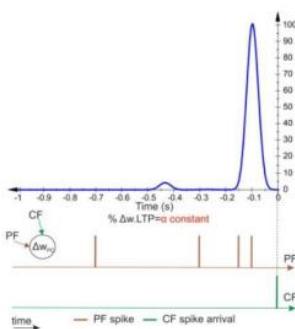
We reproduce the cerebellar architecture with an artificial spiking neural network which is composed of 100 MFs, which are connected with the 2000 granular cells of the granular layer. Each granular cell receives 4 excitatory synapses from the MFs. Half of the MFs is connected in a somatotopic way,

whereas the other half is randomly connected. There are 24 IO cells, which are 1 to 1 connected to the PC cells. PCs receive the 80% of the granular layer though the Parallel Fibers. PCs inhibit the 12 DCN, which are excited by all the MFs.

Learning/ plasticity

by correlation between PF activations and CF synaptic inputs
→ PF-PC synapses:

- Long-term depression (LTD)
- Long-term potentiation (LTP)



Changes in the strengths of parallel fiber–Purkinje cell synapses could store stimulus-response associations by linking inputs with appropriate motor outputs, following a Hebbian learning approach but with supervision of Cf discharge.

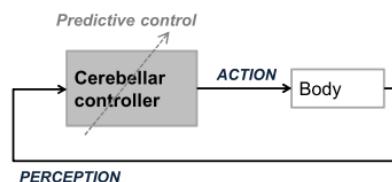
To simulate the artificial neural network, the Event Driven simulator was used.

How to check if the bioinspired microcircuit behaves as the real cerebellum?

Robotics can play a major role to this aim. Neurorobotics is the research field where neural controllers (controllers based on neurophysiology studies or on neural structure...) are embedded into the control of robots with the purpose of investigating the consequent behaviour.

For example, the previously explained spiking neural network has been embedded inside a robotic controller. How to test the emerging behaviour? Specific protocols are required.

This is easier for cerebellum than for cortex, because we know that cerebellum has a key role in learning new tasks (observations/test with ill people/people without cerebellum).



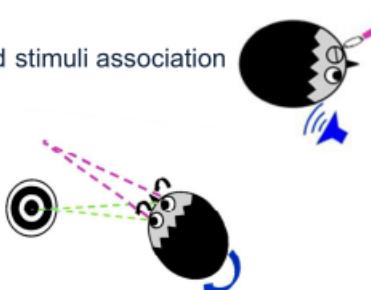
- Eye Blink Classical Conditioning (EBCC)
 - Vestibulo-Ocular Reflex (VOR)
 - Arm reaching under perturbing force fields
- **Generalized model of learning**

The cerebellar controller interacts with the body, in our case the robot, in a closed loop: receiving sensory signals, for example from the motors encoders or from visual systems, and to react to the input generating an action, changing the robot's behaviour.

We tested the same cerebellar architecture in multiple protocols in order to validate the generalizability of the model to express learning.

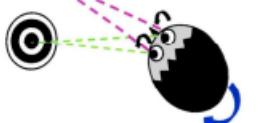
Eye Blink Classical Conditioning (EBCC)

Pavlovian associative learning of a well-timed stimuli association



Vestibulo-ocular reflex (VOR)

combined learning of timing and gain
continuous cerebellar action



Reaching perturbed by force fields

combined learning of timing and gain
continuous cerebellar action

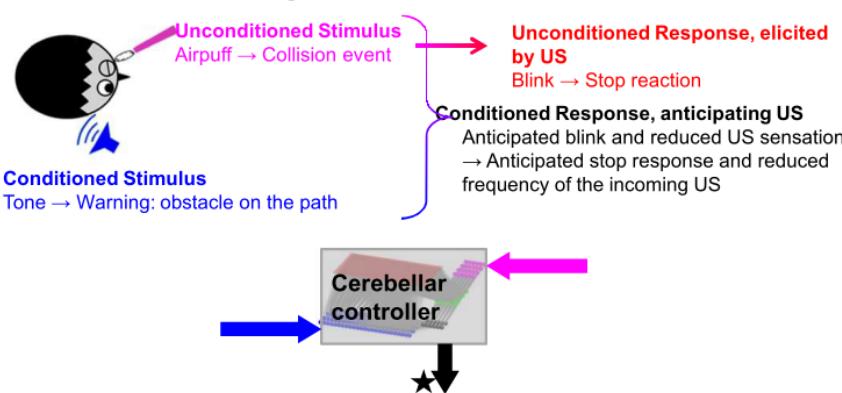


The three different tested protocols took inspiration from human physiological paradigms in which the cerebellum has a crucial role. The EBCC is a pavlovian associative learning for which the cerebellum is involved in timing association between two stimuli. The other two protocols involve both timing and gain modulation, in fact for

the VOR the cerebellar output is a continuous cerebellar action that generate the reflex, and for the reaching perturbed by force fields the cerebellar action is added to the FB controllers in order to exhibits fine motor control.

Eye Blink Classical Conditioning (EBCC) → Collision-avoidance task

Pavlovian associative learning of a well-timed stimuli association



During the EBCC physiological protocol, a human subject receives two stimuli: one conditioned stimulus, for example a tone, followed after a well-defined interval called inter stimulus interval, for example 400 ms, by a second stimulus, the unconditioned stimulus, for example an air puff toward the subject's eye. At the beginning of the protocol, the subject closes

the eye in response to the Unconditioned stimulus, this blink is called unconditioned response. After some trials of acquisition, with the presentation of CS and US paired, the subject learns to anticipate the eye closure before the US onset, generating a so-called Conditioned response (CR). We translated this protocol with a collision avoidance task.

Modelling single neurons: focus on cerebellar neurons

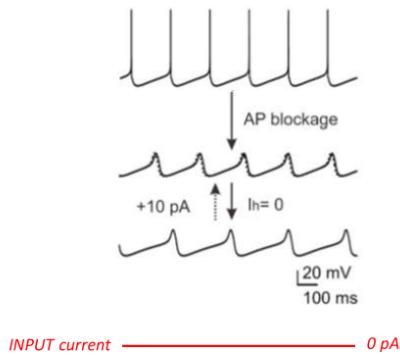
The cerebellar structure:

- Modular circuit;
- Cerebellar cortex + cerebellar nuclei;
- Distributed plasticity leading motor learning during adaptive paradigms (cortical and nuclear sites);
- It is composed by single neurons with complex dynamics.

Neurons communicate through Action Potentials (AP), change in membrane voltage depending on subcellular ion channel-mediated mechanisms. AP can be approximated with SPIKES, the basic units of neuronal coding.

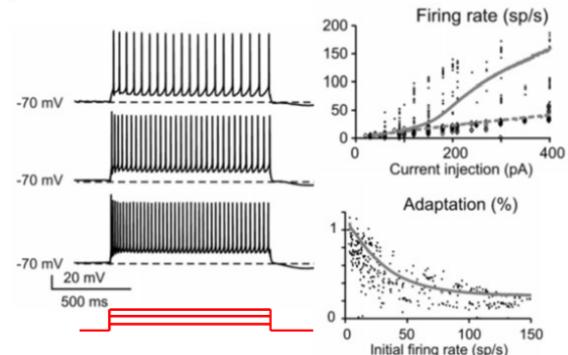
Neurons can exhibit different spiking patterns (electroresponsive properties):

- Autorhythm = spontaneous firing of neurons due to neurons' intrinsic electro-responsiveness
- SubThreshold Oscillations (STO) = sinusoidal oscillations of the membrane potential around the threshold potential value

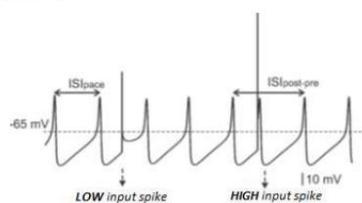


Membrane voltage of a cerebellar Golgi cell (adapted from [Solinas et al, Front Cell Neurosci, 2007a,b])

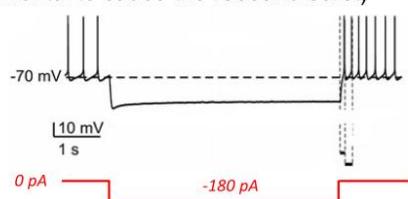
- Depolarization induced bursting = increased firing rate of neurons following the starting of a depolarizing external stimulation
- Linear current-frequency relationship
- Spike-Frequency Adaptation (SFA) = Decrease in the neuron's firing rate when stimulated with a constant input.



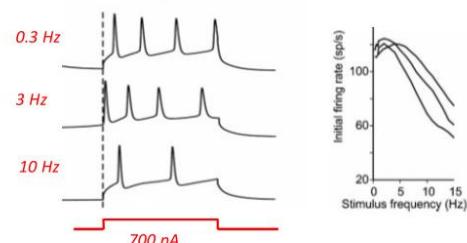
- Phase reset



- Post-inhibitory rebound burst = increased firing rate following the end of a negative hyperpolarizing input (amplitude and duration of the negative input are fundamental to cause the rebound burst)



- Resonance = maximum firing response of a neuron at a preferred frequency of the input current stimulus



FUNDAMENTAL for:

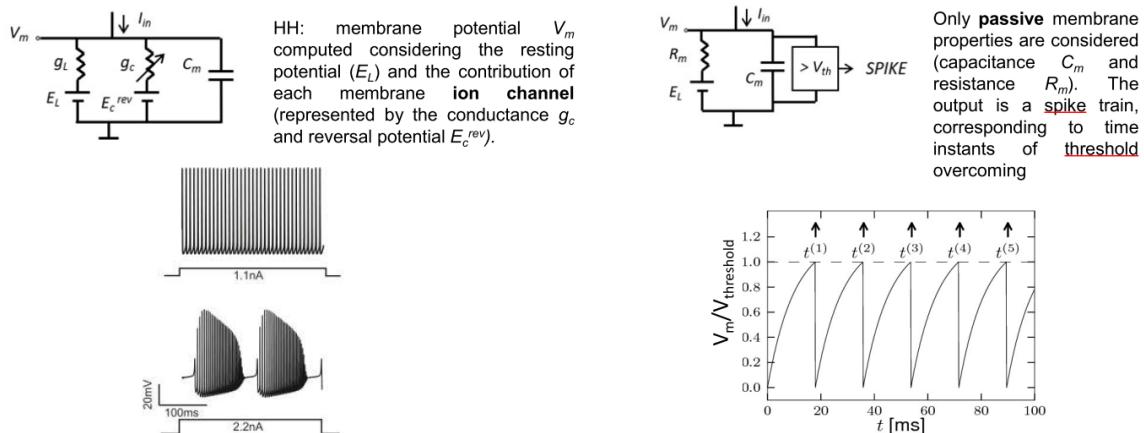
- ✓ Generating network dynamics
- ✓ Noise filtering
- ✓ Plasticity enhancement
- ✓ Communication within and among brain areas

Cerebellar cells have different dynamics in term of spiking patterns. For example, Golgi Cell fire at low frequency, instead PCs fire at high frequency.

At the input of the cerebellum, Golgi neurons (GoCs) contribute to process sensory signal coming from Mossy Fibers (MFs), shaping the activity of Granular neurons (GRs). Thanks to recurrent inhibitory loops, oscillatory and resonant properties of GoCs and GRs, the Granular layer acts as a spatio-temporal filter of sensory inputs. GR signals converge to the Molecular and Purkinje cell layers through Ascending Axons (AA) and Parallel Fibers (PFs), with a very specific geometrical organization. Purkinje cells (PCs) are the final integrators of the cerebellar cortex, inhibiting the cerebellar output that drives motor corrections. In vivo, intrinsic simple spikes of PCs are modulated by inhibition from Molecular Layer Interneurons (MLIs), while olivary inputs elicit PC complex spikes through Climbing Fibers (CFs), following sensory stimulation. Deep Cerebellar Nuclei cells (DCNs) are the only output of the cerebellar circuit, projecting to multiple brain areas, eventually contributing to the motor pathway. **Integrating the inputs from the cerebellar cortex and MFs, DCNs can modify their spontaneous firing and generate bursts to eventually tune motor commands.** They also continuously control learning processes through inhibitory feedback loops to the Inferior Olive (IO). The PC-DCN-IO loop connections are organized to form microcomplexes. The result is a modular geometrically-organized architecture, where each microcomplex integrates different sensorimotor information and operates through region-specific spiking patterns that correlate with target functional behaviour.

We can module single neurons with different level of *morphological* detail: from multi-compartment to point neuron models. **Multi-compartment** neuron models describe the activity of each neuron element (dendrites, axons, ...) taking into account morphological features. Example from the neo-cortex microcircuit. **Point** neuron models describe the activity of neurons as collapsed in a single point, neglecting compartment differences and morphological features. They represent more the computational properties of neurons, than the electrical activity and its spatial distribution.

Or we can model single neurons with different levels of *electrical* details: HH and LIF (Leaky Integrate-and-Fire).

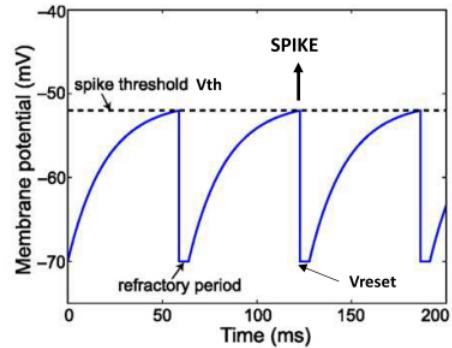


In the Leaky Integrate-and-Fire (LIF) neuron, the subthreshold dynamics of the membrane potential is modelled through a single passive term:

$$\text{Membrane potential dynamics} \rightarrow \tau_m \frac{dV_m(t)}{dt} = -(V_m(t) - E_L) + R_m \cdot I_{in}(t)$$

Spike condition → If $V_m > V_{th}$, then $V_m = V_{reset}$

- τ_m is the membrane time constant ($\tau_m = R_m \cdot C_m$, where R_m and C_m are the membrane resistance and capacitance, respectively), and it accounts for how fast the V_m curve increases
- E_L is the resting potential and it represents the steady-state value of V_m in absence of external input current
- I_{in} is the input current.
- Action potentials are approximated as **single spike instants**: whenever V_m reaches a firing threshold V_{th} , the membrane potential is reset to a fixed value V_{reset} . After the spike, V_m remains at V_{reset} value (constant) during the refractory period and it is not possible to emit spikes.



When modelling single elements of brain models, like neurons, a fundamental issue emerges: the balance between biological plausibility and computational load.

HH multi-compartment models with morphology representation has a good biological plausibility, but a poor computational load. Instead, LIF point neuron models have a poor biological plausibility and a good computational load. For this reason, LIF is used more.

There are also multi-dimensional LIF models:

- **Izhikevich (non-linear)**: it's a simplification of HH model into a 2D IF system.

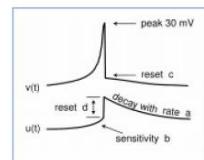
The model:

$$\begin{cases} V'(t) = 0,04 \cdot V^2(t) + 5 \cdot V + 150 - u(t) + I \\ u'(t) = a \cdot (b \cdot V(t) - u(t)) \end{cases} \rightarrow \begin{array}{l} \text{Membrane Potential} \\ \text{Membrane Recovery variable} \\ (\text{K+ activation and Na+ inactivation}) \end{array}$$

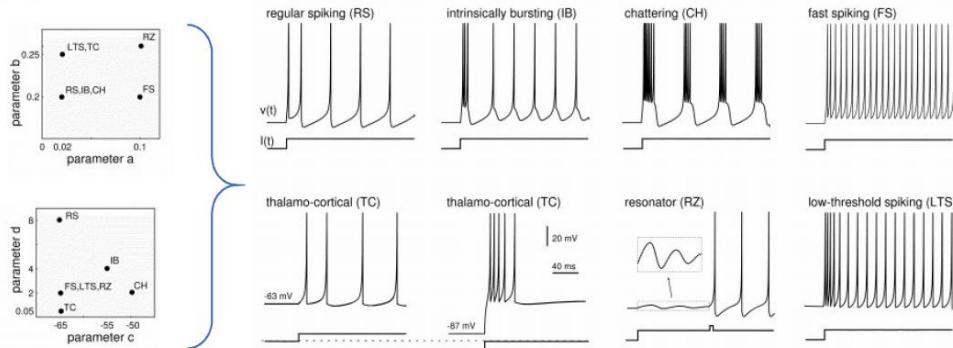
for adaptation

If $V(t) \geq 30\text{mV} \rightarrow \text{SPIKE}$:

$$\begin{cases} V(t+1) = c \\ u(t+1) = u(t) + d \end{cases}$$



[Izhikevich, IEEE Trans Neural Networks, 2003]



When neurons reach the threshold, the spike is emitted. The membrane recovery variable is summed to d . In this way we are increasing the hyperpolarization part of the equation (negative). This is a good approximation for the different behaviour of the neurons. The model has been successfully used to represent cortical and thalamic neuron dynamics.

- Adaptive Exponential Leaky Integrate and Fire (LIF) model (non linear)

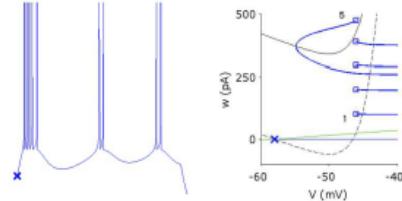
The model:

$$\begin{cases} C_m \cdot V'(t) = -g_L \cdot (V(t) - E_L) + g_L \cdot \Delta_T \cdot e^{\frac{V(t)-V_{th}}{\Delta T}} - w(t) + I \\ \tau_w \cdot w'(t) = a \cdot (V(t) - E_L) - w(t) \end{cases} \rightarrow \begin{array}{l} \text{Membrane Potential} \\ \text{Adaptive current} \end{array}$$

If $V(t) \geq V_{th} \rightarrow SPIKE:$

$$\begin{cases} V(t+1) = V_r \\ w(t+1) = w(t) + b \end{cases}$$

Regular bursting as response of the Adaptive Exponential model to a current step: left - voltage as a function of time; right - trajectories in the 2-dimensional space of voltage (horizontal axis) and adaptation variable (vertical axis). Resting potential marked by cross; sequence of reset values marked by squares. Nullclines $w'(t) = 0$ (green line) and $V'(t) = 0$ before (black dashed line) and after the current step (black line). (Adapted from Gerstner and Brette (2009), Scholarpedia, 2009)



- Properties:

- multiple electroresponsive properties based on parameter values
- replacement of the strict voltage threshold by a more realistic smooth spike initiation zone.
- subthreshold resonances or adaptation as in the Izhikevich model.

The model includes two state variables: V_m and an adaptive current coupled with V_m , which accounts for adaptation or bursting, depending on the value of the coupling constant, a . An exponential term allows a realistic representation of the action potential initiation and shape

- Generalized LIF (linear)

The model:

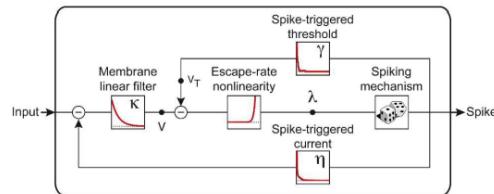
$$\begin{cases} C_m \cdot V'(t) = -g_L \cdot (V(t) - E_L) + \sum_j I_j(t) + I_e \\ I'(t) = -k_j \cdot I_j(t) \\ V'_{th} = a \cdot (V(t) - E_L) - b \cdot (V_{th}(t) - V_\infty) \end{cases} \rightarrow \begin{array}{l} \text{Membrane Potential} \\ \text{Spike-triggered current} \\ \text{Spike-triggered threshold} \end{array}$$

[Pozzorini et al., Plos Comp Biol, 2015; Mihalas and Niebur, Neural Comput., 2009]

If $V(t) \geq V_{th} \rightarrow SPIKE:$

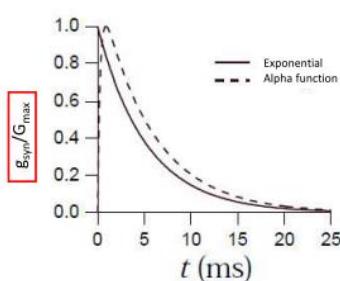
$$\begin{cases} V(t+1) = V_r \\ I_j(t+1) = R_j \cdot I_j(t) + A_j \\ V_{th}(t+1) = \max(V_\infty, V_{th}(t)) \end{cases}$$

Block representation of the GLIF model. The **membrane** acts as a low-pass filter $\kappa(t)$ on the input current $I(t)$ to produce the modeled potential $V(t)$. The **exponential nonlinearity** (escape-rate) transforms this voltage into an instantaneous firing intensity $\lambda(t)$, according to which spikes are generated. Each time a spike is emitted, both a **current** $\eta(t)$ and a **movement of the firing threshold** $\gamma(t)$ are triggered.



There are 3 state variables. The Threshold depends on the membrane potential, it's not fixed. Also, the spike threshold is not deterministic, but the neuron fire with a high probability when V is close to the threshold.

An additional current, I_{syn} , is provided as an input to the model membrane potential to model the contribution of input spikes ((conductance-based model):



Connections among simplified neuron models are usually implemented as conductance-based or current-based synapses. In the first case (more realistic), the synaptic current depends on the membrane potential of the post-synaptic neuron. At spike events, the conductance change can be modelled as an exponential or alpha function. In current-based

models instead, the post-synaptic current depends only on a kinetics function and the synaptic weight.

All these model work towards a unified point neuron model for cerebellar neurons. The aim is to build a model able to reproduce all the cerebellar electroresponsive mechanisms, while keeping:

- *Neurophysiological realism* (elements in the model \leftrightarrow biophysical mechanisms)
- *Low computational load* (\rightarrow linear and analytically solvable, to increase simulation step without losing precision within large-scale Spiking Neural Networks - SNNs)
- *Generalized features* (not fitting on single traces)
- Different sets of parameters for different cells, reproducing all the electrophysiological properties of each population, i.e. spike patterns more than sub/supra-threshold mechanisms (since within SNN)

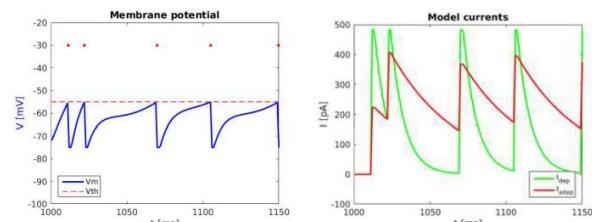
EXTENDEND – GENERALIZED LIF NEURON MODEL (E-GLIF)

- State variables:

$$\begin{aligned} \text{Membrane potential} \quad & V'_m(t) = \frac{1}{C_m} \left(\frac{C_m}{\tau_m} (V_m(t) - E_L) + I_{stim} + I_e + I_{dep}(t) - I_{adap}(t) \right) \\ \text{Spike-triggered depolarizing current} \quad & I'_{adap}(t) = k_{adap} (V_m(t) - E_L) - k_2 I_{adap}(t) \\ \text{Adaptive current} \quad & I_{dep}(t) = -k_1 I_{dep}(t) \end{aligned}$$

- Spike generation at t_{spk} :

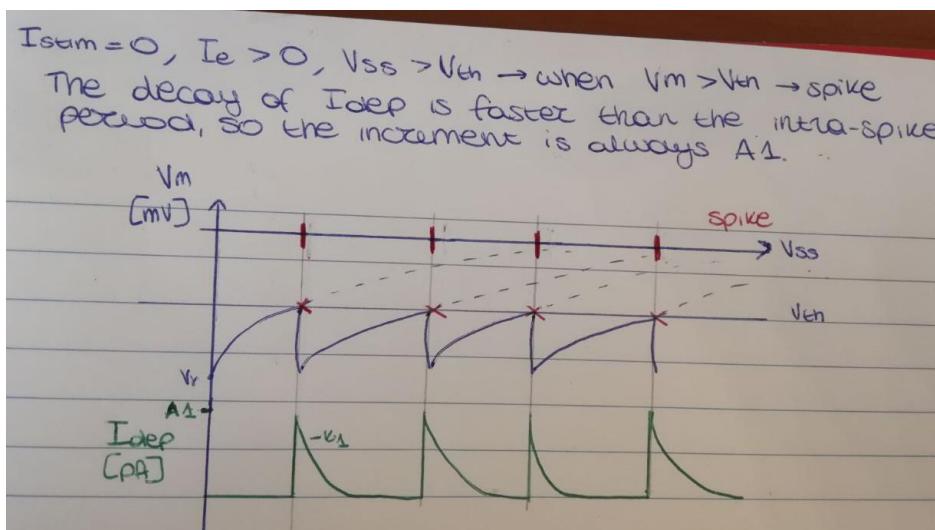
$$\begin{cases} t_{spk} \notin \Delta t_{ref} \\ rng < (1 - e^{-\lambda(t_{spk})t_{spk}}) \end{cases} \quad \begin{array}{l} \rightarrow \text{Refractory period} \\ \rightarrow \text{Stochasticity} \end{array}$$



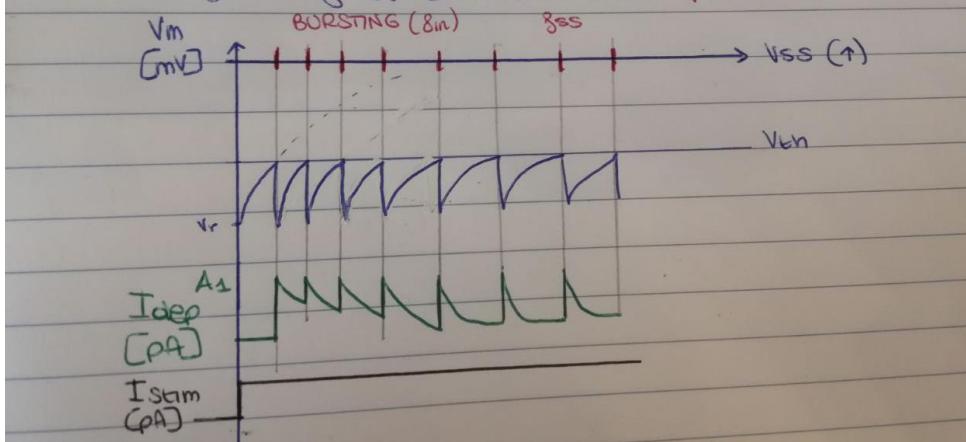
- Update rules:

$$\begin{cases} V_m(t_{spk}) \leftarrow V_r \\ I_{dep}(t_{spk}) \leftarrow A_1 \\ I_{adap}(t_{spk}) \leftarrow I_{adap}(t_{spk} - 1) + A_2 \end{cases}$$

When arrive at thr emits the spike and then go back to the start value.
[Geminiani et al, *Front Neuroinform*, 2018]



$I_{stim} > 0$, V_{ss} increases, so the rise of V_m increases and we will have a spike before one of the conductances is 0, so we will depolarize the cell. This will lead to the bursting at the beginning of the stimulus. ($f_{in} > f_{ss}$)



In addition to the leaky current term, $\frac{C_m}{t_m}(V_m - E_L)$, each one of the membrane currents defined in the model (I_e, I_{adapt}, I_{dep}) accounts for a different mechanism that can be properly parameterized: I_e is an endogenous current modelling the net contribution of depolarizing ionic currents generating autorhythmicity.

I_{adapt} is an adaptive current, usually hyperpolarizing, which is characterized by a small spike-triggered increment (A2) that decays thereafter according to k_{adapt} and k_2 . I_{adapt} models the activation of potassium channels generating a slow hyperpolarizing current. Since I_{adapt} activates slowly while I_{dep} is already decaying, the balance between the two currents generates spike-frequency adaptation and afterhyperpolarization. Moreover, by being coupled with V_m by k_{adapt} , I_{adapt} endows the model with the capability of generating post-inhibitory rebound bursting, intrinsic subthreshold oscillations and resonance.

I_{dep} is a depolarizing spike-triggered current, which has a larger spike-triggered increment (A1) and faster decay (k1) compared to I_{adapt} . I_{dep} mimics the fast (almost instantaneous) activation and deactivation of sodium channels. I_{dep} can generate depolarization-induced excitation and sustain post-inhibitory rebound bursts.

The parameters in the model include those directly related to neurophysiological quantities ($C_m, t_m, E_L, t_{ref}, V_{th}, V_r$ in blue), that are fixed for each specific cell type, and the more abstract ones related to neuron-specific functional mechanisms, that need to be optimized ($k_{adapt}, k_2, k_1, A_2, A_1, I_e$, in red).

The biological parameters (in blue in model equations) were fixed to biological values taken from literature or available from animal experiments or databases. For the other neuron-specific functional parameters (tunable parameters, highlighted in red), we developed an optimization strategy based on

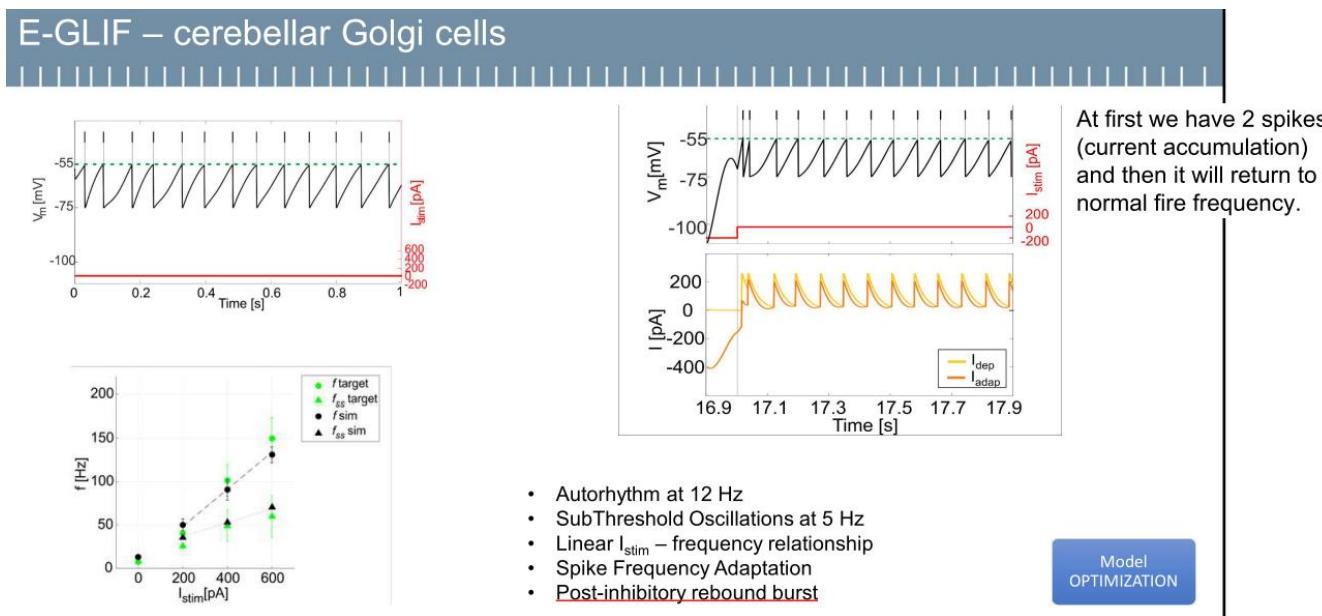
a desired input-output relationship, considering a current step I_{stim} as the input and spike times as the output.

By computing the analytical solution of the model, we were able to associate different regions in the parameter space to different system responses (i.e. exponential or oscillatory and stable or unstable).

For the optimization we need to define parameters, cost and constraints, in order to modelize the input-output relationship.

E-GLIF model and optimization were applied to reproduce the complex electroresponsiveness of cerebellar Golgi cells (GoC). GoCs are the main inhibitory neurons in the granular layer of cerebellum and are responsible for reshaping the input signals coming from mossy fibers.

In single-cell recordings, GoCs show spontaneous firing around 8 Hz, a nearly-linear input-output relationship (about 0.25 Hz/pA), input-dependent spike-frequency adaptation when depolarization is maintained, rebound bursting after hyperpolarization, phase-resetting, subthreshold self-sustained oscillations and resonance in the theta band (around 3-6 Hz).



If $I_{stim} = 0$, the neurons can fire. Even if we decrease I_{stim} , the steady state frequency doesn't change, because it depends from the initial condition.

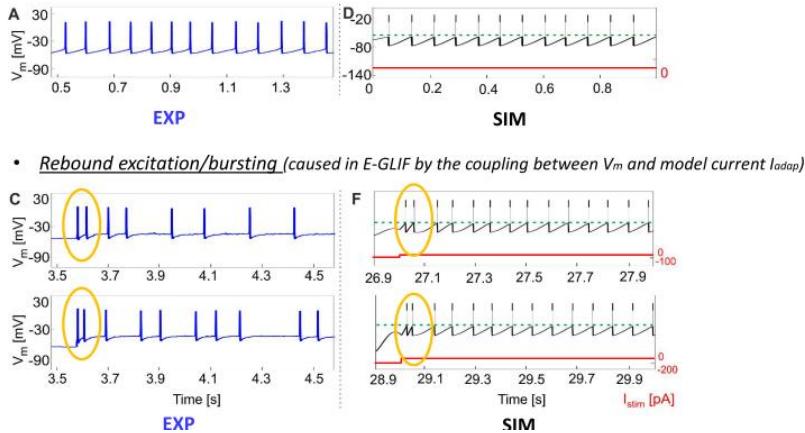
The linearity between I_{stim} and response f was maintained over multiple I_{stim} levels.

With increasing input current steps, the balance of model currents at the end of stimulation resulted in a pronounced hyperpolarization before returning to autorhythm. This effect, that was not observed in physiological recordings, was due to the high value of I_{adap} at the end of current steps required to achieve spike-frequency adaptation.

Looking at $f - I_{stim}$ relationship, experimental recordings and model behaviours evidently differed in the steady-state response rate (f_{ss}) that may be related to experimental mechanisms not modelled in

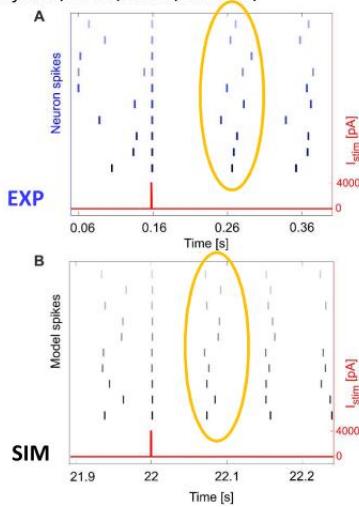
simulations. Indeed, the experimental f_{ss} values were lower than in the model, thereby reducing adaptation in the model compared to experiments.

- Autorhythm (caused in E-GLIF by model current I_e)

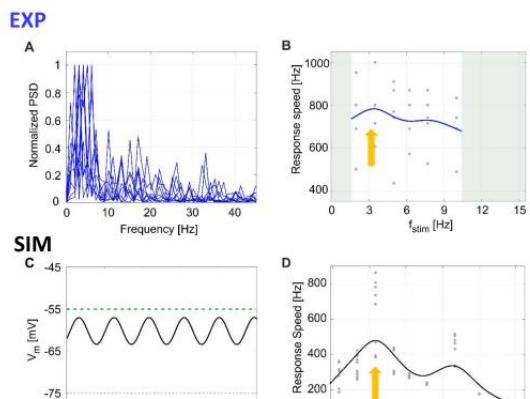


The autorhythm was stably reproduced; The rebound burst systematically occurred as a doublet after a hyperpolarization and its internal speed and latency increased, with higher absolute values of the preceding negative current steps, consistent with experimental results.

- Phase reset (after impulse current, spiking latency independent from pre-impulse spike time)



- Oscillations (E-GLIF second order dynamics) and resonance (oscillation-driven) in theta band



Response speed = mean spiking latency in resonance steps for increasing stimulation frequencies

If we give an input in the oscillation band, the response will be higher

An important phenomenon evident in GoCs (as well as in some other brain neurons) is phase-reset, which allows desynchronization within sub-circuits triggered by strong impulses (left).⁷

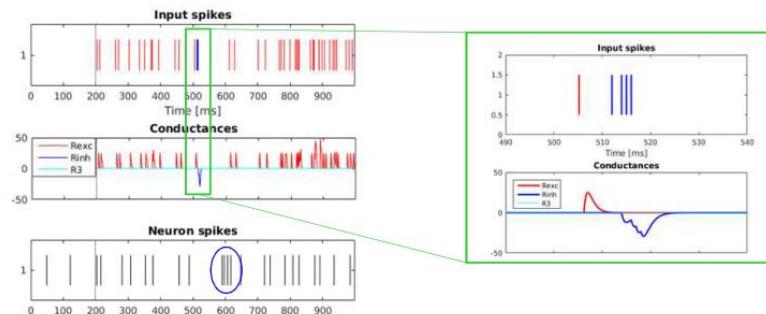
The GoC E-GLIF was able to reproduce this feature (Figure left B), thanks to the coupling between I_{adap} and V_m that caused a rapid increase of I_{adap} when V_m value raised following a huge external pulse; this blocked spike generation for the same time interval independent from the phase of autorhythm before the pulse, resetting the cell's phase of the autorhythm.

Finally, another property evident in GoCs and often observed in central nervous system neurons is the presence of endogenous subthreshold oscillations, which provide a fundamental mechanism for

efficient network intercommunication and plasticity. Oscillations are correlated to resonance, and both have been shown to depend on intrinsic membrane properties. GoCs work as a band-pass filters by amplifying the input in the theta band.

E-GLIF – synaptic inputs

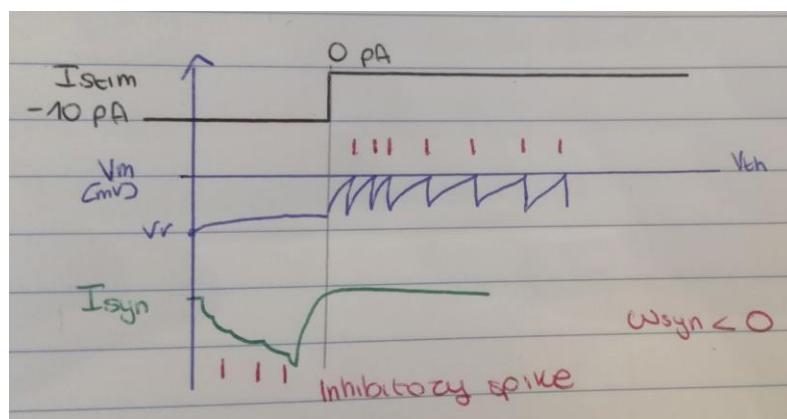
3 receptors with **alpha conductance-based synapses**, for synaptic inputs from different neural populations:



- Increased firing irregularity ($CV = 39\%$)
- Rebound burst following inhibitory input burst

The excitatory spike train increased irregularity in neuron firing, consistent with the irregular spiking of GoCs in vivo. The inhibitory spike train generated a rebound burst in E-GLIF after the end of the input stimulus, due to the intrinsic changes of the model currents. This result confirmed the ability of GoC E-GLIF to reproduce the rich variety of electrophysiological properties of GoCs.

If we have a non-constant stimulus, the spike frequency will change: during inhibition there will be no spike, then we will see the bursting and, in the end, the normal spike.

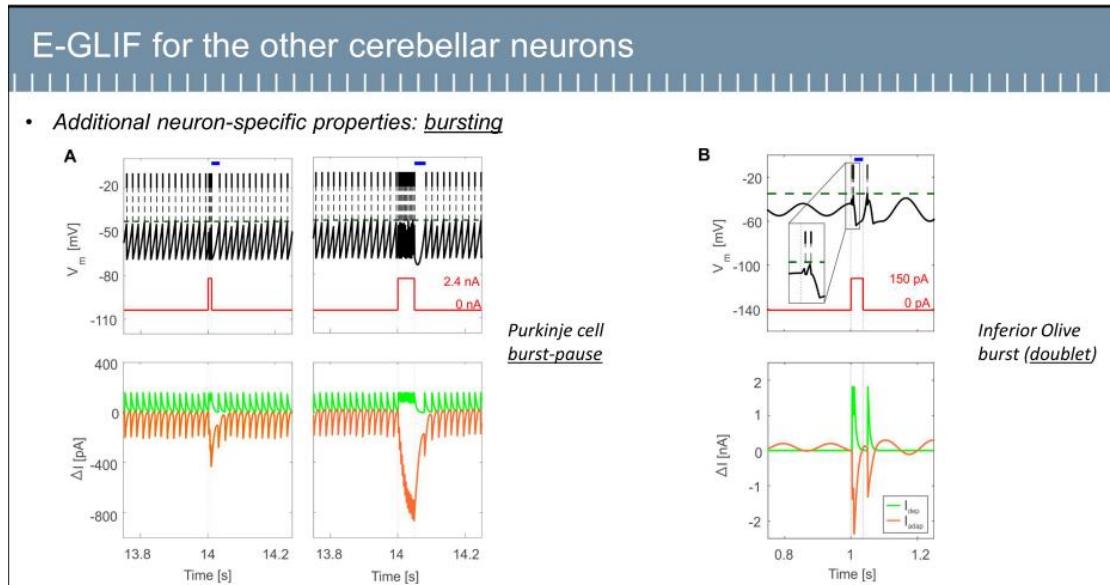


The E-GLIF was applied also to other cerebellar neurons.

In absence of external stimuli, PC, MLI and both DCN E-GLIF produced irregular autorhythm at physiological frequencies, while GRs and IOs generated STO at 6 and 7 Hz, respectively. At the end of a hyperpolarizing current step, PCs and DCNs exhibited rebound excitation (doublets/bursts), which is

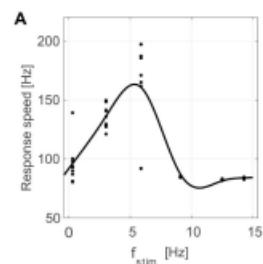
fundamental for efficient signal transmission (right). In IOs, post-inhibitory rebound spikes were generated with 50% probability, as in experiments.

All the bursting are increasing the fire rate and then go back to a normal fire rate.

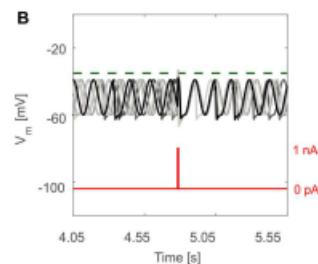


When stimulating PC with current pulses of 2.4 nA, the typical intrinsic bursting (burst-pause response) was generated. This was achieved thanks to the balance of model currents, I_{dep} and I_{adap} that accounted for subcellular mechanisms leading to PC complex spikes.

- Additional neuron-specific properties: oscillation-driven properties



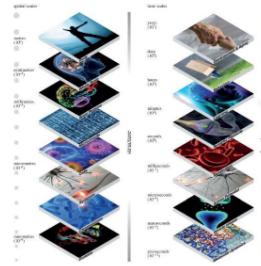
Resonance in theta band for Granule cells



Phase reset of subthreshold oscillations for Inferior Olive neurons

Multiscale simulations of the cerebellar circuit - CerebNEST

- Role of **single neuron properties** in cerebellar Spiking Neural Networks:
 - They are proved and fundamental electrophysiological properties → increased reliability of model.
 - Possibility to evaluate single neuron simulated dynamics, in physiological and pathological conditions (Geminiani et al., IJNS, 2018).
 - They generate mechanisms which are altered in pathological conditions (e.g. oscillatory mechanisms) and restored during possible treatments (e.g. neuromodulation).
- Multiscale model of the cerebellar circuit:
 - Single neuron dynamic properties in the point-neuron models represented as E-GLIF
 - Plasticity mechanisms [D'Angelo, *Prog. Brain Res.*, 2014]
 - Morphology-based large-scale network connectivity (based on a cerebellar scaffold [Casali et al., *Front Neuroinform*, 2019] and Allen Brain Atlas)
 - Extra-cerebellar connectivity → common simulation platform is fundamental! (NEST – Neural Simulation Tool) [Gewaltig et al., *Scholarpedia*, 2007]
 - Increase the realism of the model and then the reliability of simulations and the robustness of predictions based on the simulations' outcome
 - Integrate the model in the common HBP infrastructure



Based on novel experimental evidence, the update and validation of cerebellar model elements, can lead to more realistic simulations of mechanisms at multiple scales: single neuron dynamics, network responses and, eventually, sensorimotor signal encoding. This is of paramount importance if we want to use computational models to help neuroscientists understanding the neural bases of behaviour and also to explain pathological conditions.

Neuroengineering for biology

Electronical tools to interface neuronal networks

Molecules → single neuron → microcircuit → network of neurons → brain map and control system → behaviour.

In the present figure, we can see the different “functional layers” concerning the neural system. We stop our investigation at the neural network level. Why do we stop there? Because the microbiology literature has investigated the single neuron level and the interaction between single neurons in details. Those levels are well known and they are something that is firm knowledge. However, the content of information is not given by the single neuron activity, but it is given by a network of connections. It is not easy to interface the natural neural network to study information flow, and therefore we need good instruments to study at the natural neural network level.

Both the single-neuron level and the CNS-level are well known but the dynamics of neural networks is still far from elucidated. For this reason, studies at this level of analysis are essential to deeply understanding neural pathways. This question opens some very interesting technological challenges.

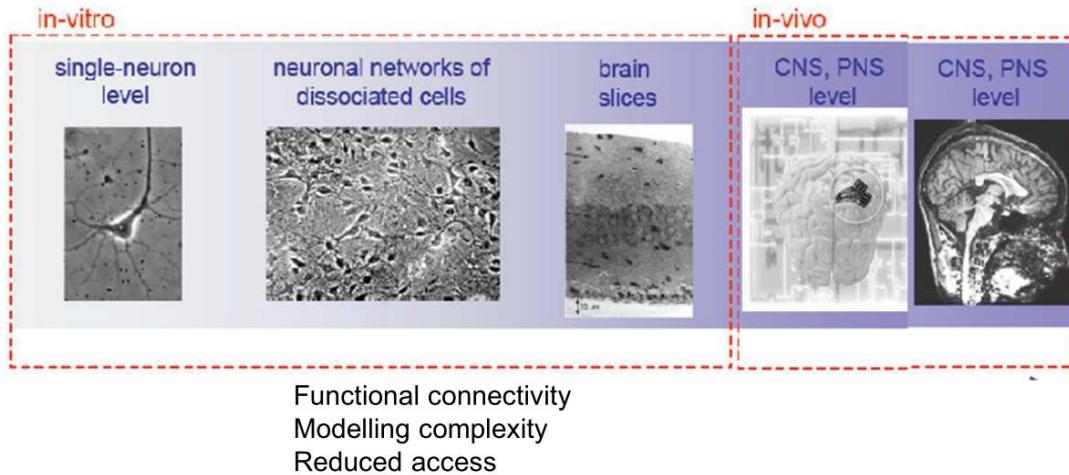
Because of the huge amount of cells in the brain what we can know by in-vivo experiments is very limited and concerns a subsystem where the boundary conditions are not under control (i.e. the activities of the other connected areas). Deep brain recording has been done on primates and animal and only very rarely on humans (and anyway only on pathological subjects as epileptic kids in order to know the area of the epileptogenic foci or on very few (a couple) experiments for controlling robot with cortex matrices of electrodes on SCI patients...).

Or we can have some deep recording from the PD patients who are undergoing DBS implants during the surgery to check for the position of the electrodes, but these are short recordings...

Anyway, deep recording is done by matrices of electrodes in the case of cortical recording or by wires with electrodes in the case of deep brain. To have a rough idea of the size of these data, we can have about 10 contacts on each wire, about a few wires implanted (about 10, max), each electrode records the signal of about a few cells (2-5)... so, at the end, we are recording a few hundreds of cells (10×10^5) in an area, where many thousands of neurons are indeed working together... the undersampling is quite strong!

The other possible source of information is by using in vitro models. It should be noted that in vitro models require suitable technologies to interface with the model itself. The method has to be respectful and not to perturb what you want to measure. While from a reading technological point of view, in vitro is simpler than in vivo, in vitro models are to be properly designed and a lot of effort of biologists has been devoted to proper culture solutions able to mimic the physiological environment and to assure that networks developed in vitro are behaving as in vivo ones.

This step can't be taken for granted, preparation of culture needs always to follow detailed validated protocols and often baseline behavior needs to be tested before performing any specific experiment. Neurons are very delicate cells, and in vitro neuronal culturing is quite challenging, especially for assuring glia cells nutrients and proper perfusion of medium, besides temperature, Ph, Humidity and so forth. Medium change is for example a procedure that perturbs the neurons activity but still it is not fully measured the impact of this perturbation...



In vitro experiments

- In-vitro experiments can be used to study the small functional cellular structures:
 - Slices: are functional naturally grown tissues extracted from the brain and then analyzed (real tissue extracted from a real brain)
 - Cultured neurons: are embryonic dissociated neuronal cells which are coltured in vitro and built the neuronal network directly in vitro. The system is then completely autonomous but it is only a model of natural functional networks. (built in vitro)
 - Human patient specific IPS cells differentiated to neuron-like... great challenge for future research!
- **Multimodal approach is pursued to get the maximal information rate usually the different approaches are consecutive in time (depending on the goal)**

Cult neurons have formed a network, which is stand alone.

In slices we cut a network, so we're looking only to a single part of the network.

IPS send back to stem cell and then ridifferentiate to another cell.

The users' requirements:

- stimulate the same population of neurons
1. Simultaneous record + stimulate of **hundreds individual** neurons
 2. Long acquisitions (days and months): maintain stable contact
 3. Monitor transmembrane potentials (-80;+30mV)
 4. SNR able to catch subthreshold transmembrane potentials ($\pm 0.5\text{--}10$ mV with a rise time of <1 ms and a slow decay time of 100–1,000 ms), and spike occurrence and spike oscillations (up to 50Hz)
 5. record APs with amplitudes of ~100 mV and duration of 1–500 ms (long APs for recording from cardiomyocytes).

Traditionally the functional properties of neurons have been investigated using conventional electrodes, such as glass micropipettes, thus allowing neurophysiologists to disclose a detailed picture about the single cell properties, e.g. the receptor sensitivity and ion channel gating.

At the single neuron level, the standard procedure is the patch clamp. The invention of intracellular recording and stimulation technologies were hallmark developments that enabled the biophysical ‘language’ by which individual neurons transmit electrical information, communicate and ‘compute’ subthreshold synaptic information to be deciphered. The power of intracellular recording systems is that they exhibit very good electrical coupling with the cell and provide accurate readout of the entire dynamic range of voltages generated by cells without distorting the readout over time. Yet, the use of sharp or patch microelectrodes is limited to individual neurons as steering of the electrode tips into target cells requires the use of bulky micromanipulators and the duration of intracellular recording sessions is limited by mechanical and biophysical instabilities.

Intracellular electrophysiology: patch clamp

The traditional intracellular electrophysiology has some advantages:

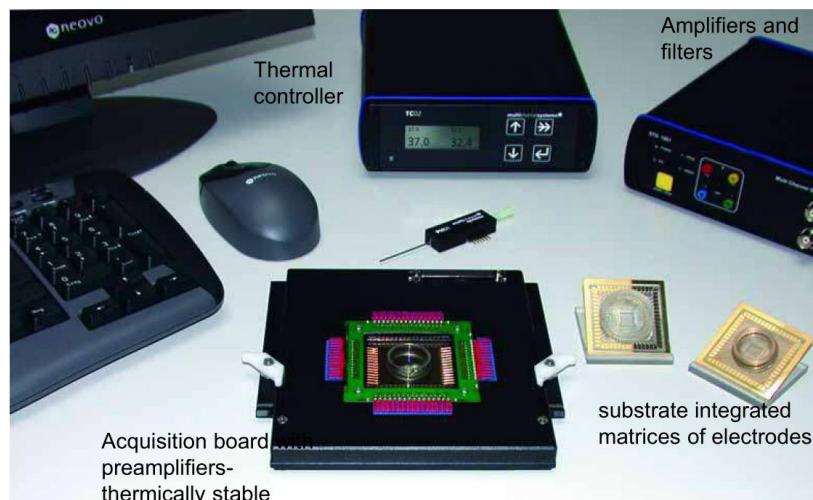
- The locally, well identified cause-effect link is perfectly known
- There is a correspondence between morphology and function
- Good SNR and dynamic response

The traditional intracellular electrophysiology has some limits as well:

- It is invasive and therefore it perturbs the neuron which dies just after the measure.
- The spatial resolution is linked to the number of clamps that are placed on the neural culture. The positioning has to be done at the microscope, and it is not possible to place more than 1 or 2 clamps. Investigation at the network level is not possible with this method.
- There is an intrinsic biophysical and mechanical instability that prevents to prolong experiments over few hours.

We know exactly who we are recording. The problem is that we can read only a part of the network; this could lead to the death of the cell because it is perforated.

Multi electrodes arrays (MEA)



The MEA-Systems record, amplify, and analyze signals from biological samples *in vitro*. The data is analyzed by the included data acquisition software. MEA-Systems are used to record from brain or cardiac slices, neuronal or cardiac cultures, *ex vivo* retina, cell lines or stem cells.

Multielectrode arrays (MEAs) or microelectrode arrays are devices that contain multiple plates or shanks through which neural signals are obtained or delivered, essentially serving as neural interfaces that connect neurons to electronic circuitry. They are based on substrate integrated matrices of electrodes for the recording of extracellular activity. There are two general classes of MEAs: implantable MEAs, used *in vivo*, and non-implantable MEAs, used *in vitro*.

We're not recording the voltage across the membrane, but the external voltage. MEA are microscopic slides where electrodes are inserted. Connections between each electrode and the board for data collection are of course isolated. The obtained measure is an extracellular measure with respect to a reference electrode, thus a voltage difference is measured. It should be noted that each electrode does NOT correspond to a single neuron. MEA has a spatial resolution that corresponds to a cluster of neurons. The standard type of *in vitro* MEA comes in a pattern of 8 x 8 or 6 x 10 electrodes. Electrodes are typically composed of Indium tin oxide or titanium and have diameters between 10 and 30 μm . These arrays are normally used for single-cell cultures (i.e. many cells but all of the same type) or acute brain slices. In case of recording *in vitro* slices, one major issue in order to obtain quality signals concerns that electrodes and tissue must be in close contact with one another. The perforated MEA design applies negative pressure to openings in the substrate so that tissue slices can be positioned on the electrodes to enhance contact and recorded signals.

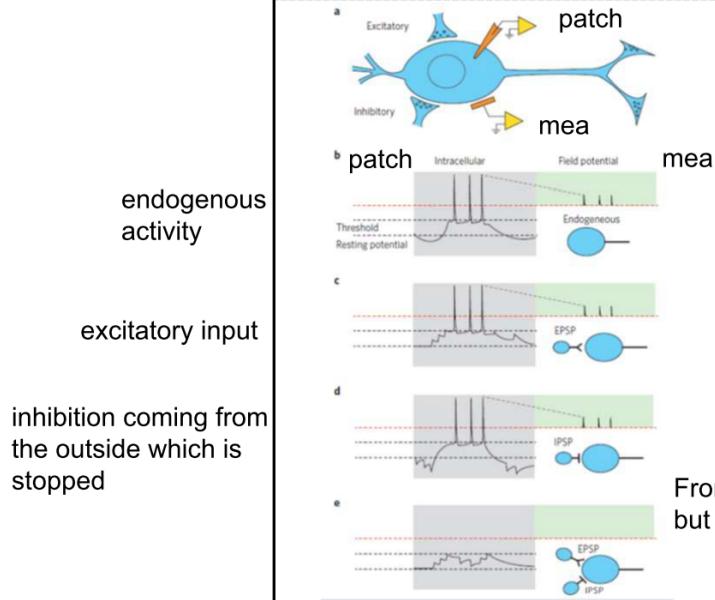
A different approach to lower the electrode impedance is by modification of the interface material, for example by using carbon nanotubes, or by modification of the structure of the electrodes, with for example gold nanopillars or nanocavities.

We're moving from intracellular to extracellular voltage recording. MEA is also recording the activity of the whole network, not only the one of one neuron.

The extracellular space is conductive as well, and although the resistance is very low, it is not zero.

According to Ohm's law ($V=R*I$), the extracellular current results in a small voltage that can be measured with extracellular electrodes. Extracellular signals are smaller than transmembrane potentials, depending on the distance of the signal source to the electrode.

Extracellular recording: the problem of dark neurons



endogenous activity
excitatory input
inhibition coming from the outside which is stopped

"Whatever sorting algorithm is applied, it remains the limit that MEA recordings could not provide information on as to whether a firing of an individual neuron is triggered by endogenous mechanisms, a barrage of incoming excitatory inputs or the cessation of inhibition... this information is typically available only to intracellular recordings across neuron membrane."

Spira and Hai, 2013
From the field potential we see the same behaviour, but if we see the inside, the behaviour is different

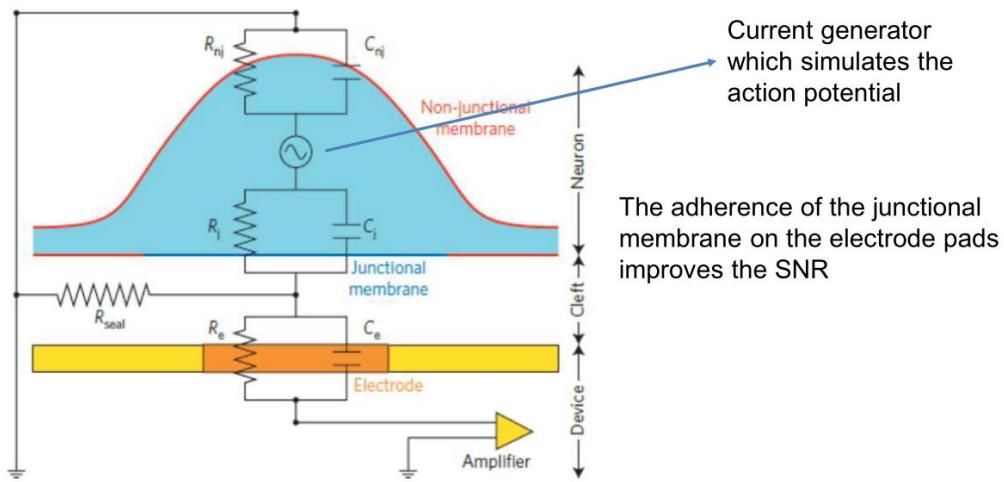
Endogenous membrane properties as well as excitatory and inhibitory synaptic inputs regulate the firing patterns of individual neurons. This is depicted in the schematic of a neuron (blue) that receives an excitatory and an inhibitory synaptic input in a Subthreshold and supra-threshold electrophysiological activity of the neuron is recorded by an intracellular (upper orange electrode) and an extracellular (lower orange electrode) electrode. The amplifiers are depicted in yellow. The intracellular recordings are shown in the left panels of b–e, and the corresponding extracellular recordings are shown in the right panels (green background).

In b, a neuron endogenously generates a train of APs (of approximately $\Delta 100$ mV) by depolarization of the membrane potential from the resting value of approximately -80 mV (bottom dashed line) reaching a threshold level to fire APs (middle dashed line) at about -50 mV, and then the membrane potential endogenously repolarizes. The extracellular electrode picks up the FPs generated by the APs (marked by vertical lines and green background). Note that the recorded FP amplitudes range between 0.01 and 1 mV and are not drawn to scale. The attenuation factor ($1/100$ to $1/1000$) is so large that subthreshold potentials generated by individual neurons cannot be recorded. Thus, the extracellular electrode is practically 'blind' to the subthreshold events (grey background, below the red dashed line).

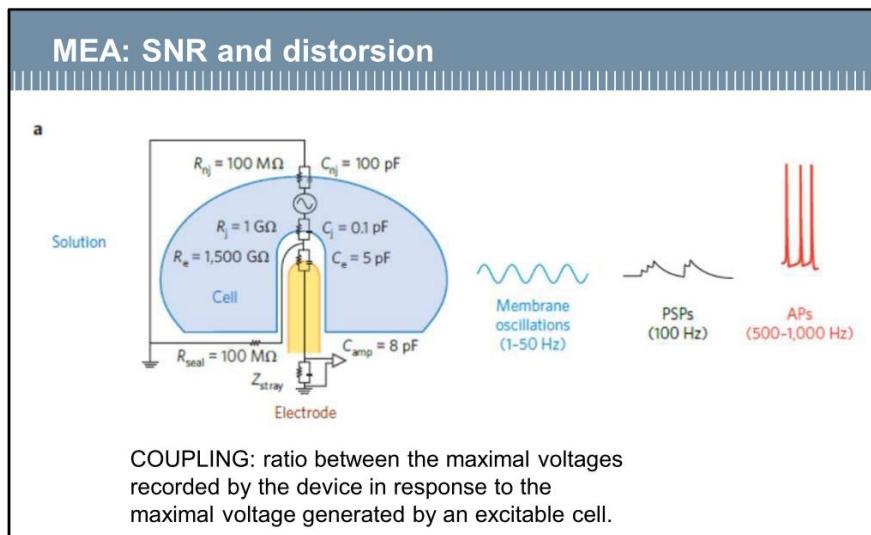
In c and d the very same pattern of APs firing is generated by excitatory (c) and inhibitory (d) synaptic inputs. Whereas in c summation of excitatory synaptic potentials depolarizes the neuron to reach the firing level, and the neuron stops firing when the barrage of the excitatory inputs stops (leading to membrane repolarization), in d the train of APs is generated by dis-inhibition (the cessation of the barrage of inhibitory synaptic inputs).

The significant differences in these mechanisms (b–d) cannot be detected by the extracellular electrode. Furthermore, unless an individual neuron is firing APs, synaptic inputs are not 'visible' to the extracellular electrodes at all (e). In this example, the extracellular electrode does not detect the presence of a neuron that receives a barrage of excitatory and inhibitory synaptic inputs. These inputs may be of significant importance to the functioning of the neuronal circuit.

How can we improve the design of extracellular recording in order to get the most of that recording? We want to reduce the junction between the electrode and the cell and have a bigger electrode.



Depending on cell's morphology around the electrode, the electrical signal measured by each electrode is composed by different signal sources. The contribution of each source to the signal recorded by an electrode is a function of the distance from the source to the electrode. The recorded signal is affected by the membrane, the medium, the electrode pad etc. which act as capacitors. The recorded signal is therefore a sum of different contributions.



The electrical coupling between a neuron and a sensing pad is defined here as the ratio between the maximal voltages recorded by the device in response to the maximal voltage generated by an excitable cell.

The surface area of the junctional membrane can be anywhere between a very small fraction of the cell surface area, up to approximately 50% in cells that flatten while adhering strongly to substrate-integrated sensing pads. This variable depends on the geometry of the sensing pad and the morphology and adhesion characteristics of the specific cell. The junctional membrane can thus be of very high resistance and low capacitance. This implies that only a small fraction of the current generated across the neuron's membrane, flows through the junctional

membrane. Reduction of the junctional membrane resistance would be very effective in improving the electrical coupling coefficient between a neuron and an electrode.

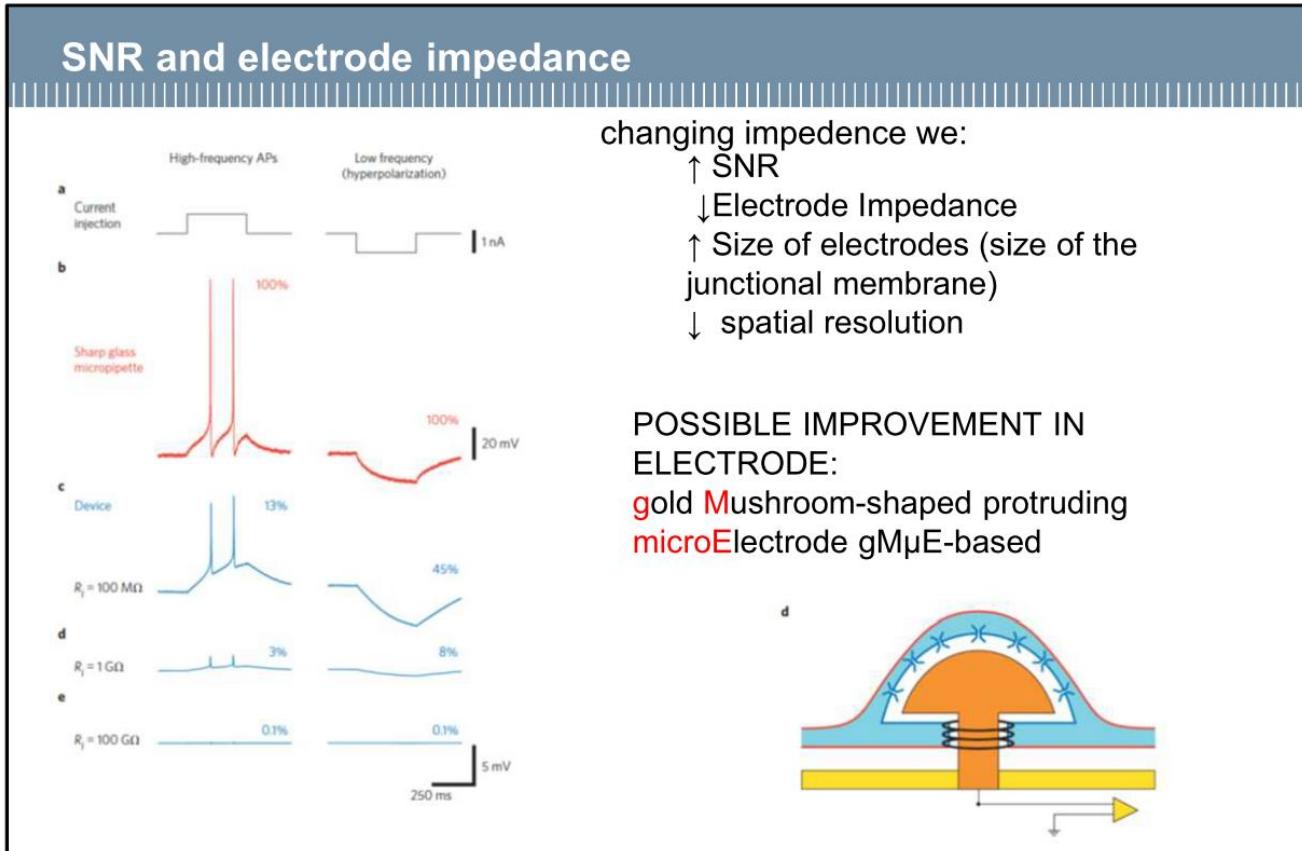


Figure 3 | Dependency of the electrical coupling on the junctional membrane resistance and pulse duration. Shown is a simulation of the cell-device coupling of APs and a long hyperpolarizing pulse at three different values of R_j ($100 \text{ M}\Omega$, $1 \text{ G}\Omega$ and $100 \text{ G}\Omega$). **a**, Schematic illustrations of the depolarizing (left) and hyperpolarizing current pulse (right) delivered to generate two APs and membrane hyperpolarization, respectively. **b**, Simulation of the ensuing intracellular potentials recorded by an intracellular electrode (red). **c–e**, The recorded potentials by an extracellular-located electrode (as shown in Figs 1 and 2) under different junctional membrane values (blue).

d, A neuron engulfing a gold mushroom-shaped protruding microelectrode gM μ E-based. Note actin rings surrounding the mushrooms stalk stabilizing the configuration. They use of a **chemically functionalized micrometre-size mushroom-shaped gold protrusion as the sensing electrode providing an increase of the neuron–microelectrode electrical coupling coefficient from approximately 0.1% as recorded by a planar extracellular MEA to approximately 50%**

Out of the five criteria to evaluate of the benefits of the approaches, the gM μ E-based MEA provided multisite, simultaneous, intracellular recording and stimulation for periods of days (which is for as long as we carried out the recordings). The filtering properties of the gold electrodes and the a.c. amplifier used do not enable the resting potentials of the neurons to be recorded. Nevertheless, the configuration successfully monitored subthreshold synaptic potentials and APs. The filtering nature of the recording system can be deconvoluted and thus unfiltered high-quality recordings of APs and synaptic potentials can be retrieved. A stable electrical coupling between gM μ E and a neuron coincided with the formation of cytoskeletal actin rings surrounding the stalks of the mushroom-like structure. Individual gM μ E enables both voltage recordings and application of

current. So far, attempts to obtain in-cell recordings and stimulation from rat hippocampal neurons and primary cardiomyocytes were unsuccessful. It should be noted nevertheless that these attempts were limited to gM μ E functionalized with poly-d-lysine rather than by the engulfment promoting peptide.

The drawbacks are that the microfabrication of such electrodes is much more complex and consequently the costs. MEA slides are not disposable but anyway they can't be efficiently used for more than 10-15 cultures because cleaning procedures and sterilization ruins the surface of the slide and SNR is worsen.

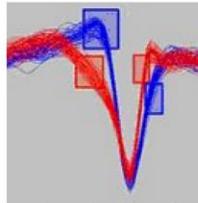
We are recording a cluster of cells, so we need to attribute the spike to the specific neuron. The electrode is sensible to all the activity of the network all around. How can we distinguish them? Hp: each neuron has a recorded shape which is always the same. The wave form depends on the geometry and it is stable if we're recording for one hour. If the wave form of the neuron is specific to that neuron, then we can use a template.

Spike sorting

Two cells afferent to the same electrode will in general have a different covered area.

Even if they cover the electrode in the same way, their spike waveform will be different because in general they have a different nature and ionic channel density (V_j).

Assumption: the shape of the spike of each neuron is stationary



However, it can be hypothesized that during registration physical characteristics of the neural culture are kept constant, and therefore each neuron fires with the same spike shape. We can therefore apply algorithms for spike recognition/spike sorting that recognize a template. If the algorithm identifies a template that is repeated in time, then the template can be linked to a source. With good post-processing analysis, it is possible to reconstruct a spatial resolution given by template recognition, but it is not possible to link the templates to the network morphology.

1. filter data
2. threshold the data
3. extract the data of each single spike (3 ms around the peak)
4. feature extraction
5. clustering

This permits us to give to each neuron a wave form. We don't know exactly where these neurons are, but we know that they're near the electrode.

From filtered data, it is possible to recognize the event "spike". From identified spikes, you need to create a template data set. With the template data set, you can identify when each neuron was spiking.

Note – the electrical shape of the spike is not important. The information is when the neuron is spiking. The different electrical shapes are used only to distinguish among different neurons, but do not carry any information content.

High density MEA (Active Pixel Sensor APS MEA)

GOAL: increase the spatial resolution

WEAKNESSES:

- worsen the signal-to-noise ratio
- Great deal of computational power to extract data and to sort them out

MEA stimulation

Stimulation is a difficult challenge. If we send a stimulus to an electrode, the stimulus will be spread all over the network. We can use light stimulation which could stimulate only one neuron.

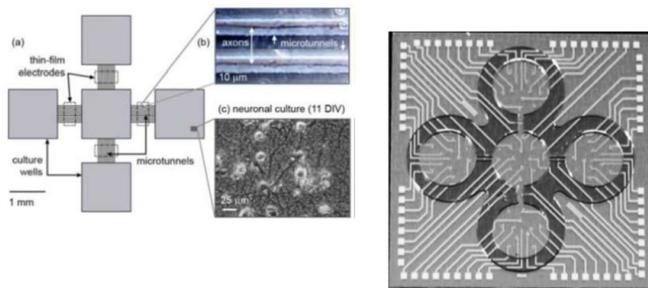
Low selectivity: medium is conductive

Clustering microfluidic solutions to confine stimulus

So the neurons are connected from a functional way, but they don't share the fluid.

Axons can go through micro channels, so the functional connection is alive.

The problem of stimulation with mea is the fact that the fluid is conductive.



It is possible to stimulate neurons in the culture to investigate the network response to a given stimulus. However, the electrical stimulation has a very low sensitivity.

In another special design, 60 electrodes are split into 6×5 arrays separated by 500 μm . Electrodes within a group are separated by 30 μm with diameters of 10 μm . Arrays such as this are used to examine local responses of neurons while also studying functional connectivity of organotypic slices. Separation between the two subcluster can also be achieved by proper microfluidic stimulation.

Pros and Cons

Spatio-temporal recordings of network activity (study the network allows to know more how the brain process data)
Large scale acquisitions (network level): modulation of local properties and impact at the network level
Long and repetitive time recordings (up to about one hour) (it depends on the environment. If the environment is controlled we can record for a lot of time because we know cells are good in that environm.)

Low correspondence between morphology and function
High temporal resolution, low spatial resolution
Low selectivity in stimulation
No registration of subthresholds potentials (low SNR) -
>Dark neurons

Optical tools for studying neuronal networks

Optogenetics: technologies used for those studies. It's a method that is based on proper cage compounds molecules that are responding to light stimulation. We can genetic put this protein inside a molecule and make it visible, when it's active. Optogenetics is better than drugs (slow) and electrical stimulation. This method was used to study epilepsy and depression. It can also be used to study the impact of drugs.

Optical stimulation of in vitro neuronal cultures

Optical stimulation
<p>ONE-PHOTON UNCAGING</p> <p>The basic approach is to cage the compound (Black) with a blocking group (red). Thus, caged compound can be switched into the active form by short UV pulses.</p> <p>This allows us to obtain high spatial and temporal control during stimulation.</p> <p>PROS</p> <ul style="list-style-type: none">• Glutamate is one of the most common neurotransmitters in the CNS<ul style="list-style-type: none">→ Physiological stimulation• The UV pulse can be highly focused<ul style="list-style-type: none">→ High selectivity

Optical stimulation uses photolabile neurotransmitters. For instance, chemists can modify the glutamate molecule, placing a compound that inactivates it. If the bond between the Glutamate and the inactivating compound is photolabile, then it is possible to separate the two molecules with light stimulation. Once the Glutamate is uncaged (i.e. the bond is broken), the stimulus to the neurons is the same as the natural one.

The question now is: how selective is the optical stimulation?

Problem of stimulation: electric is ok if we want to be accurate, but not a spatial accuracy. If we want to stimulate more neurons to see the consequences, we should use the optical stimulation. There is a caging group connected to a molecule and inactivate the molecule (no glutamate). If you focus a light on this molecule, the cage compound is uncaged and as soon as it is cut, there's a release of the glutamate. We can confine the point of light stimulation. only where it is pointing, we have the release of glutamate, so to activate the neurons in that specific area. We prepare the medium so the whole area is active, but we decide the point to active by pointing the light.

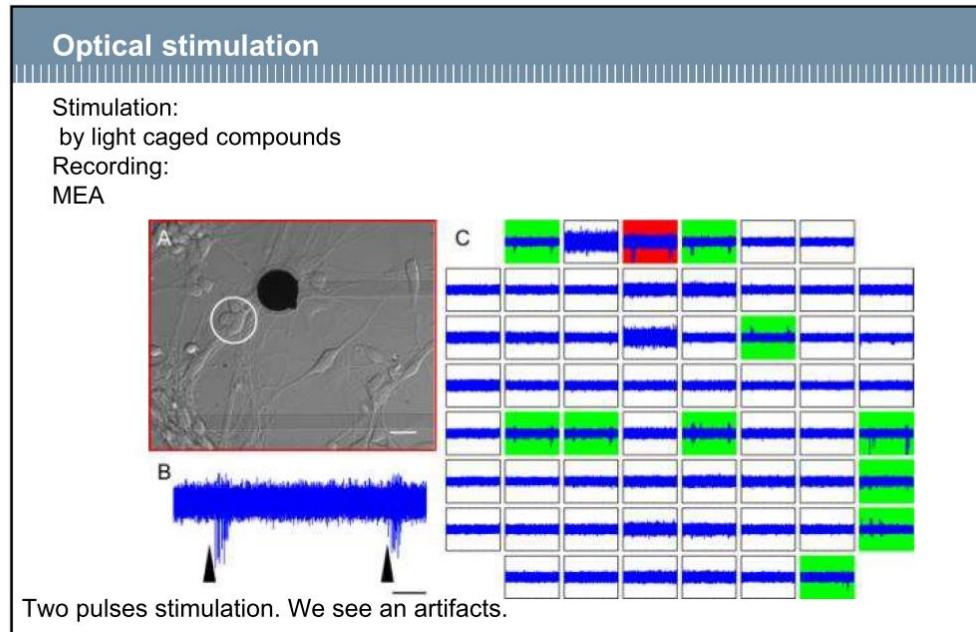
We know where we are stimulating, so we have a morphology-function relation. Also, we can have a high spatial resolution.

Technological ingredients: the center is a microscope, with a camera that allows to see the culture, an illuminator, couple of MEA systems (record electrical activity) it's inside the microscope. The MEA board and computer are outside. The control unit control the length of the light, when and where it's directed.

We have 2 fiber optics aligned (one UV and one on visible spectrum). In the medium we put the cage compound that goes everywhere. We activate first the visible spot to check where is oriented the fiber optics and the size of the light we're producing. We turn it off and we start the UV pulses. The petri has the MEA system under it ->

recording electrical activity at network level. Everything is inside the microscope. Focused optical stimulation + recording of all network activities.

white: stimulation; black: electrodes. The most close electrodes is the one with the red background. There are also correlated activities (green) bc stimulation are seen by other electrodes which are far from the stimulation point -> connectivity of the network of neurons.



Local stimulation of a site of the neuronal network and detection of the resulting activity on the whole network. (Panel A) The optically stimulated area near one of the sixty electrodes is marked by a white circle. (Panel B) Activity recorded in the nearest electrode after two optical pulses of 50ms. The application of the pulses is indicated by the black arrow heads. (Panel C) Activity recorded from the entire network. The red box highlights the trace of the electrode very close to the stimulus area. The green squares represent the areas where there is correlated activity. We can observe that the synchronous activity doesn't reflect the passive spatial morphology [e.g. electrical field decreasing with distance]. This demonstrates that the culture has a network activity that can be recorded: we have a functional recording.

Depending on the length and power of the pulses we can make the stimulation more or less spread. Changing the level of duration of UV pulses, we can activate small or broader portion of the network.

Optical recording of in vitro neuronal cultures activity

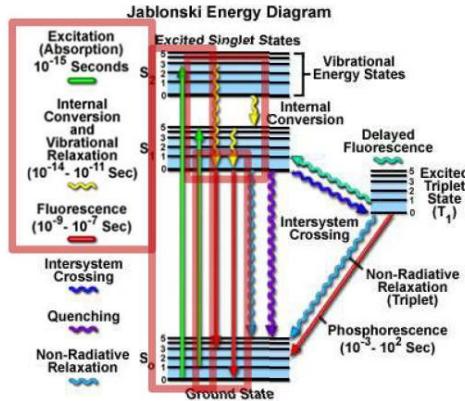
We can record the activity with optical means, not MEA. THIS IS OPTICAL RECORDING, not stimulation. It passes through microscopes.

The physical principle is fluorescence. It is the phenomenon that some molecules can be excited by a light and they change their state, emit light at higher wavelength and then returning to the initial state. The emission is an immediate process. Fluor can be done by many materials: organic and inorganic. It can be a property of the material itself. We can prepare are mean in order to give him some specific fluorescence property. We can drive the mechanism that we record by choosing the proper fluorochrome.

Fluorescence

Fluorescence is governed by three events:

- Excitation (or absorption)
- Vibrational relaxation
- Emission

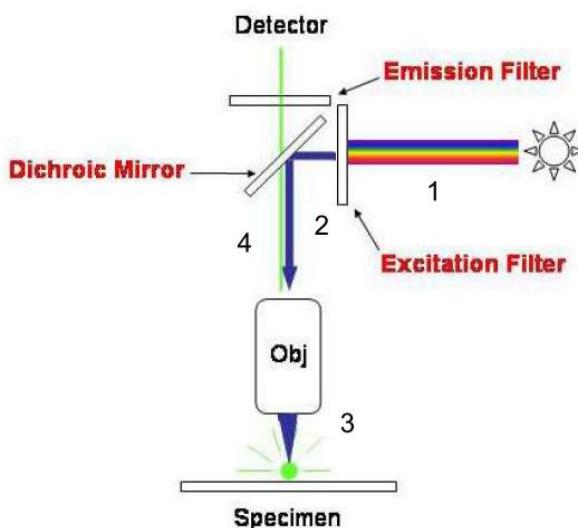


Fluorescence is a quick process: it is measured in billionths of seconds

Energy loss in vibrational relaxation
causes emitted photon to have less
energy than absorbed one

Emitted photon has a different
wavelength ("color") than absorbed one

Fluorescence microscopy



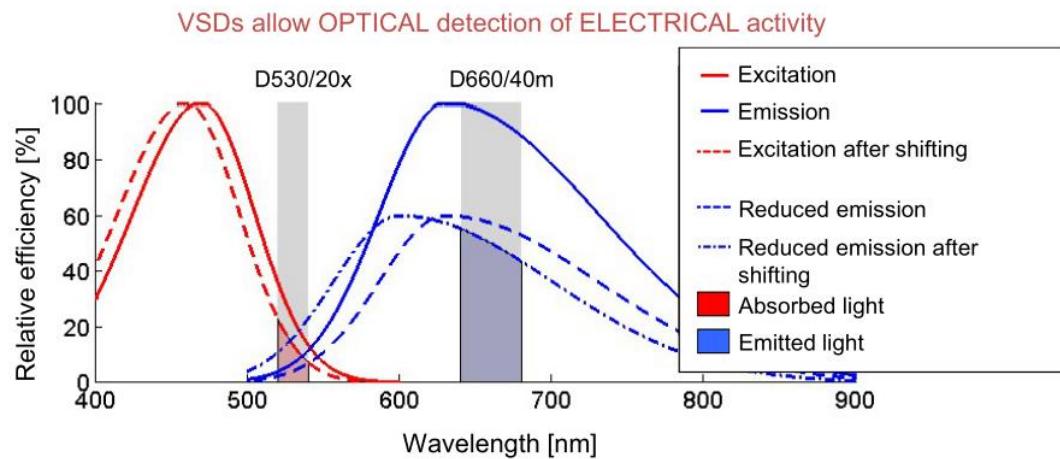
Fluorescence microscopy:

1. White beam from source to excitation filter
2. Monochromatic beam ($\text{wavelength} = \lambda_1$) from excitation filter to sample
3. Absorption and monochromatic beam emission ($\text{wl} = \lambda_2, \lambda_2 > \lambda_1$)
4. Monochromatic beam from sample to detector

Schema: light source, which pass through filter (selected only some wavelength) and then goes to a mirror, which reflects the light and illuminate the culture. It receives the excitation light and emit light in a different wavelength. The emission pass through the object and the dichroic mirror let the emission light pass so it can reach the detector. We want to find a specimen that has properties similar to the neuron network. We want a fluorochrome that is able to detect the abilities of neurons.

Epi-fluorescence microscope equipped for both transmitted and reflected fluorescence microscopy.

VSDs: principle of working



VSDs efficiency is a function of local electrical voltage.

If excitation light is constant, output light intensity depends only on sample electrical properties.

The specimen that we can use to record the activity of neural network is VSD (Voltage Sensitive Dyes). Its excitation properties depend on the state of neurons (active or not). Solid lines: red, excitation, means that neurons are excited if they're stimulate inside the red spectrum. When they're stimulated, they emit in the blue spectrum (higher wavelength). If the neuron is spiking, we have a shift of the excitation form (dashed red). When we excite, we know the specific wavelength and for that we use the filter (not a single wavelength, but some of them). If we pass from excitatory to the rest state (continuous line) to the active state (dashed), in the emission we will pass from the continuous line to the dashed one (less energy) and we also have a shift in the emission form.

At first we have a reduction to the excitation and a left shift. So, the emission will drop.

We have selected the excitation wavelength (red column) and also the emission wavelength (blue column). This corresponds to the excitation filter and emission filter. VSD are connected to the membrane of the neuron. As soon as we put them inside the mean, they go inside the membrane of the neuron.

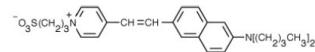
NEURON SPIKING: excitation shape is shifted to left. Also, the emission form is shifted and reduced in amplitude. We have a huge drop in the emission. The real blue column is the reading of spike.

The curves «after shifting» refer to the shift of the optical properties of the dye when the membrane voltage is modified by the spike. If the spike is on, there is a double effect. First, a left shift of the excitation curve (red dashed curve), which results in a low absorption at the defined wavelength (530nm). The low absorption by itself implies the reduction of the excitation light (blue dashed curve). Further, the spike event induces a second mechanism which is a shift of the excitation curve (dot-dashed blue curve). The design of the microscope is then optimized to maximize the difference between the emitted light in presence and in absence of spike.

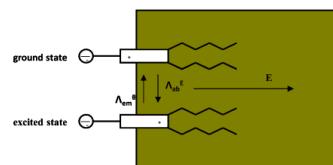
VSDs: principle of working

In order to perform its activity, a VSD molecule needs three parts:

1. a hydrophilic head
2. a rigid middle-section
3. a hydrophobic tail

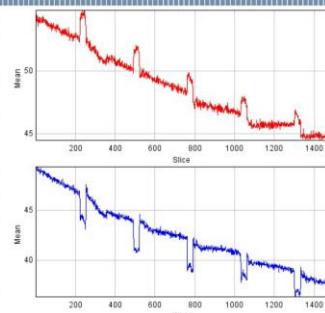
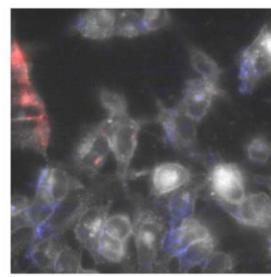


An electrical field changes the amount of energy lost between absorption and emission.



Emitted photons shift their wavelength as a function of membrane potential

HeLa



Complex geometry in the culture causes opposite variations in different regions of the image, instead on opposite sides of the same cell.
(areas marked in blue highlight depolarization, while those in red hyperpolarization)

Comparison of technologies for Neuronal culture reading

	Patch clamp	Mea	VSDs
Temporal resolution	+++	+++	Depends on camera, could limit the spatial resolution
Spatial resolution	Single neuron or compartment neurons	Pool of neurons around the selectrodes (post proccesing improvable)	Single neuron ...depends on the trade-off with the field of view (objective)
Field of view	Max few neurons (1 or 2)	Full coverslip culture	Trade off with spatial resolution
SNR	Subthreshold potentials	Spikes (dark neuron)	Spikes (on neurons compartments) looking at the membrane, so we can see the membrane
Link activity and morphology	Perfect for the recorded neurons	NA	Good in the field of view of the axon.
Difficulty	+++	++	+

in cameras temp resol is connected to spat resolution.

in case of VSD we are looking at the membrane, so we can see the membrane

high for all electrical recording, spike is a very short phenomenon

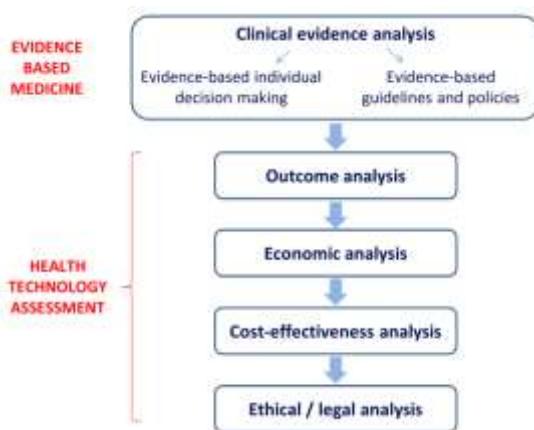
VSDs: if we want to see single neuron, we need a lens (high spatial resolution, but small area). It's a trade-off between the FoV and how much neurons we want to see.

Rehabilitation Robotics

Design experimental studies in rehabilitation

Progress in health care, an increase in levels of wealth, improvements in standards of living, and better nutrition, combined with reduced fertility rates, have contributed to an increase in the number of older people. According to recent projections, the number of Europeans aged 65+ will almost double over the next 50 years, from 85 million in 2008 to 151 million in 2060. This caused a positive correlation between prevalence of neuro-motor diseases (e.g. stroke) and age and so an increasing demand for novel and cost-effective solutions to improve the outcome of the rehabilitation process.

FROM RESEARCH TO DECISION-MAKING

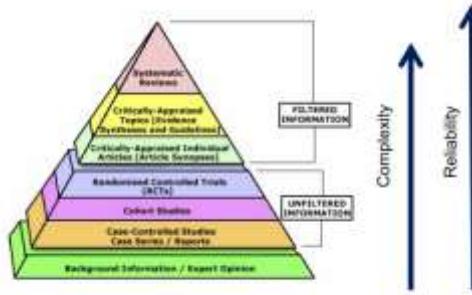


The process is characterized by 5 main stages:

- Evidence analysis: a systematic evaluation of evidence for a technology – this stage corresponds to evidence-based guidelines
- Second stage is outcome analysis: it consists of an estimation of the magnitude of the effects of a technology on the desired clinical outcomes (the benefits) and on potential harms (the risks) – it determines if the benefit-risk ratio is sufficiently high to justify the technology

- Analysis of costs
- Compare the clinical effects against the costs to determine if the ratio is sufficiently high
- Last stage is the analysis of the ethical and legal implications of the technology

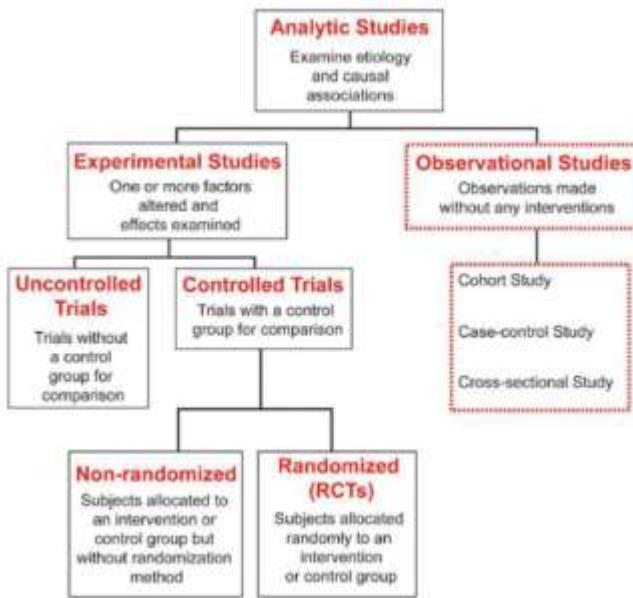
Evidence Based Medicine: decision making in medicine, not only the decision about the individual, but it's also the base for guidelines and policies. It is necessary only for the clinician to decide, but also to the system to provide guidelines. We have to consider many analysis (outcome, economic, ethical/legal, cost-effectiveness). The target of evidence-based medicine is the patient. We also need proper measurement about the efficacy and a careful application. The doctor needs to see the patient, make an analysis and then decide using the evidence-based medicine.



Evidence goes through different levels. We can start from a general expert opinion up to cohort studies, up to randomized controlled trials (putting together several events in a controlled way), then there's a gap and we can do filtered information and put together different information. The more we're going upward we're increasing the complexity.

More systematic observation ▶ better evidence

STUDIES DESIGN



The differentiating characteristic between observational and experimental study designs is that in the latter, the presence or absence of undergoing an intervention defines the groups.

By contrast, in an observational study, the investigator does not intervene and rather simply “observes” and assesses the strength of the relationship between an exposure and disease variable.

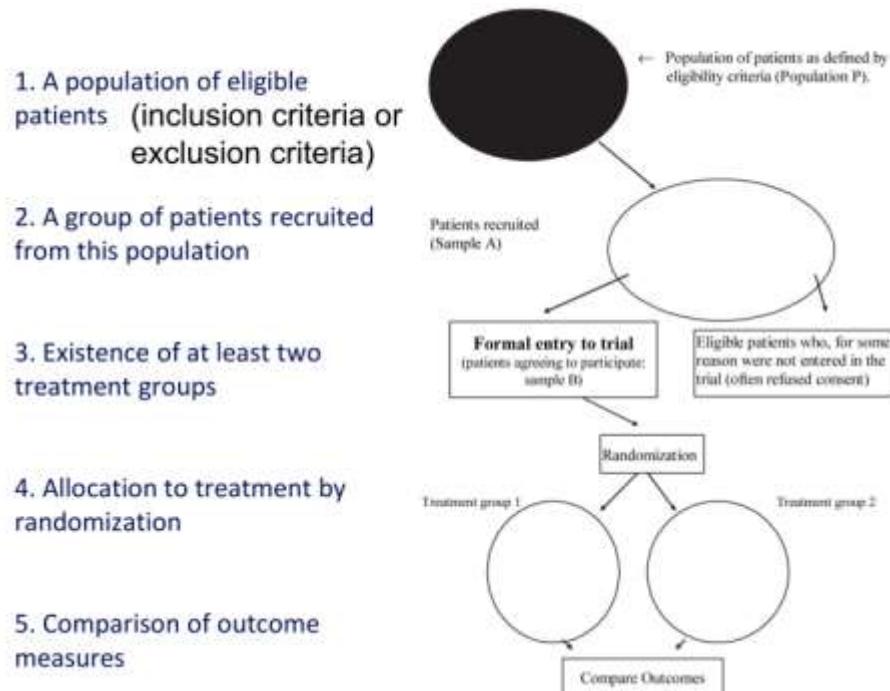
Case-control and cohort studies offer specific advantages by measuring disease occurrence and its association with an exposure by offering a temporal dimension (i.e. prospective or retrospective study design). Cross-sectional studies, also known as prevalence studies, examine the data on disease and exposure at one particular time point. Because the temporal relationship between disease occurrence and exposure cannot be established, cross-sectional studies cannot assess the cause and effect relationship.

Randomized Controlled Trial (**RCT**) is a type of scientific experiment used to test the efficacy and efficiency of a service in healthcare, such as a new technology, methodology, treatment or drug therapy in a well-designed target population following a rigorous methodology.



Clinicians and policymakers often distinguish between the efficacy and the effectiveness of an intervention. Efficacy trials (explanatory trials) determine whether an intervention produces the expected result under ideal circumstances. Effectiveness trials (pragmatic trials) measure the degree of beneficial effect under “real world” clinical settings. Hence, hypotheses and study designs of an effectiveness trial are formulated based on conditions of routine clinical practice and on outcomes essential for clinical decisions.

The schematic diagram of an RCT is:



We need to define primary outcomes (one or two) and secondary outcome. Before starting we need to define the outcomes that are most important.

Randomization refers to the participant's allocation process to the treatment(s) or control group.

Random allocation will equalize individual differences between groups allowing as far as possible the treatment effect to be established uncontaminated by other potentially competing factors. In other words, the aim of random allocation is to minimize the effect of possible confounders, leading to a fair comparison between the treatment(s) under investigation and the other procedure chosen as control.

The use of randomization as an attempt to ensure that subjects have an equal probability of assignment to experimental groups, and hence to reduce the likelihood of known and unknown confounders affecting the results.

ETHICAL ISSUES

The World Medical Association establishes the Declaration of Helsinki in 1964 to provide ethical universal rules to conduct clinical medical research. Since 1964, it has been reviewed several times lastly in 2013. No valuable medical journals publish studies whose design is not based on its principle.

To guarantee the transparency and the independence of the ethical committee from the interests of a single category (e.g. the researchers or the sponsor) it is composed by individuals with different competences, not related to the research. It includes clinicians, biostatisticians, patients, expert in medical devices, bioethics and insurance issues.

The ethical approval is mandatory before the beginning of any clinical study. At the end of the study, the researchers have to submit to the ethical committee a report summarizing the main results of the study.

The declaration of Helsinki states that:

- The experiments have to be performed in compliance with the respect of the patient
- A treatment known to be inferior shouldn't be given to any patients
- The privacy of patients has to be guaranteed
- Participation is voluntary and the patient can withdraw from the study in any moment
- An informed written consent has to be signed by each participant before enrolment
- Research can be conducted only if the importance of the objective overtakes the risks
- A clear research protocol should be established

A **bias** is a systematic error that may induce misleading conclusion about the efficacy of the intervention. There are different types of biases and all of them are elements used for evaluating the reliability of the trials.

- SELECTION BIAS: It occurs when the person, who enrolls the patients in the study, knows in advance the allocation to the treatment group. Indeed, in this case he/she can be influenced in the decision to enrol the patient. The randomization is not done properly;
- ALLOCATION BIAS: The randomization procedures should be designed to balance groups at baseline with particular attention to prognostic factors that can influence the outcome;
- ASSESSMENT BIAS: it can occur when the assessors are not blinded to treatment allocation, and thus can be influenced in his/her evaluation, mainly when outcome measures characterized by low sensitivity, high subjectivity (such as patient reported outcomes) and low inter and intra rater variability. Assessment bias can occur also when patients are not blinded to treatment allocation, since he/she can behave differently during the assessment;
- PUBLICATION BIAS: The ultimate goal of any medical research is to influence clinical practice. Thus, the findings of a RCT have to be published in scientific international journals. To be published papers undergo a peer-review process, and often papers with positive findings are considered more likely to be published than papers that do not show any statistically significant differences -> this can lead to a publication bias as medical research not equally reports studies with positive and negative results. We want to share the results through a publication in a

scientific journal. Positive results have more probability to be published. So, the publications are biased because negative results will not be published;

- STOPPING RULES: A-priori sample size is not trivial. Recruitment too few patients may prevent the achievement of a definitive result on the superiority of a treatment over the other. On the other hand, recruiting more patients than necessary may become unethical as the surplus patients could be exposed directly to the superior treatment.

If we have 60 sample and after 50 people and all of them are going into one direction, then we should stop the trial. This has to happen even if the 50 people have good or bad results.

Consort stands for Consolidated Standards of Reporting Trials. The CONSORT Statement has been developed to improve the quality of reporting of RCTs. It was firstly published in 1996, and then revised in 2001 and 2010. It is focused on the most common design, that is the two-group parallel trial. It includes a checklist of 25 items that should be included in reports of RCTs.

TRIAL DESIGN

- PARALLEL DESIGN: Each participant is randomized to one of the intervention arms. The parallel design with two groups is by far the most used and this is why we will more concentrate on this design during this lecture;
- CROSSOVER DESIGN: Each participant is exposed to each intervention in a random sequence. This design is appropriate only for chronic patients;
- FACTORIAL DESIGN: Each participant is randomly assigned to a group that receives a particular combination of interventions or non-interventions; e.g. no treatment vs treatment A vs treatment B vs treatment A + B;
- CLUSTER DESIGN: Pre-defined homogeneous clusters of individuals (e.g. clinic 1 and clinic 2) are randomly allocated to different study arms (i.e. intervention or control group);
- SPLIT BODY DESIGN: Body parts (e.g. upper limb or lower limbs) within each participant are separately randomized.
- SUPERIORITY TRIAL: To determine a clinically relevant difference between two interventions;
- EQUIVALENCE TRIAL: To determine whether an intervention is neither worse nor better than another intervention;
- NON-INFERIORITY TRIAL: To determine whether an intervention is not inferior to another intervention.

Were participants recruited from primary, secondary, or tertiary healthcare or from the community? It has to be clear if the study was conducted in one or several centres (multicentre trials).

The primary outcome is the pre-specified outcome considered to be of greatest importance; it is used for sample size calculation. Information on outcomes should be sufficient to allow others to use the same outcomes. If outcomes are assessed at several time points, it should be specified the time point of primary interest. Other outcomes of interest are secondary outcomes (additional outcomes) - there may be several secondary outcomes. It could be useful to specify who assessed the outcomes. When appropriate, the use of previously developed and validated scales should be reported.

We need to define our primary outcome, so our parameter should have a minimum detectable change. We need to be aware of the limit of that parameter.

SAMPLE SIZE

The **sample size** calculation requires:

- Estimate of the clinical importance difference between the intervention groups
- The significance level or α (type I) error level: rejecting the null hypothesis when it is true
- The statistical power or β (type II) error level: not rejecting the null hypothesis when it is false
- For continuous outcomes, the standard deviation of the outcome
- Dropout rate

There are different ways to define the sample size, for example using a normally distributed variable:

$$N = \frac{2\sigma^2(z_{\beta} + z_{\frac{1}{2}\alpha})^2}{\tau_M^2}$$

The more the outcome is noisy (high σ) the larger the trial needs to be. The smaller is the difference in treatment means (low τ_M) the larger the trial needs to be.

RANDOMIZATION

- Random allocation: each participant has a known probability of receiving each intervention before one is assigned, but the assigned intervention is determined by a chance process and cannot be predicted.
- Methods of sequence generation: random-number table or computerized random number generator.
- Type of randomization: simple or restricted.

BLINDING

There are 3 figures: participant, assessor and operator (who is doing the therapy). In case of rehab it's difficult for the operator to be blind, because he's perceiving the treatment, instead for pharma this is important. Sometimes it's difficult to blind the patient (if we use a robot).

- Assuring a complete blindness of an RCT means that participants, those administering the interventions, and those assessing the outcomes have to be completely unaware of group assignment
- Double blind = participants and assessors are unaware of group assignment
- Single blind = only participants or only assessors are unaware of group assignment
- In some studies (e.g. surgical interventions, rehabilitation), blinding of participants or care providers is difficult to obtain, but blinding of assessors can be achieved. Blinding is an important safeguard against bias.

STATISTICAL METHODS

Describe the statistical method used to analyze the data:

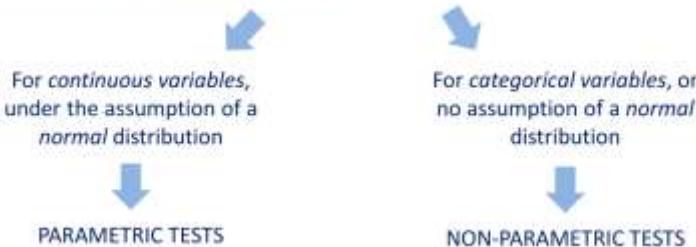
- Estimate of treatment effect, that is the contrast between the outcomes in the comparison groups, and its 95% confidence interval
- Provide statistical significance of findings (P-value), that is the probability that the observed data could have arisen by chance when the interventions did not truly differ (P-value < 0.05)

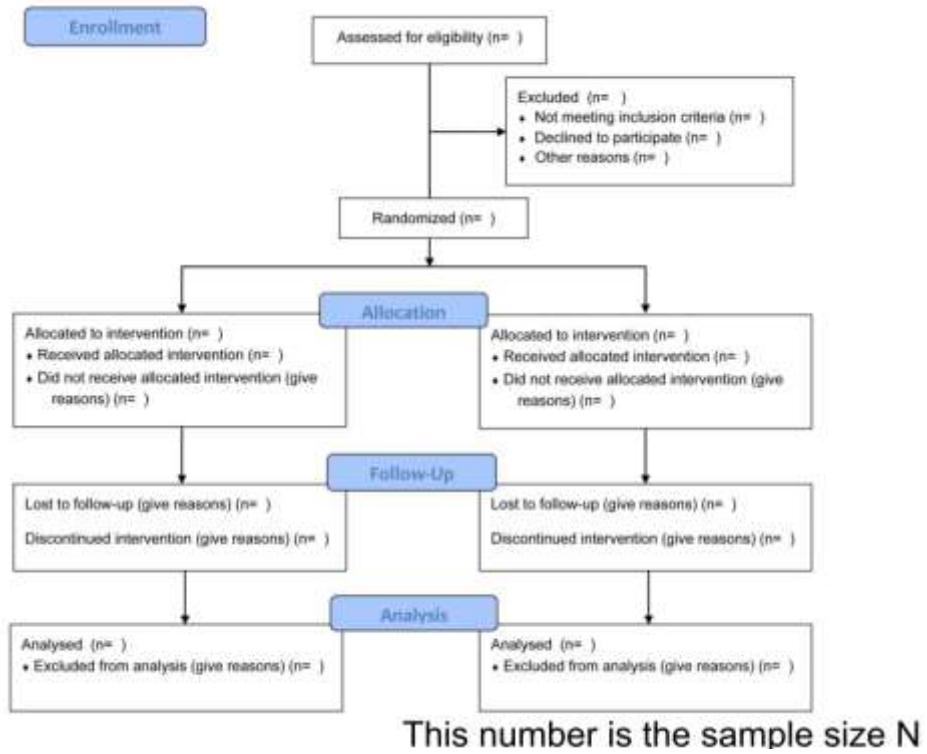
Comparison of baseline values

- Significant tests of baseline differences (e.g. student's t-test for independent samples) are common
- These tests assess the probability that observed baseline differences could have occurred by chance
- These tests are not needed since we already know that any differences are caused by chance



Longitudinal methods of analysis to examine repeated, correlated observations within and between subjects over time



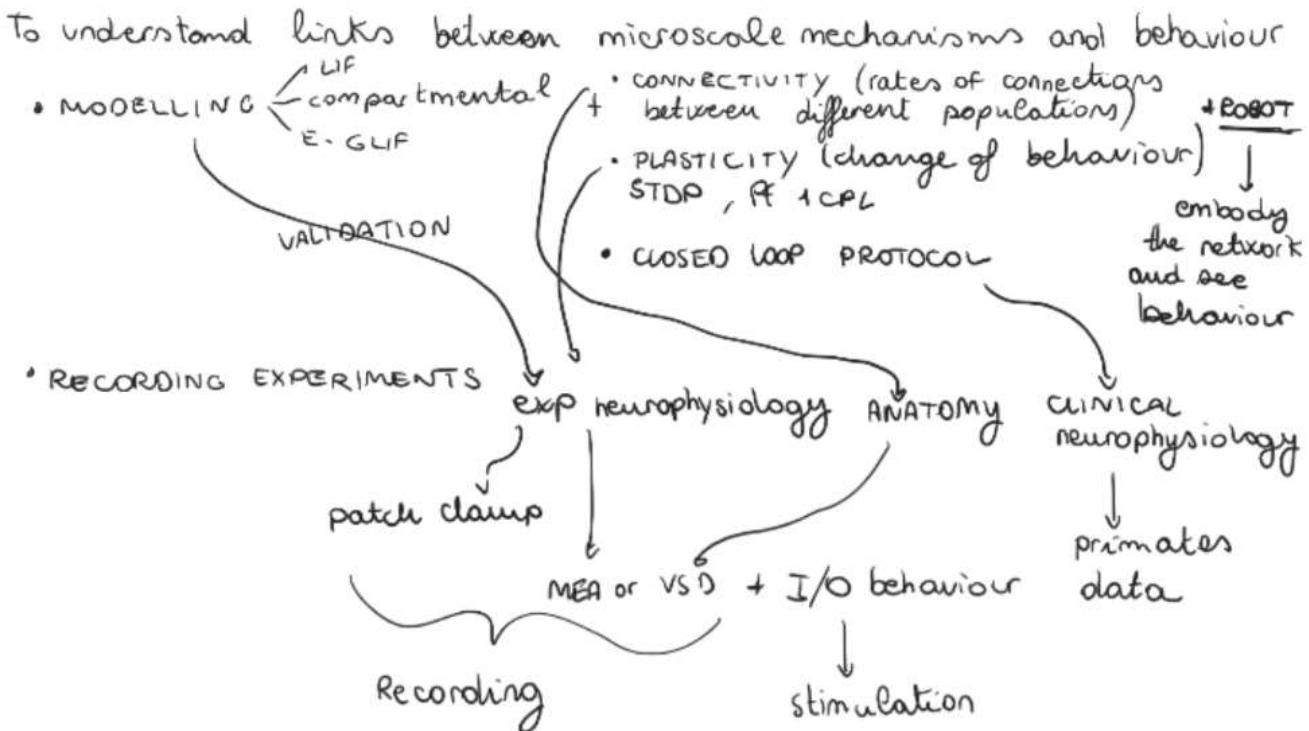


This number is the sample size N

Robots for rehabilitation

All the problems about computational neuroscience and neuro for biology are attempts to understand the micro scale phenomena (behaviour phenomena). In order to understand the link between neurons brain areas and behavioural variables we can: model and make experiments.

Modelling: develop model starting from neurons models (LIF, compartmental, E-GLIF), connectivity (raise of connections between different population), then we need plasticity (change of behaviour across time as STDP), then close-loop protocols.



Do not loose into details but have a clear overall picture

Neurons validate experimental neurophysiology, connectivity validate anatomy, plasticity \rightarrow experimental neurophysiology, close loop protocols \rightarrow clinical neurophysiology. Then we can put a robot to study behavioural variables. For neurons model we need patch clamp; For connectivity and plasticity \rightarrow MEA or VSD (recording) + stimulation (cage compound). Clinical neurophysiology \rightarrow primates' data.

NEURO-ROBOTICS

Blending of research in robotics, neurology, and artificial neural networks

The motivations are:

- Enable direct neural control of robotic limbs and bodies. In order to interpret neural signals, we need to be able to model how they work;
- Support re-learning of motor control in neurological disorder;
- In order to rehabilitate, we need to facilitate motor exercises, associated with active participation AND task completion;
- Model robot motor systems on the methods used in humans and animals: enable fast & fluid movement;
- Contrast static & dynamic stability in humans and today's robots.

Ageing society + improvement in acute care imply that the number of people with chronic disability is increasing, resulting in a higher request of rehabilitation.

This change in the demographic distribution of ages happens all over the world. In Africa the expectation of life has increased more than in developed country.

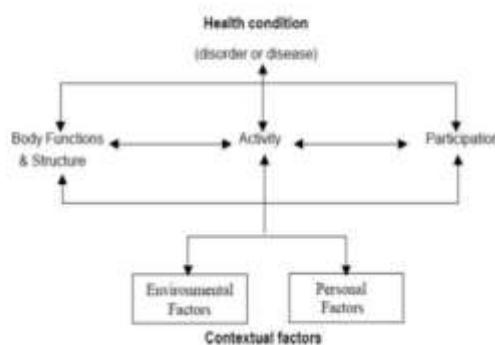
The decline of the ability of a patient is connected to his training. We want to have a longer life but also a higher quality of life. Rehabilitation and technology play an important role in order to have a healthy living person. The most important element is the education, so people will eat better, exercise more and improve the quality of life.

Now solutions are more related to chronic care, not only acute care. A stroke could lead to some disabilities that will be there for all the life of a patient, so we need to study chronic care.

Rehabilitation robotics are tools to assist the clinicians in promoting rehabilitation of an individual so that he/she can interact with the environment unassisted. This technology promotes the re-learn and stop the therapy.

Orthotics aim at improving functions in people with a weak limb due to a neurological disorder who cannot properly control it when interacting with the environment (Assistive technologies). Orthoses are designed to work in cooperation with the intact body and either control or assist movement. The goal is not the final recovery of that function, but to assist the function.

International Classification of Functioning, Disability and Health (ICF)



As the functioning and disability of an individual occurs in a context, ICF also includes a list of environmental factors.

A **stroke** is a fast-cerebral functionality loss due to a improvise blood flow interruption or haemorrhage.

Immediately after the stroke, 80% of the patients is affected by hemiparesis (loss of muscular tone), 35% maintain a partial disability even after the rehabilitation treatment. Functional deficit is contralateral in respect of the cerebral lesion. Motor functional recovery has an exponential trend. Initial recovery is due to resolution of local ischemia, anoxia, diaschisis, and edema reabsorption. Afterwards functional recovery occurs at the same time with a dynamic process of cortical and subcortical reorganization (post- acute phase - about 6 months after the accident). Chronic phase (low and slow recovery).

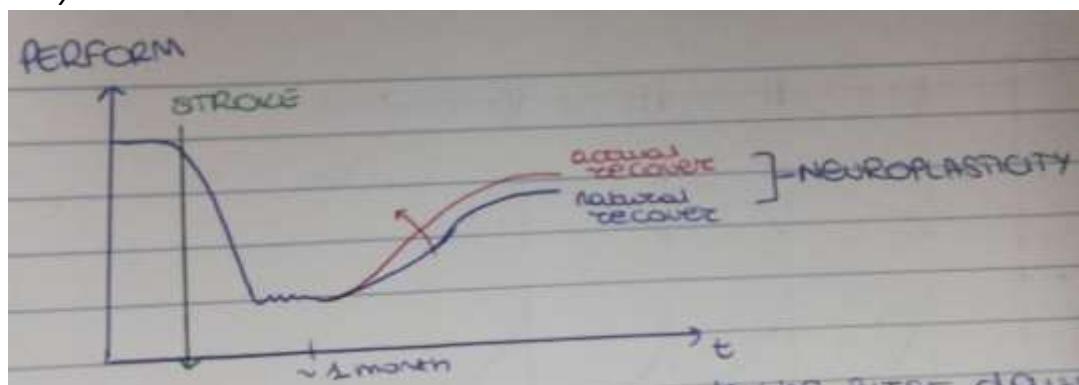
Stroke happens in a moment. In the first days there's a drop of performances, then they're the same and after one month they can increase fast because the tissue is recovering naturally (penumbra).

Over this period and after there's a good possibility to recover the function, without going upper the initial performances.

Neuroplasticity are all the modifications in the organization of neural components occurring in the central nervous system during the entire life span of an individual. This is the basis of recovery in rehab.

The **key ingredient for motor re-learning** in case of neuroplasticity are:

- *Amount of practice;*
- *Early intervention:*



The change of the recover curve depends on the rehabilitation. Natural and actual recover are related to neuro plasticity: neurons that died during the stroke stay dead, but the connections around them can replace their functions. We say that in the brain we have redundant connections: there a lot of ways inside the brain to go from A to B; some can be less efficient, but they work. To change the curve the rehab should start as early as possible.

- *Functional and goal-oriented training:* ability to perform movement and goal of the movement. It's better to train for functional task;
- *Biofeedback and Augmented information:* it's about having information about what we're doing. Provide a continuous feedback about the movement is a key element for training. E.g. the stroke will lead to a lateralize impairment. If we ask a patient to pedal and the healthy leg will do an

extra job. This is a functional rehab, but it will not lead to re learning because the patient will use mostly the healthy leg. We should provide a bike that give the information of how much he has to push with legs. This is the feedback that he needs to re learn the movement.

- *Repetitive training, 'rewarding, interactive and engaging task'*: train needs repetition (learning comes from errors), but this can be very boring especially if the patient has small capabilities. It's also important to reward and encourage the subject, so, doing a rehab (robot) with all these things is very important.
- *Volitional contribution*: if we have some residual capabilities of the subject for the task, it's important that the patient is conscious, and he tries to do the moment (instead of a passive movement) because the brain is doing synapses, so the residual brain capabilities connected to the task are activated.
- *Individualized training*: every patient is different, so we need to propose a custom rehab that will change with the change of its performances.

Increased practice leads to greater skill, as long as practice is challenging, progressive, and skill based. The key factors for motor recovery are:

- Functional training
- Active participation
- Self-initiated movements
- Training intensity: regular
- The more practice, the greater success

The limitations of conventional therapy (physiotherapist) are:

- Poor motivation (depending on personal interaction with therapist)
- Limited by availability of therapists (low dose)
- Limited number of repetitions of exercises
- Unclear feedback regarding therapy progress
- Limited modulation of therapy

We want to translate the neuroplasticity in the design of robotics. We need continuity care, and this is a problem that we need to solve. Continuity care is mainly done by monitoring, exercising and being adherence to therapy. The latest is one of the main problems in care because people don't take always the medicine or they do, but not in the right moment. This impact the continuity of care. It's a wide spread problem that affect 30% of all people that make a therapy. There are a lot of devices that advise you when to take the medicine, or boxes that open only when it's time to take the drug and can say if the drug was taken out of the box, but there's no a way to see if the patient actually took it.

The **design** and **clinical translation** of safe, simple, immersive and functional devices is needed for assuring the maximal recovery during the hospitalization as long as in the continuation of the rehabilitation after discharge (at the point of need) and eventually at home.

Evidence-based assessment of rehabilitation therapies' alternatives (including neurorobotics assisted therapy, training adopting neuroprostheses and hybrid assistive devices as well as conventional treatments) is a milestone in the view of the customization of treatments on single patient.

Rehabilitation robotics is a field of research dedicated to understanding and augmenting rehabilitation through the application of robotic devices. Rehabilitation robotics includes development of robotic therapies, and the use of robots as therapy aids instead of solely as assistive devices.

When we talk about rehabilitation robotics, we need to talk about COMMERCIAL DEVICES:

- **END-EFFECTOR:** apply mechanical forces to the distal segments of the limbs. Only one part of the body is controlled. The advantage is that it is easy to setup and control. The disadvantage is that there's no control of proximal joints, so there's the possibility of abnormal movement patterns. It's an easier robot;
- **EXOSKELETON:** robot axes are aligned with the anatomical axes of the subject. The advantage is that we have a direct control of individual joints, so minimization of abnormal posture. The range of motion of joints can be fixed following the volition of the patient, for example, if he's in pain doing some movement, the range can be changed. They're much more expensive and complex;

There's another class of rehab robot, the **soft robotics**. They don't have rigid components on the robot-human interface or minimal rigid components that will not impose physical restraints on joint motions. The subject has a big box on the back cabled to other parts of the body. It's more similar to natural movements because of the cables, which permits more freedom, but one problem from the operational point of view is that a little misalignment could provoke a problem on the joint.

Rehabilitation in the past 20 years has made big steps.

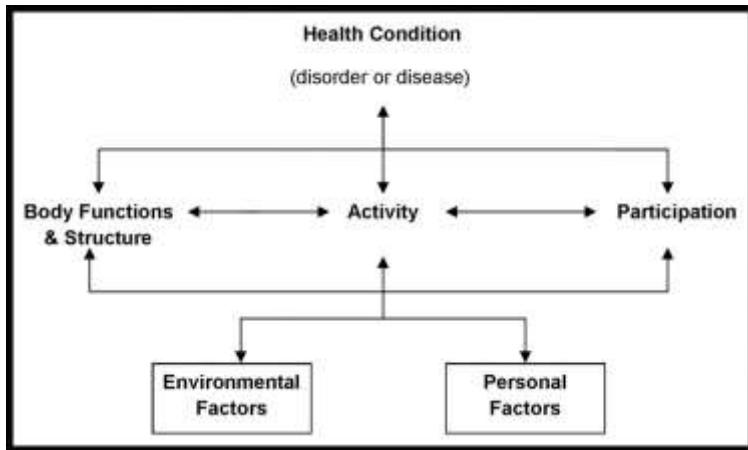
There are a lot of different approaches which are the result of the combination of patient needs, marketing, business strategy...

There are robots that work on all the body, instead there are others that permit to exercise specific parts of the body. Also, there are some robots that contain different robots which permits different patients to work with them all together at the same time.

Are these products producing benefits to the clinic?

RATULS trial highlighted that MIT-manus based robotic training led to improvement in upper limb impairment compared with usual care (Body Structure and Function domain) but not improvements in upper limb function or ADL (Activity domain). Enhanced Upper Limb Therapy, EULT, in which training specifically focused on daily activities and functional tasks, led to improvements compared with usual care at the end of the intervention period (at 3 months) for both Body Structure and Activity domains.

Let's look at ICF.



- Body functions & structure: it's about a specific functioning. How much do you move your joint?
- Activity: it's about the activity that you can actually do. Now that you can move your elbow, can you brush your teeth?
- Participation: it's about using the activities in social environment. Now that you can eat with a spoon, can you eat with a spoon in a restaurant?

The paper was about studying Body functions & structure and Activity of the robots. The results of the paper are that robotics work well on body functions, but they don't have a good impact on the activities. The EULT have better results in the activity domain. MIT-Manus doesn't work well in the activity domain.

A very recent review paper proposed a long-standing view that in clinical settings robotic therapy should focus on impairment training, combined with therapist transition-to-task training, to translate the impairment gains into function.

Let's talk about a specific rehab robotic: the ReWalk.

This is a different kind of exoskeleton in which the control of the gait is made on the crutches. This was proposed for spinal injured people, but wheelchair remains the best solution because it's more stable and safer. The only problem of wheelchair is the immobility of the joint, which means demineralization, devascularization, risk of thrombosis... Also, you never talk to people in the same-level, which is a psychological issue which is very important for the person. The idea of this exoskeleton is not to abandon the wheelchair, but to train the subject for an hour and to be used during some important events. ReWalk helps with the education and permit injured people to train in a non-boring way.

There are guidelines for treating post-stroke people. For US system guidelines are the base of assurance. Guidelines classify the treatment in levels and classes.

Level A: multiple population evaluated; Level B: limited population evaluated; Level C: very limited population evaluated. Classes are differentiated based on how much the benefits are higher than the risk.

Paper: “Control strategies for robotic movement training after neurological injury”

Now that we know what rehab robotics are, we need to understand how to build them.

The focus of this paper is the rehab robotics after a stroke, so the use of robots to physically interact with participants.

There are 3 directions of R&D:

1. Mechanical design: high number of degrees of freedom, less light, less bulky, portability, wearability...
2. Devices for activity of daily life: portability, safe use, easy use, wearable;
3. Control strategies: define how the device interacts with the participants (Human Robot Interaction).

The final goal is to improve the motor recovery (functional recovery). To achieve the final goal, we need to promote motor neural plasticity through the robotic therapy. The underpinning mechanisms of neuro-plasticity is still under scientific studies, which use a combination of rehab practice, neuroscience and motor control theories.

There are 4 categories of robots:

1. *Assistive controller*, also called active assistance: the robot is helping the subject in achieving the task;
2. *Challenge-based controllers*: the robot is making the task more difficult in order to achieve the task;
3. *Haptic simulation with Virtual Reality*;
4. *Non-contact coaching*: the robot is not worn by the user.

Active assist rehabilitation rehab paradigm: the robot helps the participant to move the limb in the desired pattern. The robot is giving physical assistance to the patient. The rationale:

1. The robot is doing a stretching exercise, preventing stiffness of muscles and reduce the spasticity (very important problem for people with stroke);
2. The robot can help the subject doing the correct task. Moving the limb, neurosensory stimulation is giving to the brain. This helps the brain in re-learning the function.
3. Physically demonstrating the task. In this way repetitive pattern are given and this helps the subject in the repetition of the task.
4. Safety, it's a key requirement. You can propose the subject to have a walking task even if he doesn't have a good equilibrium because the robot is safe.
5. Progress in task difficulty: the robot can change the difficulty of the task, by just changing some parameters.
6. Psychological benefit: the subject need to be rewarded. We need the patient to do the task, only in this way he will do it again. If he can't he will get frustrated and he won't do it again.

The possible problems that come against this concept:

1. You're physically guiding the movement in a specific pattern and the robot assist the subject, so without it he will not be able to do the same movement. Physically guiding a movement change the dynamic of the task. The subject will not learn the final task, but the task made with the robot. We need to modify the "help" given by the robot to make the subject perform the movement by himself;
2. Using desired pattern is limiting motor assistance to discover new patterns to do the task. For example, post stroke people have poor control of dorsi flexion, so they have a very risky walk and inefficient. So, we would like to help them walking, making them up the knee when they have the "drop foot" in order to walk.
3. Slacking hypothesis: subject ride the robot because they understand that if they don't do anything the robot will do the task for them.

Assistance-as-needed

The goal is:

1. Encourage user's participation;
2. Assist the patient in order to accomplish the task.

There are 4 categories for assistance as needed:

1. *Impedance based controller*: when the participant moves along the trajectory, the robot should not work; If the participant deviates from the trajectory, the robot should give assistance giving a restoring force that will take the subject to the right trajectory; Assistance forces increase as the participant deviates, the more the deviation, the more the assistance. The force is proportional to the error: $F = -k(error)$ (impedance controller in robotics).
 - a. Restoring force;
 - b. Back wall: if the subject stops, there a back wall that pushes the subject to the final goal. In this case: $F = k(P(t) - P^*(t))$, where $P^*(t)$ is the desired trajectory.

The major problem of designing this strategy is the desired trajectory. We can use design trajectory by using:

- a. Mathematical models of stereotyped trajectory, minimizing the third derivative of the trajectory;
- b. Physiological stereotypes experimentally: we record the trajectory from 100 healthy subject and then we teach it to the robot;
- c. Pre-recorded trajectory: the physiotherapist can guide the end effector (with the user inside) and move them from A to B and record the trajectory. Limit robot for personalization.
- d. Bilateral trajectory: after a stroke one side of the body is injured and the other one is almost healthy and we can use the almost healthy side to make the movement and the other side will mirror it. Usually the daily activity is not mirroring.

2. *Triggered assistance*: in order to start the movement, we need the subject to start doing the task. When the performance variable is higher than a threshold, than we have:

- a. Impedance based
- b. Force or torques, kinematic (angles, velocities)

Every time we have a triggered assistance, we need to define a *elapsed time*: after a certain time the robot will trigger the movement even if the patient didn't. This assistance can be used at the starting point of the movement, or every 10% of the trajectory.

3. *Counter balance based*: can be done by passive robots (devices which doesn't have motors). The robot is just giving an anti-gravity support of a certain percentage, but to make the movement the subject doesn't need the help of the robot. The task is fully governed by the user, there's no a desired trajectory. On some movement the gravity affects the 70% of the movements, so if we help the patient with these tools, he will use only the 30% of his muscles. Going on with the therapy the help of the robot should be reduced in order to let the subject do the real movement. We can also use *active devices*, the so-called transparent robot (the robot is moving following you). They're compensating the weight of the robot, which means that the subject will not feel the robot weight. At this point we can add a gravity compensation. We always have a passive component for safety reasons: if there's no power supply the robot keeps its position and don't fall down.
4. *EMG-based*: we're grouping EMG_trigger and Myo Control. The first one is a specific solution of (2) based on some activity muscles. The therapist can decide the muscles that he wants to use. Myo control generates a force which is proportional of the EMG recorded signal. The advantage is that the subject doesn't ride the robot and we don't need a desired trajectory, the robot will follow the subject, which is good if the subject has a good control. We will not use it in subject with severe control. The problems are electrode placement, skin impedance... the EMG has worse SNR. We need to calibrate the system every time. Another problem are spasm, which occur in neurological-damaged people, which can result in risky movement in the robot falls. We need to choose the use of EMG based strategy when we are very confident about the situation of the subject.
5. *Performance-based*: adaptive controller. We want the robot to be able to follow the subject. The robot has to modulate the assistance: you adapt the parameters in order to follow the contribute of the patient. As soon as the patient is doing well the assistance has to decrease or it has to make the task more difficult, instead if the patient is struggling, then the robot should help him more or make the task easier. We're modulating the assistance: $P_{i+1} = f * P_i + g(e_i)$. f is the forgetting factor (make the assistance reduce) and P_i is the assistance at iteration i . if the error is increasing, we're increasing the assistance.

This performance based is answering to minimal assistance and a minimal error. This is the cost function.

Challenge-based controllers

The robot is resisting the task or amplifying the error. We learn more to do something if we have a bigger error or if we do more effort. This approach is in continuity care of assistance. When the task is feasible, we can make the task more difficult and don't help him to finish it.

Haptic stimulation

We integrate the robot as a haptic feedback from a videogame. We're in a virtual environment with virtual feedback. It can be cognitive difficult, because it's not a real situation, so the subject need to understand how he has to move in order to move the player. The advantage is that they're more interesting.

Non-contact coaching

The robot is in front of the subject and supporting him and checking if the person is doing the task (NAU ROBOT).

The most important thing is to understand the requirements of the users.

Neuroprostheses

Fundamental of Functional Electrical Stimulation

"FES is the stimulation able to induce the contraction in a muscle without its neuronal control, in order to obtain a useful functional movement".

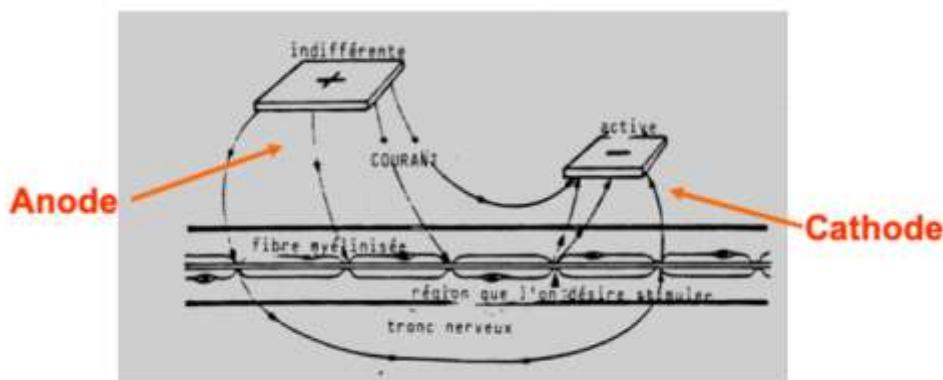
"Functional electrical stimulation (FES) is the technique of applying safe levels of electric current to activate the damaged or disabled neuromuscular system in a coordinated manner in order to achieve the lost function. Neuro-prosthesis is a device that uses electrical stimulation to activate the nervous system. These initiate a physiological-like stimulation in the intact peripheral nerves, providing functional restoration of various body organs in the neurologically impaired individuals."

The first definition was most tailored to spinal injured people. The second one is much broader.

FES has broad applications in the clinic, with different objectives. FES increases muscular strength and fights atrophy and this also has a systemic impact; for example: it helps venous return, increases peripheral vascularization, reduces risks of sores, facilitates their healing loads the bone, with the effect to reduce demineralization and risk of fracture...

Long term immobility has systemic effects that can eventually influence a lot the health and the quality of life of people.

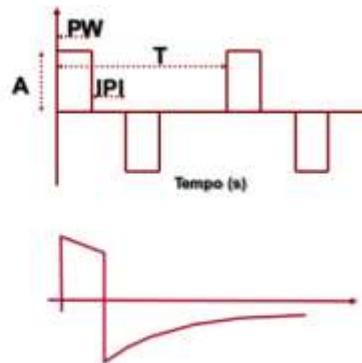
As we start the contraction, muscle needs blood and reactivate the vascularization. Also, the skin and connective tissue benefit of the stimulation.



There is an anode and cathode couple between which some current is flowing (ion movement). Underneath, ions move producing a depolarization of the nerve or muscle. Usually nerves are more superficial and they have a lower threshold and therefore usually we stimulate the last motor neuron. If the induced depolarization overcomes the threshold a spike is generated, which propagates in the axon to the muscle point. This mechanism generates artificial muscle contraction with exactly the same dynamic as the natural contraction (muscle depolarization due to motor neuron stimulus). Only the innervated muscles can be stimulated.

Different stimulation patterns can be identified:

- **Current Amplitude [A]**
(it excites the nerve; there is a current threshold)
- **Pulse width (PW, [μ s])**
(the alternative parameter able to adjust the charge)
- **Tension [V]**
(it supports the current erosion)
- **Stimulus Frequency (1/T) [Hz]**
(it regulates the force of the mechanical action)
- **Stimuli shape**
(it limits possible tissue damage)



- **Current amplitude.** Most stimulator are current controlled rather than voltage-controlled, because peaks of current are most dangerous for the subject. Indeed, imposing the voltage may produce unknown peaks of current depending on variability of impedance due to many possible factors (for example: electrode-skin coupling that is not determined and could be not stable). Typical current amplitude values are 15 up to 130 mA (highest values are applicable only to neurological subjects, with sensorial deficits, indeed the stimuli activate also sensorial afferent fibers provoking pain if too high...).
- **PW** is the duration of the single pulse, it is usually set between 0.1 and 0.5ms
- **Stimulus frequency** depends on the force of the mechanical action that you need to produce and it correlates quite linearly with the induced muscle fatigue
- **Stimulus shape** is usually biphasic in most clinical applications because we do not want to leave unbalanced distribution of ions inside the tissues.

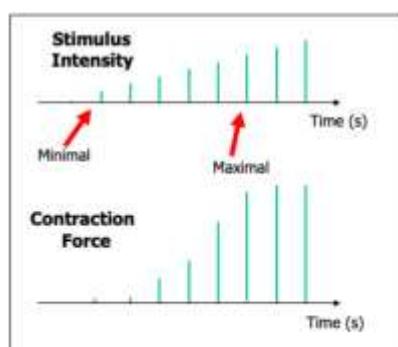
There are three kinds of electrodes that can be used: surface, percutaneous and implanted. Use of surface electrodes is mandatory for any application which is temporary and repeated over time (all rehab settings). Implanted or percutaneous electrodes are used for assistive devices such as sensorial neuroprostheses in amputees. For example, implanted electrodes are used for sphincteric or cardiac stimulation.

NATURAL MUSCULAR CONTRACTION

FORCE GENERATED BY THE MUSCLE

$$F(t) = \sum_{i=1}^N F_i(t)$$

i: index of motor units
N = total number of motor units



Spatial summation

The overall force generated by a muscle is the sum of the contribution of the force generated by each single motor unit. Note - We should always remember that we are dealing with a non-linear system even if it is externally triggered.

In fact, we can observe from the recruitment curve in the slide that there is a minimal threshold under which there is no effect (dead zone), and there is a maximum stimulation intensity threshold above which nothing is changing in terms of contraction force (saturation). In between the behaviour is quite linear: the higher the stimulus, the more the fibers are recruited and the larger is the force produced.

Recruitment curve for FES stimulation shows spatial fibre summation. The increasing of stimulus intensity is achieved by increasing the quantity of charge delivered to the electrodes, the quantity of charge is the product between the stimulus amplitude (current amplitude) and the stimulus duration (pulsewidth), so charge can be increased either by increasing the current amplitude and/or the pulse width.

Muscular contraction induced by natural stimulus is different from the one induced by an artificial stimulus even if the single fibre activation mechanism is the same (stimulus arriving electrically from a motor neuron).

Characteristics of muscular contraction induced by a NATURAL stimulus are:

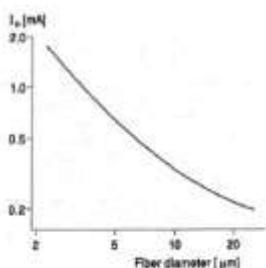
- Asynchronous activation:
 - o Force modulation
 - o Turn over in the fibre activation: in order to keep the same force, different fibers are activated by the brain in an asynchronous way. When we stimulate with FES we make a synchronous activation.
 - o A continuous force is obtained with $f=10$ Hz. When we stimulate with synchronous activation that frequency will be higher.
- Progressive activation depending on the type of fibers:
 - o Type I Fibers
 - o Type IIa Fibers
 - o Type IIb Fibers

The first type of fibers that natural stimulus contract are type **I fibers**. This is because type I fibers are more resistant to fatigue than other fibers thanks to their aerobic metabolism, even if they develop less force. Then, the natural stimulus progressively activates type **IIa** and **IIb** fibers that develop more force but are less resistant to fatigue.

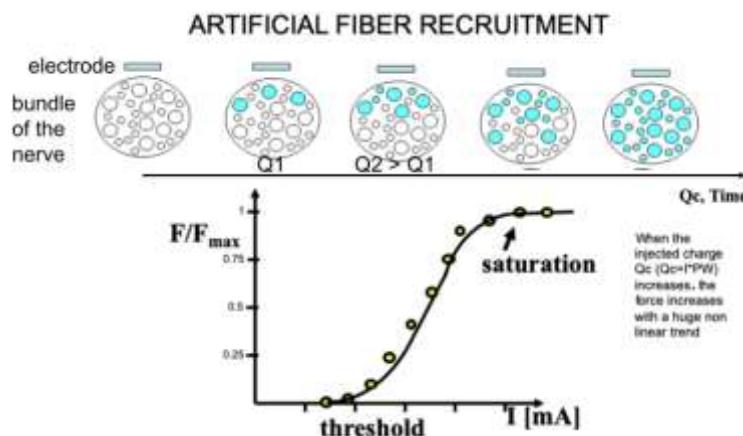
Type IIa have an anaerobic and aerobic metabolism, so they have medium recovery periods and medium resistance to muscle fatigue. Type IIb have anaerobic metabolism, they have long recovery periods and fast muscular fatigue.

ARTIFICIAL MOTOR CONTRACTION

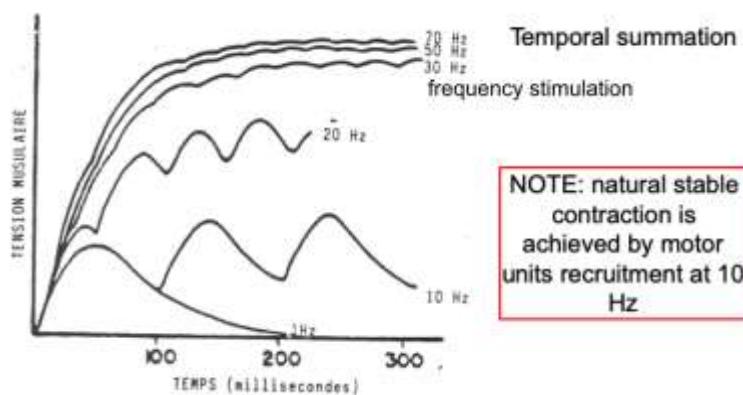
The neural control responds to the proper recruitment of anatomic characteristic. The brain works perfectly in tuning this mechanism. When we put electrodes, we're activating the real AP, but



everything is governed by the threshold. So, there's a huge dependence on who we're activating. Threshold of neurons is inversely proportional to the fiber diameter, so, if a fiber goes over the threshold it will be activated. The artificial stimulation recruits fibers in a non-selective and spatially-fixed way and synchronously.



Artificial muscular activation fiber recruitment depends on two variables: physical distribution and fiber diameter (larger fibers have lower activation threshold). Thus, the first fibers to be activated are large diameter fibers closest to the surface where electrodes are (natural muscular contraction would instead activate small diameters fibers and with spatial selectivity). With electrical stimulation, we can't obtain spatial selectivity, especially in-depth direction.



Here we can observe the effect of temporal summation to the inducing fibers recruitment. The stimulus frequency affects the capability to sustain a stable contraction over time. To avoid twitches we need to increase the stimuli frequency so that fibers' successive spiking results in a continuous muscular force, thanks to the mechanical inertial properties of the muscular tissue. We can induce maximal force development at a 30-70 Hz stimulation frequency.

This aspect is one of the major differences with respect to natural contraction. To avoid twitches, the CNS recruits each single motor unit at 10 Hz rotating across multiple motor units. This is possible thanks

to the non-synchronous recruitment induced by the CNS. To achieve the same results with a system that induces only synchronous activation of motor units, the required frequency is multiplied by a factor of at least 3. This huge difference impacts a lot on the effect of fatigue in the artificial contraction.

ARTIFICIAL

- Synchronous fiber activation
- There is no "turn over" of motor units
- Recruitment order spatially fixed and (II - I)

PHYSIOLOGICAL

- Asynchronous fiber activation
- There is a "turn over" of the motor units
- Recruitment order (I-IIa-IIb)



ARTIFICIAL ACTIVATION LIMITS

- Muscular fatigue
- It is difficult to modulate contractions

We usually want to stimulate muscles that are not healthy (e.g. atrophied muscles) and the more the muscle is deteriorated the more the fatigue is a predominant problem. In the paretic muscle there's a conversion of fibers from type I (slow) to type II (fast). Therefore, training is needed before applying electrical stimulation to increase the developable force and resistance to fatigue.

Controller for Neuroprostheses

The aim of FES control is to modulate the current stimulus during the movement according to the characteristics of the biological systems to control (non linearity and time variability) so to achieve an accurate, smooth and robust task completion.

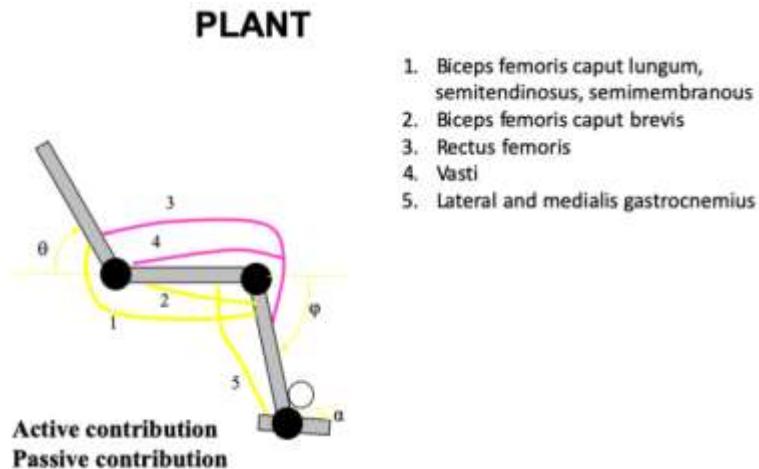
There a lot of existing control strategie: feedforward, feedback, model based, adaptive controllers, controllers based on ANN.

When testing or demonstrating a new control strategy, we have to select a simple movement. When the demonstration has been done on a simple paradigm, then we can move to a more complex motor task, eventually adding some control variables.

Concerning experimental trials, a controller is always previously tested on healthy subjects. Healthy subjects represent a more stable system and the feasibility and reliability of the controller can be assessed. It has to be noted that it is not obvious that a controller that works well on healthy subjects will perform well on neurological subjects as well. There are many different factors, for example: for healthy subjects, it is difficult to stay passive during FES, meanwhile patients that are not able to move can remain passive during stimulation; further fatigue could impact very differently on healthy trained subjects wrt people with neuromotor diseases.

After the *simulation trials* and *experimental trials* on healthy subject we can move to experimental trials on pathological subject.

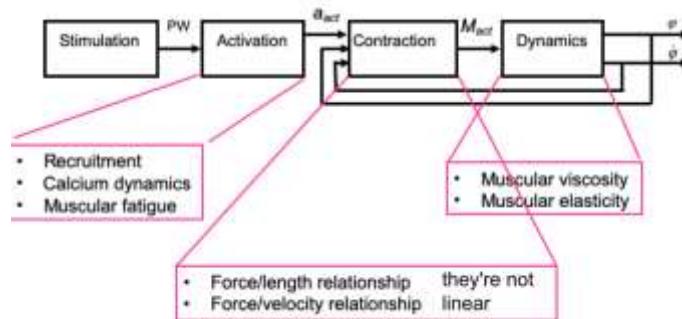
SIMULATION



EXAMPLE – flexion-extension of the knee

As often in engineering, the starting point is simulation. The plant is a biomechanical model that is our reference. In this case for example, all muscles that act on the knee joint are represented. To have a valid and reliable biomechanical model you need to model each single muscle, with its geometrical parameters (origin and insertion), length, maximum exertable force, ...

Simulation: biomechanical neuro-muscular model

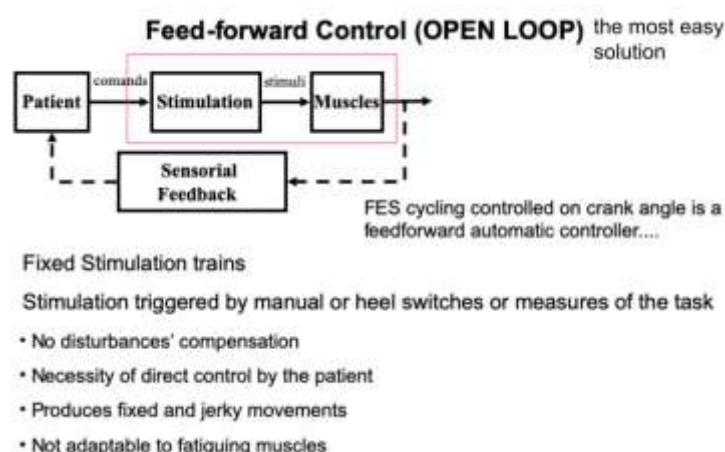


A simulation of the stimulation should include, beside the stimulation paradigm itself, the simulation of the muscular activation, the consequent mechanical transduction (muscle contraction) and the dynamics of the muscle movements, including passive characteristics as well.

The activation needs to include parameter modelling fatigue (recovery time constant), the contraction module needs to convert the level of activation (a_{act}) in variation of length of the muscle and velocity of variations. Then these parameters are converted by Hill's equation into Force.

Once the force exerted by a muscle is defined to sum the contribution of each muscle, you convert the force into a Moment, considering the specific geometry of the system (distance between muscle insertion/origin wrt joint center of rotation).

Once, you have all the muscles' contributions to the joint torque, you have to properly sum them up and include in the overall sum also the passive and viscous properties of the joint itself... alternatively, you can associate the passive and viscous properties per muscle and make a net torque for each muscle and the sum them up at joint level.



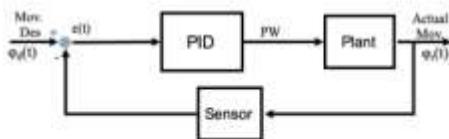
We need to define stimulation parameters to obtain the desired movement. With a feed-forward control, the stimulation train is fixed and it is not changed with respect to the executed movement (e.g. in the case the movement was not the one expected).

The start command for the stimulation is somehow provided either by the subject or by a control based on kinematic parameters (e.g. 1. tibialis anterior stimulation for foot drop during gait is activated when the subject lifts the heel, 2. cycling control is based on pedal position as measured by angular sensors on the crank etc.).

The problem with feed-forward control is that it can't respond to unattended events, because it produces always the same (often jerky) motor patterns.

Note - There is a delay between stimulation and muscle contraction that can be measured, but that can be compensated only in automatic systems, not in case of manual control.

PID controller



Stimulation changes according to the error between the desired and obtained signal
Compensation of unforeseen events

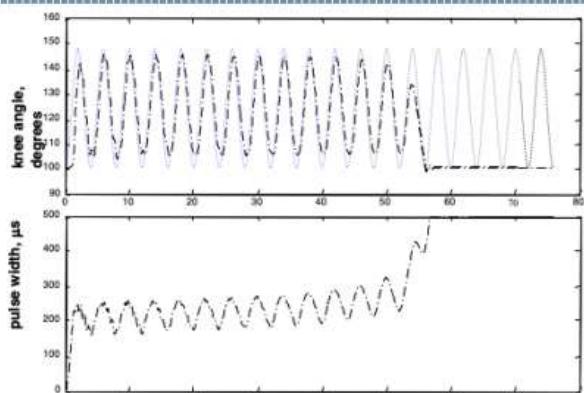
$$pw(t) = K_p \cdot e(t) + K_i \cdot \int e(t) dt + K_d \cdot \frac{de(t)}{dt}$$

We can design a feed-back controller to overcome the problems previously described. We would need some kind of sensors that can measure the effectively executed movement and therefore can assess the difference between desired and actual movement.

The PID controller works on error correction on three aspects: proportional to the current error (P), integrative with respect to the error history (I) and derivative that refers to error changes (D).

This controller, after error estimation, tries to compensate for it. The problem is to set the three K parameters. There are experimental procedures for the correct setting of the constants, but it can be complex for biological systems, most of all when the considered biological systems are human beings. So, what we usually do is to determine a priori constant setting and then correct them while using the controller. Alternatively, we can use settings that have been determined with the corrected procedures and published in literature.

Feedback controller



LIMITS

- Linear controller to pilot a non linear and time variant system
- Difficult identification of the controller coefficients
- Delay in the control due to the dominant pole to stabilize the feedback
- Impossible to compensate fast disturbances

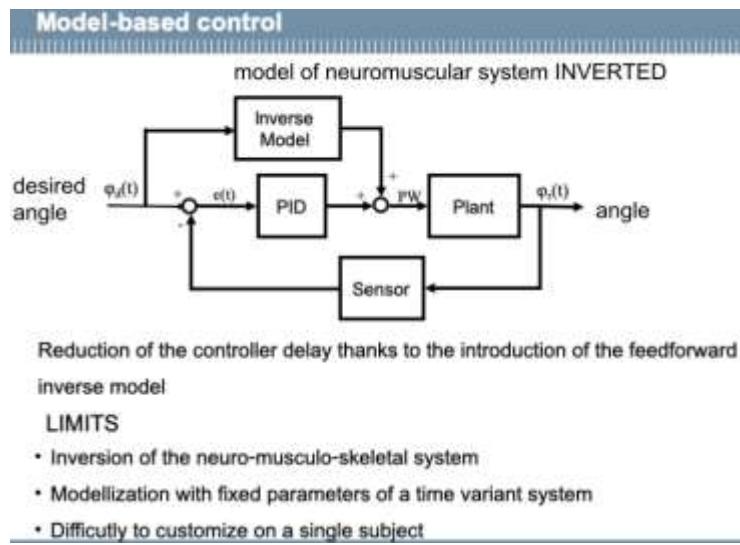
LEGEND:

Upper panel – angle at knee joint in time
 - blue line: desired angle at knee joint
 - black dotted line: actually, measured angle at knee joint

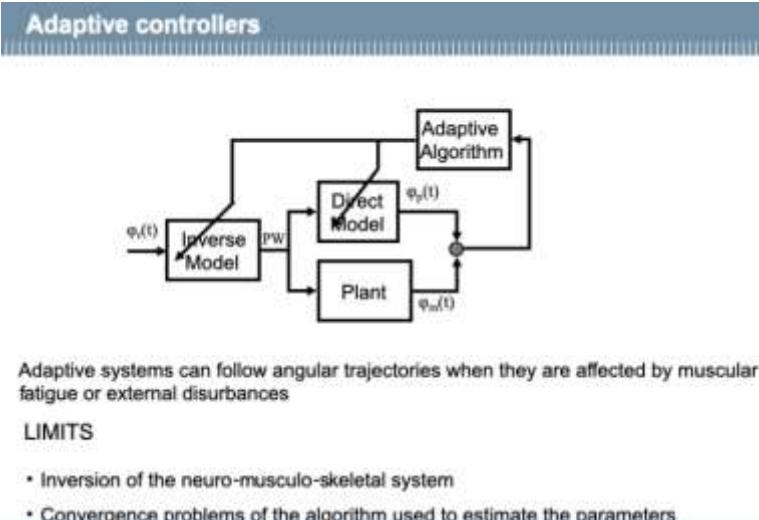
Lower panel – pulse width that induces knee angle (black line) in the upper panel.

As we can notice, PW is initially set as 0 and therefore we have an initial delay in the measured angle while the controller corrects the "error". The problem is that there is a continuous delay between error

detection and correction implementation and this determines the system to never stop stimulation because the system never recognizes the rest angle. Thus, the fatigue problem is amplified. Moreover, a fast or instantaneous event can be corrected, but with a delay. Delay in the feedback comes from the poles of the control system, to assure stability (i.e. negative feedback), you need the first pole of the system to be soon enough to prevent instability which could derive from the second pole (the second pole shifts the phase to $-\pi$ and to have a stable system at $-\pi$ phase shift it is required that the module is below 0 db, Bode criterion for system stability).



setting it up, inverting the model is extremely difficult and induces a complicated set of algorithms... Furthermore, it should be different for each subject... and even for different sessions of the same subject. This is practically impossible; thus any model is a very rough approximation.



Neural controllers (ANN) are the one used more now because they have:

- Black box approach;
- Good generalization;

Another possible approach is to use a feed-forward model that maps the basic behaviour and to use the PID controller only for the feed-back loop. This configuration for example cuts the first pursuit phase where the PW starts from zero and has to increase until reaching the desired angle (inducing delay).

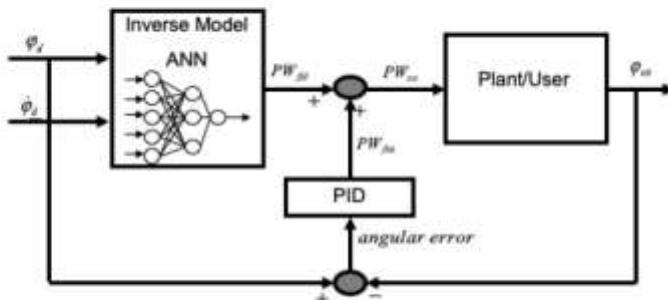
The problem here is that the feed-forward part of the controller should be the inverse model. A straight model is really complicated to set (plant, biomechanics, non-linearities...) and even if we succeed in

A further alternative is an adaptive controller. The point here is to take into account that external system parameters can change during time (e.g. fatigue is occurring). This approach avoids for example the saturation process described in slide 9. Of course, this adaptive controller should be on-line.

- Good adaptability;
- Identification and control of non-linear and time variant system.

The only problem is the availability of training set data. Can we collect a good training set?

Neural Inverse Model



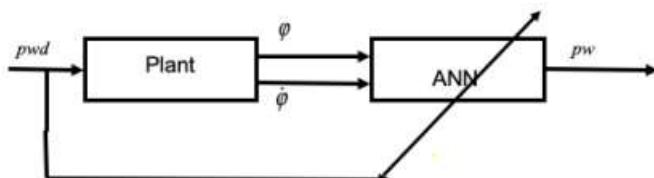
Artificial neural network as feed-forward component (neuro-PID). May we identify a network that acts as an inverse model? This network will have the desired joint angles as the input and the stimulation waves that correspond to the input angles as the output.

- ✓ Quadriceps Stimulation for knee flexion/extension
- ✓ GOAL: assure repetitions of knee flex/ext for muscular conditioning
 - ✓ Problem of fatigue controls more than trajectory tracking!

- ✓ Can we collect a training set ?

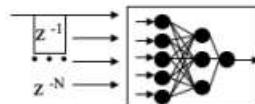
Inverse model identification: NN training set collection

TRAINING



NETWORK CHOICE

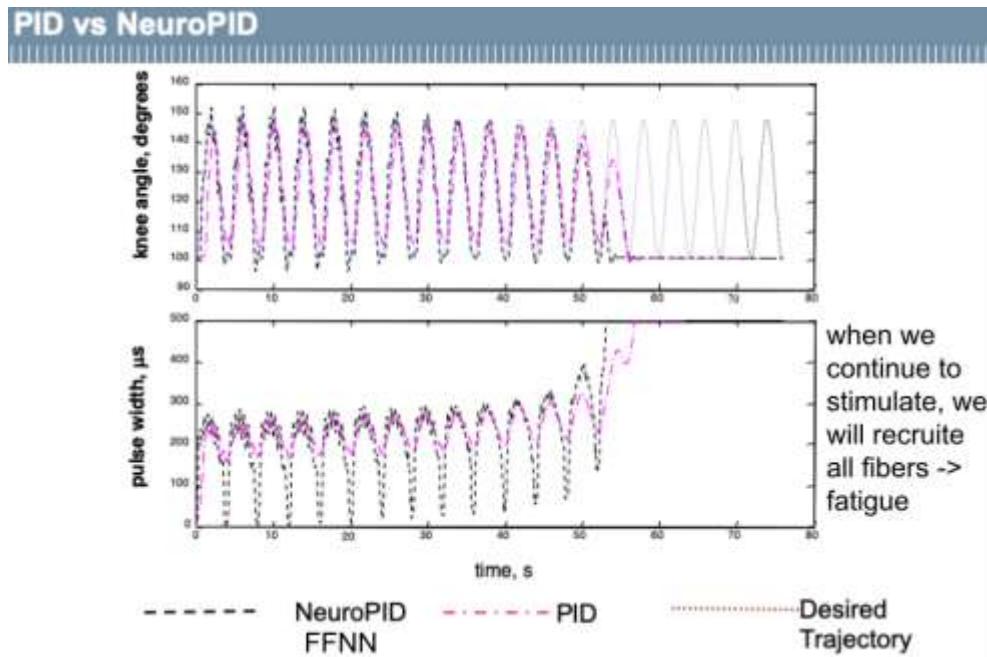
- ✓ Multi layer perceptron with time delay in the inputs in order to identify the system time variability



Supervised neural network: we need couples of input-output data as the TS (training set). We can apply different stimulation waves to the subject and measure the output joint angles induced by the stimulation. In this way, we obtain the TS and we can train a network to learn the relationship between PW and angles. The limit to this approach is that the correct functioning of the controller depends on the exploration of the stimulation

space. The range of the TS should include the range of the application we expect. From an operative point of view, though, it is very easy to be implemented.

Note – this controller result is subject-specific. The collection of the training set is a training of the subject over a similar task... no complicate experimental procedures.



We can notice:

- NeuroPID does not have the initial delay that PID has.
- There is in any case a saturation of the stimulation driven by the PID when fatigue occurs and the PID tries to correct the error.
- The NeuroPID avoids the continuous stimulation leaving a period of non-stimulation in between the repetitions (very good for fatigue recovery)

NeuroPID comprises a neural inverse model and a PID as a feedback controller:

- Reduces the delay effect of the PID controller
- Overstresses the system in order to follow the desired trajectory
- Overcomes the problem of intermediate solicitations

However, a huge improvement with respect to the PID alone is not obtained and instead the training of the ANN inverse model is required.

In case of post/stroke patients for rehabilitation purposes, the main goal is to have repetitive tasks prolonged over time, more than assuring a reliable accuracy of the target... – the PID is based on error correction. If the goal of the clinical exercise is to persist in a movement more than its accuracy, then a PID error correction is useless.

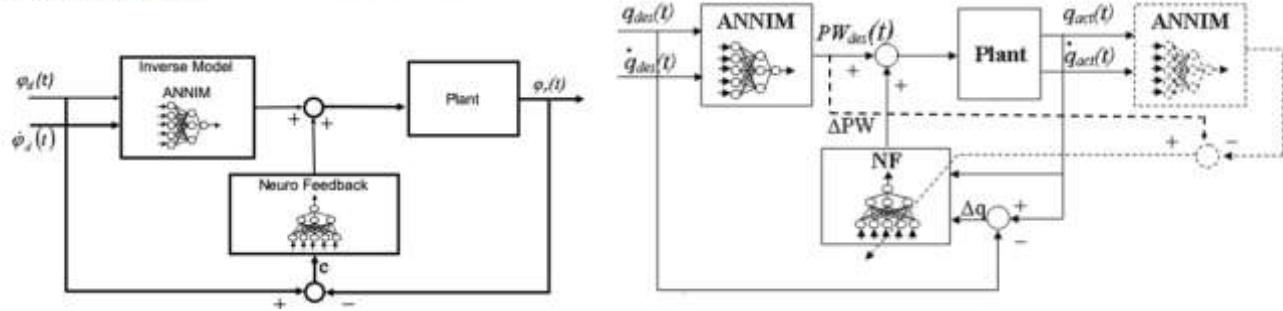
This reasoning may be not applicable to other target populations or if considering assistive devices...

We can insert a network in the feed-back controller as well so as to be able to model fatigue. For this network the input should be the estimated error and the output will be the compensation of the stimulation parameters.

Neurofeedback

Training Problem

Control the stimulation patterns adapting the **time variability** to the muscular properties of a **single subject**



We build the forward neural network mapping values for non fatigue conditions. The problem in building the feed-back neural network is to collect a TS that can map fatigue. We can add the inverse model again after the effectively executed movement so that it can How do we test the system?

a) Tracking ability – ability to track the desired trajectory (e.g. desired angle at the knee joint). In nominal conditions the EMC (feed-forward/feed-back neural networks controller) perform similarly to the Neuro-PID. Both of them cancel the initial delay that the pure PID controller has (this is a trivial effect of inserting a good

feedforward block in the controller).map the actual angle in PW that would have produced the angle in nominal conditions. Thus, if the subject's muscles are not fatigued, the PW used to stimulate coincide with the PW needed to produce the movement estimated by the second inverse model. When the measured angle is reduced because of the fatigue, there will be a mismatch between the calculated PW (second inverse model) and the nominal one (PW applied to the subject). In this way, the difference in PW maps the fatigue and we can train the feed-back network to compensate for the current fatigue level. We no longer need to bring the error to be zero (PID controller).

How do we test the system?

- Tracking ability** – ability to track the desired trajectory (e.g. desired angle at the knee joint). In nominal conditions the EMC (feed-forward/feed-back neural networks controller) perform similarly to the Neuro-PID. Both of them cancel the initial delay that the pure PID controller has (this is a trivial effect of inserting a good feedforward block in the controller).
- Fatigue condition.** Across multiple repetitions, EMC has a profile of PW increase far less than neuro-PID and PIDAW (PID with Anti-wind up configuration). During extension maintenance in each repetition, EMC maintains the same PW, while the other two controllers try to compensate the error with a continuously increasing PW. As a whole, EMC is stimulating less (smaller profiles of PW) – lower panel- with an angle profile -upper panel- which is similar in the first repetitions to the other controllers' ones and then becomes much less degraded as the exercises is prolonged and fatigue occurs. EMC maps the fatigue during the entire extension period and therefore has a more effective correction (less fatiguing).

- c) **Unattended events.** One of the problems of any controller is how it reacts to unexpected situations, as we have a feedback controller some reaction to perturbations is foreseen. Nonetheless, we do not expect a better behavior of the EMC controller in managing unattended events because we didn't train the network to face them. EMC maintains in time an analogous trend as the other two controllers, keeping its better performance in terms of error quantification.
- d) **Reaction to spasm** (sudden maximal spontaneous contractions). The type of reaction and correction to spasm is not better for EMC, but it is definitively analogous and not incorrect. Note – we may think of adding some spasm examples to the training set and this could improve the EMC answer to those events.
- e) **Intra-subject variability.** There is of course a trade-off between generalization capacity and recalibration time.

In order to test the generalization capability of the developed model we need to simulate changes in the parameters of the plant and see how the controller behave.

- a) **Robustness.** Some parameters are not influencing the final performance of the controller (Damping and Trec). We observe that for the mass value there is a quite important worsening of the controller, in this case we should define a threshold on when we have to re-train the system. For example, we can assume acceptable an increase of error of up to 10 degrees, in this case we will need to retrain the network if the mass of the leg has reduced for 45% or has increased of 10%, the advantage is that the muscular mass of the leg can be easily estimated by the diameter of the thigh, for example.
- b) **Single session calibration.** After an electrode replacement, the current amplitude used by the controller has to be re-calibrated.

Biomimetic controllers for FES

Another alternative to build the feedforward controller for FES complex tasks (e.g. multiple muscles) is to design biomimetic controllers. They control the stimulation parameters trying to mimick the physiological motor strategy used by healthy subjects to obtain the same movement.

The objective is to propose rehabilitative clinical protocols based on FES:

- Making the obtained movements smooth and similar to the physiological ones;
- Decreasing the effect of muscular fatigue;
- Increasing the duration of movement and the repeatability.

The motor tasks are: cycling, sit to stand and reaching.

We have an optimized mechanism in our brain which ends up in a stereotype movement. In specific tasks we can collect typical muscular activation in healthy subject and then use these data as the starting point for the stimulation. This is important when we want a coordinated motor task and when we want to retrain the subject.

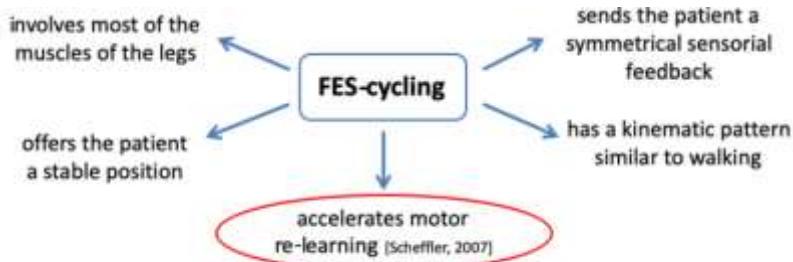
We need to cope with target populations needs.

For SCI (Spinal Cord Injured) the obtained movement will be smooth and energetically optimized because it mimicks the physiological one.

For stroke patients, the biomimetic controller gives the patient a proprioceptive and skin afference of the task that is similar to the physiological afference («normative reference of sensorial feedback» including proprioceptive). Therefore, the biomimetic controller should enhance motor re-learning of the physiological strategy used to obtain that movement.

The recovery of walking ability is the main goal of post-stroke lower limb rehabilitation. Clinical evidences suggest that functional and cyclical movements induced by FES enhance the motor re-learning process. Within this framework, pedalling could become a safer and more economical alternative for the recovery of locomotion after stroke.

In term of relearning the possibility to walk again is trained with cycling, because the movement is similar to walking (coordination of legs, control of muscles, cancel the problem of equilibrium).



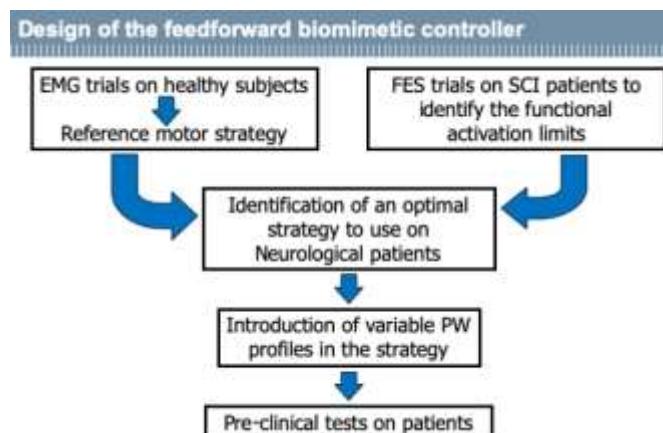
Recent studies evaluated the feasibility of FES-cycling on post-acute [Ferrante et al 2008, Sezci et al 2008] and chronic hemiparetic patients [Janssen et al 2008, Alon et al 2010].

However, up to now:

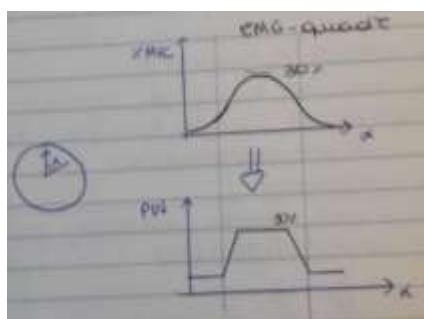
- There are no standard protocols
- Different categories of patients (chronic, post-acute)
- Few patients involved

The specific requirements for controller design are:

- Control strategy aimed at obtaining a movement:
 - Prolonged on time minimizing fatigue
 - Symmetric and human-like
- Development for a user-friendly GUI for clinical applications.

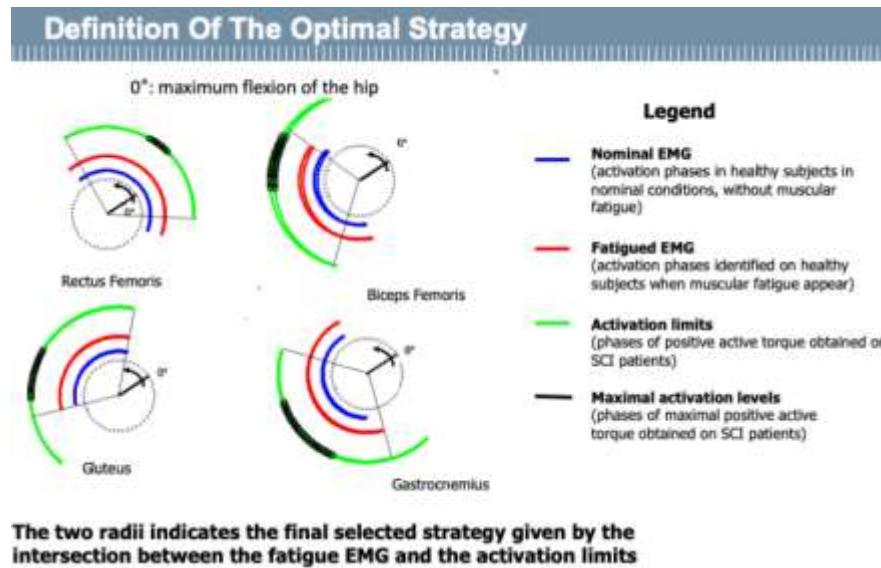


With healthy subject we have a huge repeatability, which is used as ground for the stimulation on non-healthy subject. At every angle of pedal, we can record the level of contraction of every single muscle through an EMG on healthy people. We want to convert it into pulses profile for stimulation.



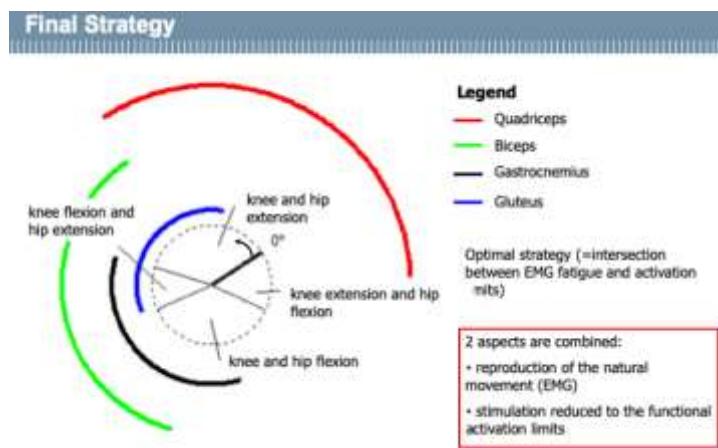
We convert the percentage of the MVC (Maximal Voluntary Contraction) in a PW. When we do this conversion, we need to consider that in the first graph we have healthy subject and natural contraction, instead in the second one we have a FES that have to be applied on an impaired patient. We know that when passing from one situation to the other the fatigue is exploding. How can we deal with it? We consider the FES only when the stimulation is facilitating the task. During a task we can use one muscle to do the task or we can have a muscle that stabilize the joint. When we flex the knee the quadricep is not doing the flexion, but

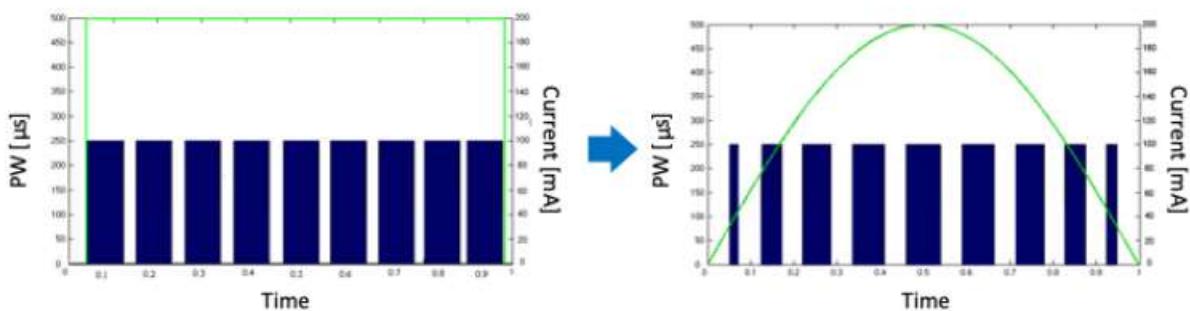
it is active in order to flex it slowly without making the leg fall. So, when we flex, we also need to activate quadriceps. We can stimulate the muscles of a very paretic person one by one during a cycling exercise to understand when the stimulation is pushing or pulling. With this experiment we can understand how to convert the %MVC into PW. We also need to consider the contribution of gravity.



It is observed that the initial instants of activation of each muscle group coincide with those identified by the activation limits and the final instants with those defined by the EMG in fatigue conditions. An exception is for the biceps femoris, in fact the initial instant coincides with that of the EMG in fatigue conditions and the final one with the end of the activation limit, the inverse compared to what happens for the other muscle groups.

We have chosen to use the identified strategy in the case of tired muscle conditions because they are the ones that best reflect the situation of artificially stimulated muscles. The reverse recruitment order is indeed one of the first causes of muscle fatigue. In light of the above, the final strategy was obtained by following the following rule: the angular range resulting from the intersection of the activation limits was chosen with the values obtained from the electromyographic signal under fatigue conditions. The optimal strategy uses functional ranges for movement.





The PW varies in a sinusoidal way within the angular ranges defined by using the optimal stimulation scheme

Unlike the strategies in the literature, we propose the use of a variable pulse width (pw) within the angular range of interest.

Analyzing the data obtained from the tests on the myelolesi subjects, a repeatability was found in the angular incidence of the maximum torque applied during the movement: in all the tests, performed for both subjects at different speed and duration of the impulse, this variation was kept below 10 °.

We therefore chose to use this parameter in order to make the stimulation session more efficient by minimizing fatigue. To do this, we decided to use a pulse width that gradually increases until it reaches a plateau at the maximum phase. muscle function and then decrease until the end of the range.

We chose to vary the pulse width linearly and sinusoidally.

The neural bases of FES re-learning process

Sometimes, after using the device for a while, patients may report that there is a ‘carry-over’ effect. This may be short-lasting or long-lasting. There is a need to explain how a peripheral stimulus could restore the deficit resulting from a central lesion; and this ‘enigma’ of carry-over has been commented on and reviewed.

Physiotherapy helps, but FES seems sometimes to have a specific ‘carry-over’ effect in addition. We aim at studying the specific feature of FES that can promote this carry-over beyond conventional PT.

The idea is to design better FES and get into on who would benefit and target only on those patients.

Three possible ‘peripheral’ mechanisms might come to mind, but none seem to resolve the enigma. The point is that none of these mechanisms is applicable only to FES.

1. Possible ‘peripheral’ mechanisms of therapeutic benefit from FES
 - a. *Muscle strength and fitness.* Firstly, FES might improve the fitness and strength of the remaining motor units to which the patient has voluntary access, by means of a training effect on them. But if the patient has access to them, then appropriate exercise training should be just as good; adding electrical training of paralysed motor units should not improve voluntary strength at all, unless it also brings them under voluntary control.
 - b. *Muscle length and connective tissue stretch.* Secondly, FES might improve the flexibility and range of motion of the affected limb, so that the voluntary efforts become more effective. But if that were the case, then passive physiotherapeutic stretching of the affected limb should be just as good. Physiotherapy helps, but FES seems sometimes to have a specific ‘carry-over’ effect in addition.
 - c. *Muscle spasticity.* Thirdly, FES might be reducing the amount of spasticity in the muscle and improving function in that way. FES does improve spasticity in some patients, though this is a complex, short-term and not a consistent effect. Also, FES ‘carry-over’ can occur in muscles which are weak without spasticity. On both these grounds, relief of spasticity can be dismissed as the basis of the ‘carry-over’ effect.

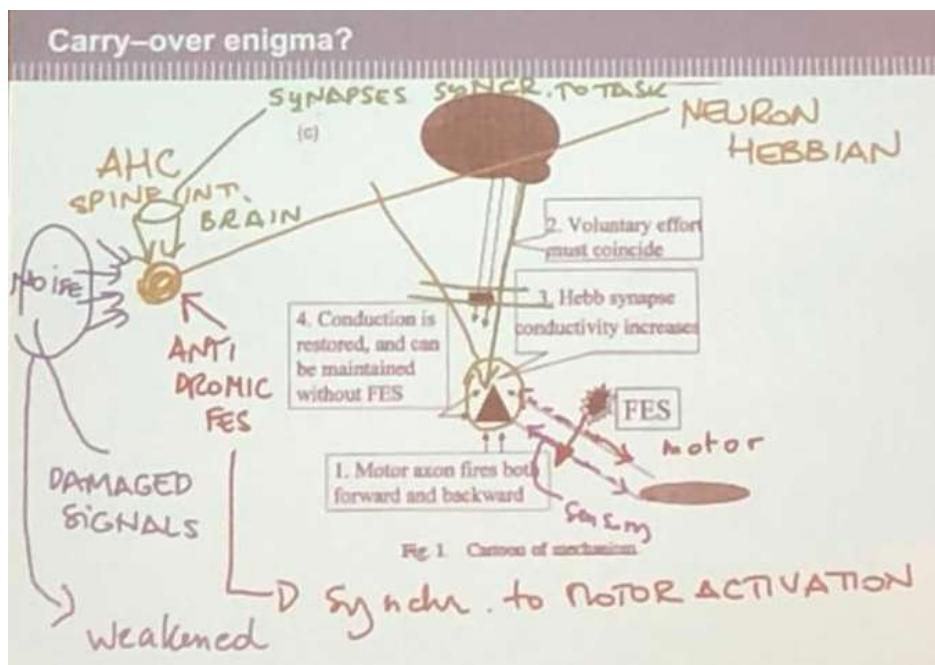
None of the ‘peripheral’ explanations can account for the carry-over phenomenon.

2. Possible mechanisms of central therapeutic benefits of FES. None of the obvious peripheral explanations can account for the carry-over phenomenon. How otherwise could FES have a therapeutic effect? Could FES in some way modify the connections of damaged central pathways? There is evidence that suprasegmental (including cortical) mechanisms can reorganise following injury, and this has attracted most attention.
 - a. *Cortical reorganisation.* There is imaging and neurophysiological evidence that cortical reorganisation can occur following stroke and recovery from stroke (as it can during the normal learning of special skills), and that processes of neural plasticity can be modified by drugs such as amphetamine or methylphenidate. There is some evidence that the ipsilateral hemisphere and the uncrossed corticospinal tract take over a role in

movement control in the recovery from hemiplegia. Forced repetitive active movement of a paretic limb appears to facilitate the recovery from hemiplegia, although for this to work well there must be a residue of active movement to build upon. It is presumed that forced active repetitive movement facilitates cortical reorganisation and the utilisation of ipsilateral pathways. There is evidence for this, but the precise mechanisms involved are likely to differ as between cortical and subcortical strokes.

- b. *Possible central effects of FES.* FES, particularly when applied through surface electrodes, activates both motor and sensory nerve fibres. High-frequency sensory stimulation may in itself be capable of modifying cortical connectivity. Evidence has been put forward in the rat that this might occur through the expression of the c-fos gene, both in the brain and in the dorsal horn of the spinal cord. In these contexts, FES may be enabling simulation of the sensory effects of paralysed movements, and a correspondingly appropriate sensory context to facilitate cortical reorganisation. The evidence for and likely mechanisms of cortical plasticity in response to electrical stimulation in hemiplegia have been the subject of recent review and will not be dealt with at length here. There does not however seem to be any obvious mechanism by which FES could be uniquely effective in promoting cortical reorganisation. Could FES alter connectivity at segmental level?
- c. *Segmental reorganisation.* Following injury to the corticospinal tract by accident or disease, changes in reflex function occur at the segmental level, over weeks and months. Spasticity, clonus and spasms may develop. H-reflexes change [18], as do cutaneomuscular reflex patterns. These changes are thought largely to reflect alterations in the connectivity of the anterior horn cell (lower motor neurone). Could FES itself modify the connectivity of the anterior horn cell in a favourable way? Is there any unique property of FES treatment which might enable it to do this? **Antidromic firing could be this critical event, because it is unique to electrical stimulation.**
- d. *Antidromic firing.* Electrical stimulation of a motor nerve fibre, whether in the nerve trunk or in an intramuscular branch, generates both an orthodromic (centrifugal) and an antidromic (centripetal) impulse. This ‘backfired’ antidromic impulse is usually ignored, except by neurophysiologists, who can record a reflection of that impulse. This is seen because the impulse, having reached the anterior horn cell, depolarises and somehow circumnavigates it, and in a minority of cases the impulse returns down the motor axon. This causes a second small clinically identifiable electromyographic response called the ‘F’ wave. No other form of therapy is capable of activating the anterior horn cell repeatably, predictably and at high frequency on demand.
- e. *Hebb synapses.* Now, why might antidromic discharge of the anterior horn cell be beneficial? One possible explanation harks back to Donald Hebb, who long ago proposed that some modifiable synapses would be strengthened if presynaptic firing coincided with or was shortly followed by postsynaptic discharge: success breeds success. Conversely, insofar as presynaptic and postsynaptic activities are uncorrelated, the connection is weakened. Terminals compete for space and contact on the dendritic tree.

Those that are effective become stronger, while those that are ineffective lose out. Hebb-type synapses have since been discovered in many species and in many locations. They have been of particular interest in the brain, in the context of learning and memory. The underlying mechanism is thought to be what is described as 'Long-Term Potentiation' (LTP), which was originally described in 1973 in the hippocampus of the rabbit and was later identified as a phenomenon occurring in many other areas of the mammalian CNS, including the anterior horn of the spinal cord. Anterior horn cells integrate the spinal reflexes and brain signal. During FES, the same cells receive orthodromic and antidromic stimulus. In a natural situation, electrical stimulus travels in a single direction just because the previous nerve tract is in refractory period. In FES, the stimulus starts from a point "in the middle" of the nerve and therefore can spread in both the orthodromic and antidromic directions. This means that antidromic stimulus is temporally aligned with brain intention of movement and therefore Hebbian synapses are reinforced. FES reinforces synapses that have activation synchronous with the executed movement with "damage" to the synapses that are not synchronized to the movement (e.g. clonus).



Carry-over is facilitated by FES because what happen at the AHC (Anterior Horn Cell). At that level we have many input (brain damage -> the signal is modified or stopped before reaching the AHC) coming from the CNS and also noise (damaged) signals and the antidromic signal coming backward. The antidromic FES is perfectly synchronized with the motor evolution of the task (contraction), because is the same signal. This is a Hebbian Neuron, we have a signal (antidromic FES) which give the pulse and is strengthening all other signals that are synchronized with it. Over time, we have that signals that are not synchronized (noise) are weakened. Assumption: there must be still some residual good connection coming from the brain and we need the subject try to do the task even if he can't do it by himself, in a way to reactivate those signals.

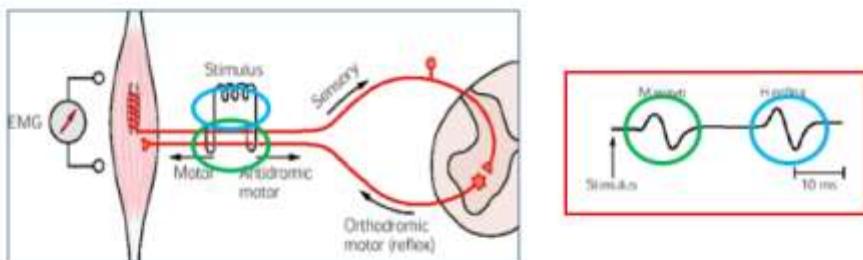
Design of myocontrolled neuroprostheses

EMG controlled stimulation is a big challenge because:

- Background
- EMG signals during hybrid muscle contractions
- Devices for EMG recordings during electrical stimulation
- Filters to estimate the volitional EMG component
- Control strategies for myocontrolled neuroprostheses
- Example of applications on neurological patients: EU project MUNDUS, EU project RETRAINER

The stimulus acting in the middle of the fiber goes to the muscle and the sensory fiber. After the stimulus we have the M-wave (direct reaction of motor activation) and then the H-reflex (arc reflex). The EMG profile will see first the stimulus, then the M-wave and then the H-wave.

Neuromuscular Electrical Stimulation (NMES) has been used for assistive and rehabilitative purposes in neurorehabilitation for the last forty-years [Scheffler & Chae, 2007].



NMES induces muscle contractions through the depolarization of motor axons (**peripheral recruitment**) and sensory axons (**central recruitment**). [Bergquist, 2011].

When muscles are not completely paralyzed, EMG signals of the paretic limb can be used to control the timing and the intensity of the stimulation. WHY SUCH A CONTROL SCHEME IS ATTRACTIVE?

1. ASSISTIVE PURPOSES: Myocontrolled NMES augments the force of the paretic muscles allowing the users to directly control the execution of the movements.
2. THERAPEUTIC PURPOSES: NMES co-incidental with the voluntary drive enhances motor re-learning.

When this kind of control is not adequate?

1. In case the subject has a very low residual EMG it could be possible to use it for triggering but not for more complex controls
2. In case the subject has a poor control of the muscle recruitment, amplifying his residual contraction can be negative

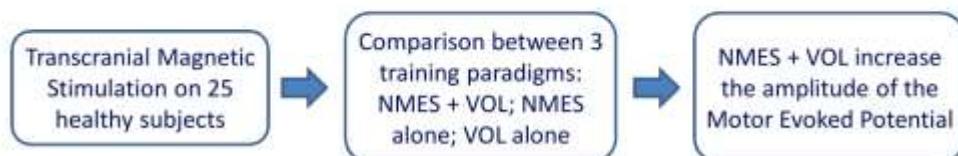
3. In case the subject has frequent spasms, it could be dangerous to apply a EMG controlled stimulation

What are the reason for EMG-controlled NP?

NEUROPHYSIOLOGICAL HYPOTHESIS FOR IMPROVED MOTOR LEARNING

CORTICAL LEVEL

- 1) NMES-augmented voluntary activations increase cortical excitability with respect to voluntary activations alone or passive NMES [Barsi, 2008]



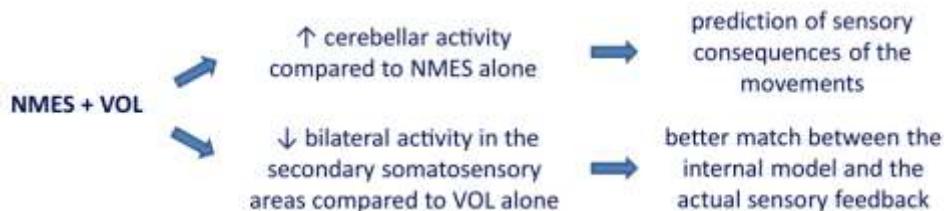
Cortical effects of FES and FES+VOL

NEUROPHYSIOLOGICAL HYPOTHESIS FOR IMPROVED MOTOR LEARNING

CORTICAL LEVEL

- 2) NMES combined with voluntary effort improves the prediction of sensory consequences of motor commands [Iftime-Nielsen, 2012]

fMRI study on 17 healthy subjects to compare cortical activity induced by NMES + VOL, NMES alone and VOL alone.



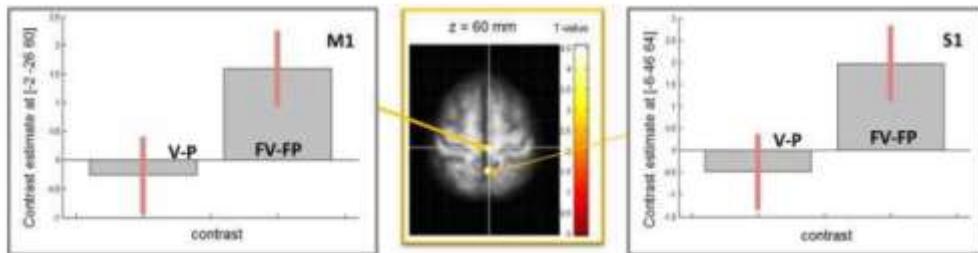
These findings indicate that during the VOL condition the cerebellum predicts the sensory consequences of the movement and this reduces the subsequent activation in SII. The decreased SII activity may reflect a better match between the internal model and the actual sensory feedback. The greater cerebellar activity coupled with reduced angular gyrus activity in FESVOL compared with FES suggests that the cortex may interpret sensory information during the FES condition as an error-like signal due to the lack of a voluntary component in the movement.

Neurophysiological hypothesis for improved motor learning

CORTICAL LEVEL

- 3) The NMES- augmented proprioception in the context of volitional intent produced a higher activation than NMES-augmented proprioception in the absence of volitional movement [Gandolla et al, 2014]

- ✓ fMRI study on 17 healthy subjects during ankle dorsi-flexion
- ✓ 2x2 factorial design, with volitional intention and NMES as factors:
 - V: only volitional; P: only passive; PV: passive + NMES; FV: volitional + NMES



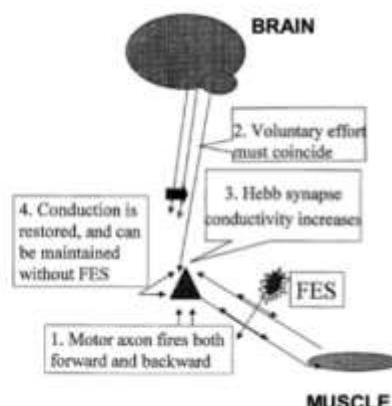
- 4) The NMES- augmented proprioception in the context of volitional intent produced specific activations in Carry-over responders wrt (with respect to) Carry-over non responders. The ability of a patient to plan the movement and to perceive the simulation as a part of his/her own control loop is important for the FES carryover effect to take place.

Before the treatment it was seen with FES + VOL that responder activate the area, instead non responder did not activate it. Subject that experience FES in a passive way did not re learn, instead the one who experienced active FES improve their abilities.

Neurophysiological hypothesis for improved motor learning

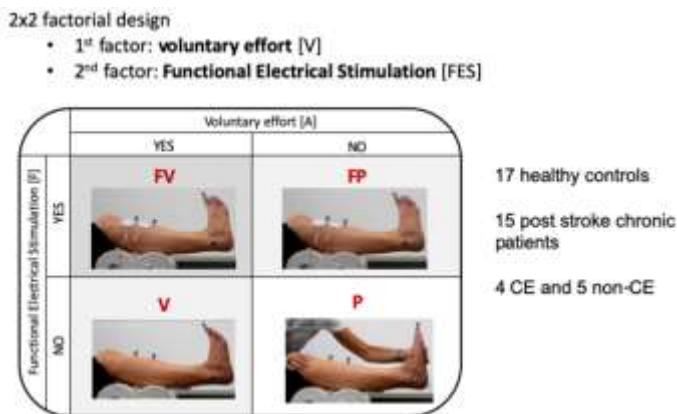
NMES antidiromic impulses combined with coincident voluntary effort synchronize pre-synaptic and post-synaptic activity of the anterior horn cells


Restorative synaptic modifications at spinal level [Rushton, 2003]



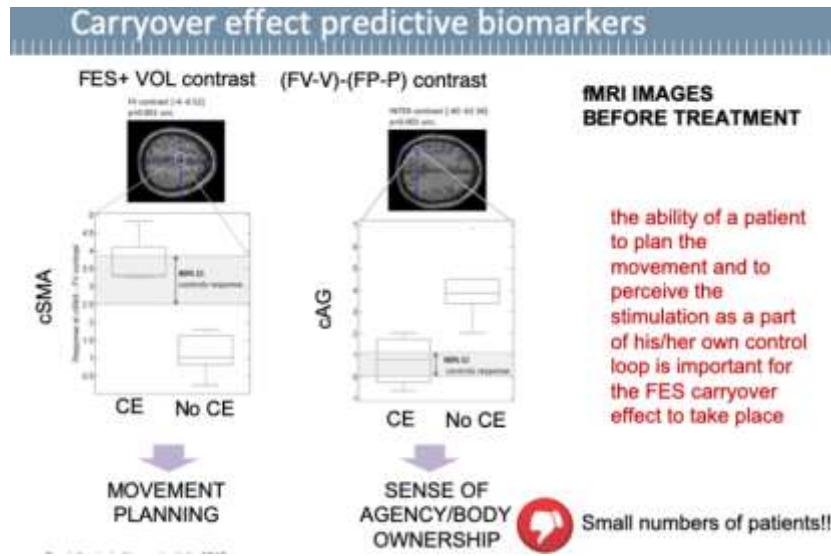
One of the most important clinical data of FES is that for some patients the benefit is mainly peripheral, the contraction, although artificial, fights muscle atrophy and reactivates peripheral vascularization and obtains function. In some patients, however, there is a motor learning effect that lasts over time. Even once the stimulation is over, this is called CARRYOVER. A major question in the scientific literature since the 1980s was the mechanism of this carryover and which patients actually benefited from it. This was mentioned as CARRYOVER ENIGMA. Here, we wanted to understand this enigma, or at least to help understand it above all to be able to identify possible markers able to identify whether a patient was a good candidate for this relearn or not ... Some patients, around 45% treated with FES during the walk had a benefit even after the stimulation system was removed while others were not... CARRYOVER ENIGMA. The hypothesis from which we started is that this effect was linked to brain correlates of the use of FES, because no peripheral clinical (muscular or sensory) data was able to explain the different outcome. On the other hand, various studies had already shown that in these patients the fall of the foot could not be recovered by conventional physical therapy.

At this point we were ready to investigate the **carryover enigma**. In fact, we wanted to compare 4 conditions that integrated two factors differently - the presence of attempted voluntary control by the subject - the presence of the artificial stimulus. In this way a 4-condition matrix is built, only voluntary movement, only FES, FES + voluntary movement, passive movement (no FES and No VOL).



I summarize some steps:

- We studied healthy subjects first
- We therefore recruited a group of chronic post-stroke subjects with the problem of falling foot, we measured their motor capacity through various clinical tests with particular attention to the problem of drop foot and we acquired them with the functional resonance protocol
- These subjects used an FES system for the correction of the falling foot at least one hour a day for a month according to specific indications given by the clinicians at the end of the month we reworked all the initial measurements including the functional resonance and through appropriate algorithms and the opinion of expert clinicians we divided them into patients with and without carry over.



We suggest that the mechanism of action of FES carryover is based on movement prediction and sense of agency/body ownership. In other words, the ability of a patient to plan the movement and to perceive the stimulation as a part of his/her own control loop is important for the FES carryover effect to take place. Although we point to abnormal responses in SMA and AG as indicators that a FES carryover effect is unlikely, it might be that in future a behavioural questionnaire devoted to the evaluation of self/non-self perceived FES-induced movement might be useful in predicting the carryover effect in routine clinical settings.

Hence a robotic system for upper limb rehabilitation was born that tries to integrate these aspects into a paradigm of exercises (European RETRAINER project with various European univ and industrial partners). It is an exoskeleton: mechanical system that is mounted on the patient's arm and partially (or totally) supports the weight.

Fixed to the chair or wheelchair

- A FES system that is controlled by the patient's voluntary activity in the muscle itself: that is, if the patient begins a minimal muscle contraction, the FES of that muscle is activated and amplifies the gesture
- A graphical interface shows the subject the exercise to be done which is mainly a sequence of movements to reach objects or positions on the table.
- An RFID identification system of each object with a special antenna mounted on the exoskeleton allows to identify the achievement of the target and to give the patient the new target. In this way the objective of the movement is evident and guided by the subject, who participates in it, the muscles are activated via FES in combination with the residual voluntary control and the subject can make an intensive sequence of exercises with partial supervision by the physiotherapist, expanding its daily rehabilitation work.
- Clinical data say that indeed patients who intensively use this system (3 days a week treatment half an hour a day for 9 weeks) have a much better recovery of arm functionality than the control group that performed traditional treatments at equal intensity.

There are strong reasons to support the development of neuroprostheses with FES.

There are two different solutions of **myocontrolled neuroprostheses**:

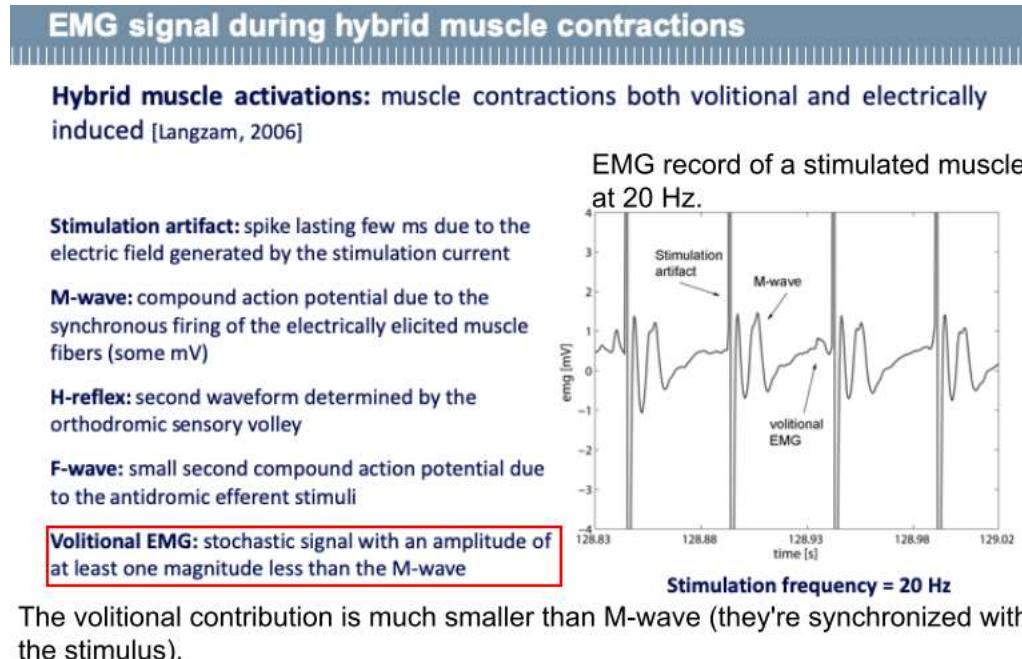
1. EMG-TRIGGERED NMES: residual volitional EMG is used to trigger the onset of a predetermined stimulation sequence applied in an open-loop modality to the same muscle used for control. We need to read the EMG before simulation to start.
2. EMG-CONTROLLED NMES: residual volitional EMG is used to modulate the stimulation intensity in a closed-loop modality to the same muscle used for control. Record during stimulation and modifying stimulation according to EMG.

TWO DIFFERENT SOLUTIONS OF MYOCONTROLLED NEUROPROSTHESIS

	EMG-TRIGGERED NMES	EMG-CONTROLLED NMES
PROS	Simple to implement → EMG signal is measured only before NMES starts	Assure the synchronization between NMES and voluntary effort
CONS	No guarantees about the synchronization between NMES and voluntary effort	More complex technological solutions are needed for the design

In the case of EMG-triggered NP, there is no check about the actual contribution of the subject during the execution of the task (risk of Slacking). In the case of EMG controlled NP, a continuous monitoring of volitional EMG is required to check for subject's participation, but the problem

of stimulation artefact needs to be solved.



The volitional contribution is much smaller than M-wave (they're synchronized with the stimulus).

Depending on the frequency of stimulation, the H-reflex and the F-wave could be masked by the following pulse and therefore are not visible.

Devices for EMG recording during FES

Standard amplification unit for EMG recordings can not be used in the presence of NMES



The **stimulation artifact** is the result of a potential difference produced by the stimulation current between the EMG electrodes → it **can not be rejected by the differential amplifier**



Since its amplitude is one to three orders greater than the M-wave, it can **saturate or even damage the amplifier of a standard EMG circuit**



Different solutions have been proposed to face the problem of the **suppression of the stimulation artifact**.

The first solution is to make a **blanking**. We know when we are going to deliver the pulse, we can stop recording the EMG in that period, so the saturation is avoided. In this way we are losing the signal in the blank window, which has a duration of 100 micro seconds. We can lose the part of the artifact. We're disconnecting the EMG every time we are giving the pulse in order to eliminate the artifact and avoid the saturation.

The second solution is to use the **low gain amplifier**. It assures that the artifact is not saturating the acquisition amplifiers and, at the same time, a proper high resolution ADC on the other hand allow a good accuracy in the reading of the volitional (small) components of the signal.

Devices for EMG recording during FES

RECORDING AND STIMULATION ELECTRODE

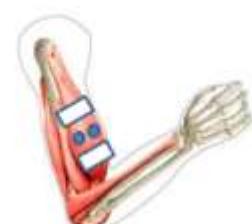
Standard solution: separate recording and stimulation electrodes

The relative placement of the electrodes affect the capability of the system to suppress the stimulation artifact

Regular placement for
EMG recordings
(SENIAM guidelines)



This placement is
preferred in the presence
of NMES [Frigo, 2000]



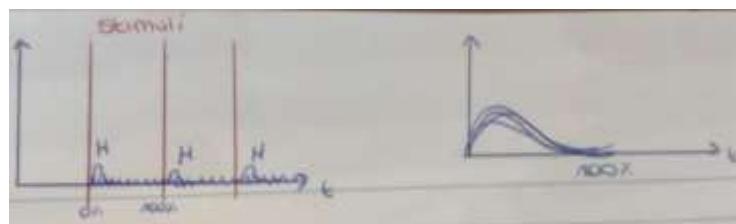
Higher common mode
component of the
stimulation artifact

- Stimulation electrode
- Recording electrode

There are two possibilities to extract the volitional EMG:

1. *Blocking window.* The signal is zeroed for the first 20 or 25 ms of each inter-pulse period. The volitional EMG is estimated from the remaining part of the inter-pulse period. → The M-wave is not completely removed;
2. *High-pass filter.* Assumption: 20-30 ms after the stimulation pulse, only low-frequency electrically-induced components superpose the volitional EMG- Blocking window + high-pass filter with a cut-off frequency between 200 and 330 Hz. Because M-wave has a specific frequency content, we can cancel it out with a high pass filter in order to extract the volitional EMG.

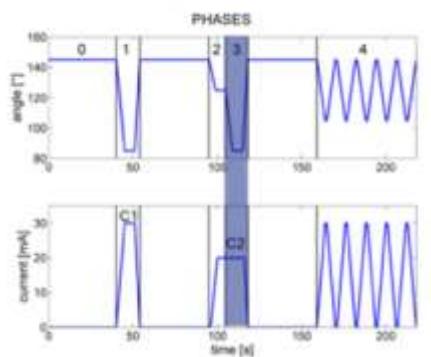
We assume that the volitional EMG is stochastic (band limited Gaussian signal) and the M-wave is repeatable and time-variant. To extract the volitional EMG, we use a short blocking window, to have a low gain+ high resolution EMG recording and apply the *linear prediction adaptive filter*.



We superimpose all the inter-pulses, making a mean. The stochastic part (volitional) will disappear and we obtain the M-wave. We subtract the wave to the original signal, obtaining the mean level of volitional EMG during each inter-pulse interval.

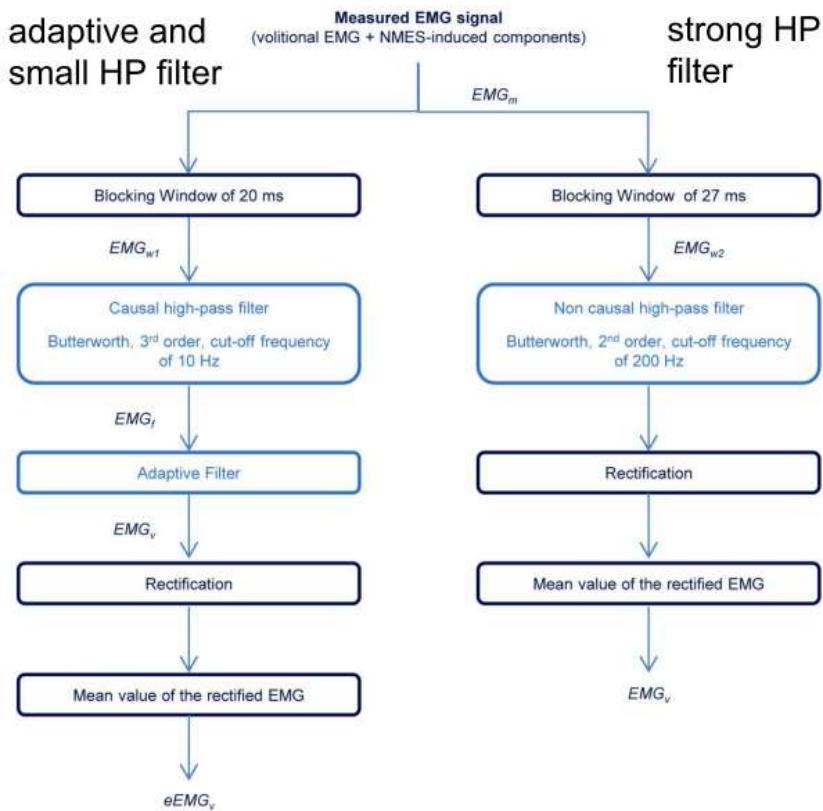
HOW TO IDENTIFY THE BEST FILTER ?

Comparison between a time-domain (**adaptive filter**) and a frequency-domain method (**high-pass filter**) on dynamic EMG signals acquired on **healthy subjects** (N=10) and **neurological patients** (N=8)



phase 0: rest
phase 1: stimulation C1, no volitional
phase 2: stimulation C2, no volitional
phase 3: stimulation C2 plus volitional
phase 4: variable stimulation, no volitional

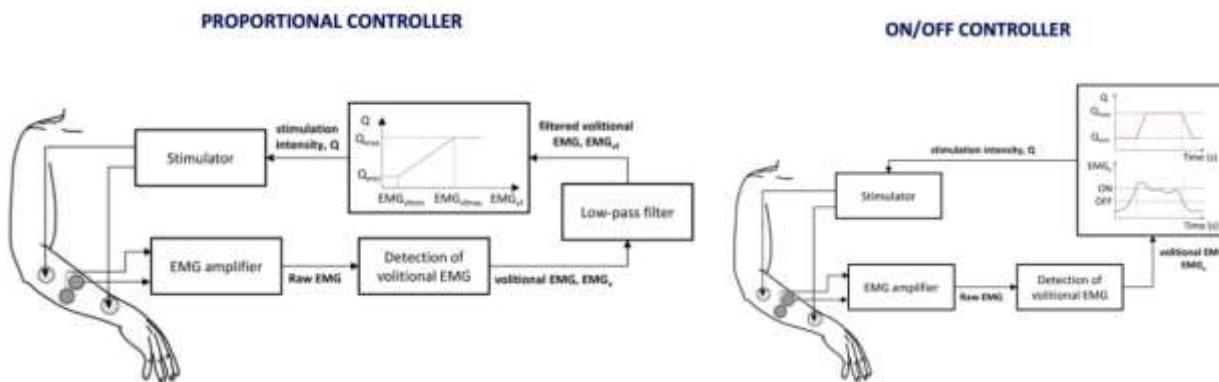
The goal of those filters is to distinguish phase 3, when we ask the subject to move, so when we aspect to see the volitional control. The two possibilities are:



The capability to extract the volitional component is higher in adaptive filter than HP.

How do we design a myocontrolled NP?

We need to convert the volitional EMG into the command of the stimulation. This is what we need in a mycontrol stimulation, because we want to follow the volitional contribution. We need to transform it into pulses. There are 2 possibilities: the proportional controller and the ON/OFF controller.

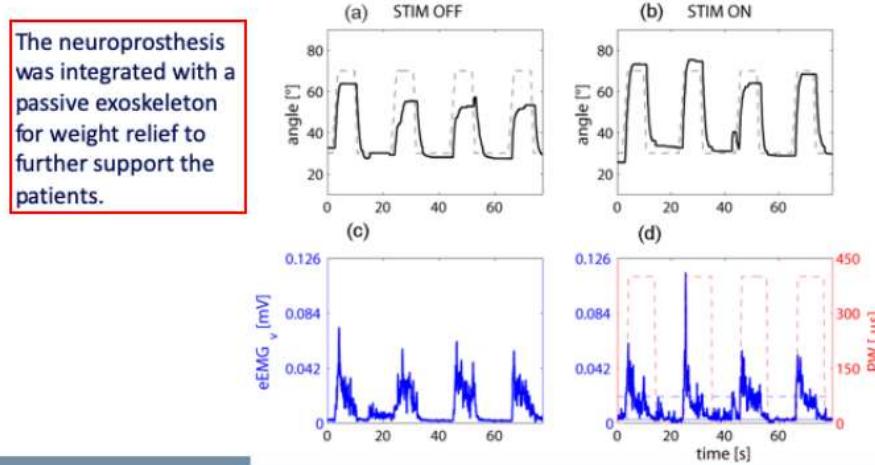


Example of applications

Test of the ON/OFF controller

Task: elbow flexion-extension with and without myocontrolled-NMES support

Participants: 2 healthy subjects and 3 people with Spinal Cord Injury

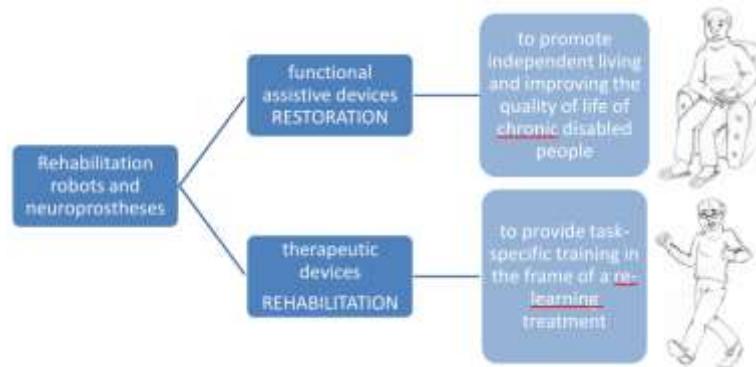


In rehabilitation we should use the EMG triggered and then extract the volitional, because we want to give the subject a feedback about how he is doing the task. This is better than the on-off controller, because it's difficult and it take time to decide the thresholds.

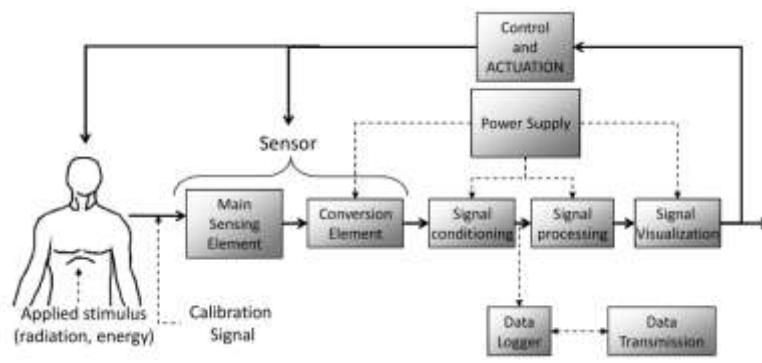
When do we stop the FES? When the subject finishes the task, the stimulation is turned off and when he wants to start again the new task, the stimulation is started (triggered).

Interfacing neuroprostheses and robots to subject intention

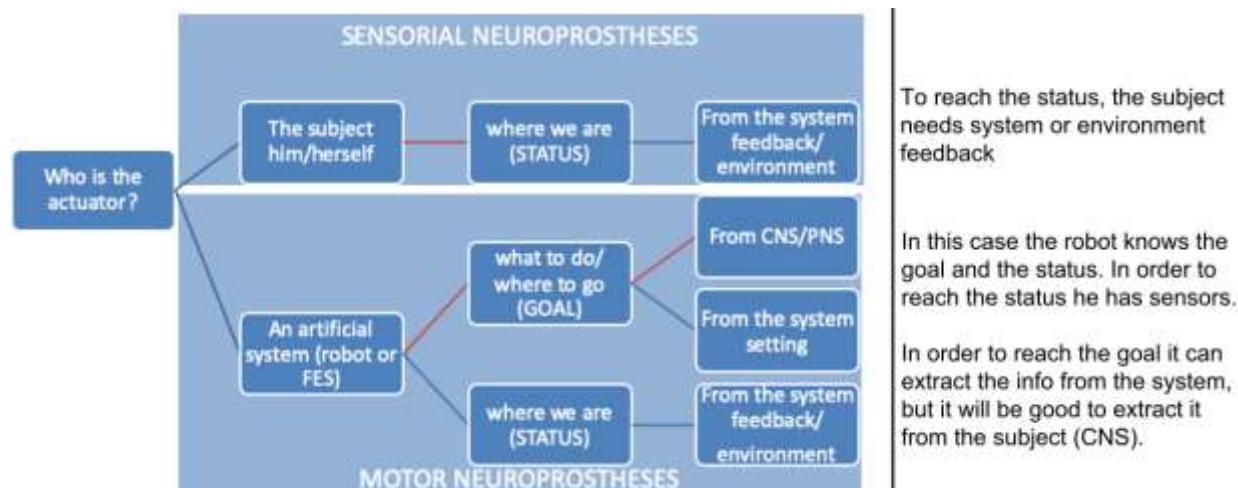
Now we consider together neuroprostheses and robotics. They both share the 2 different targets: restoration and rehabilitation.



They both need a signal coming from the subject to be controlled.



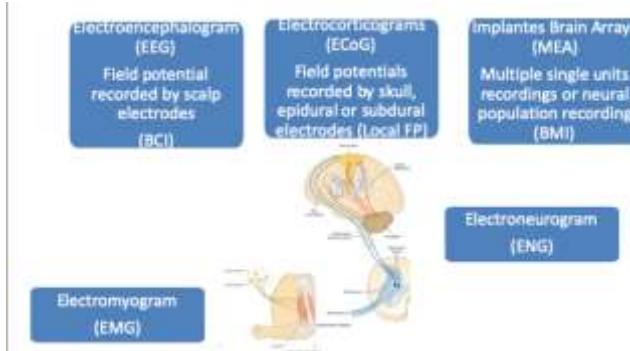
Actuation can be either done by Robots or by FES we are not here distinguishing these two possibilities.



Sensorial prostheses are implanted device where information recorded by the sensor are sent back to the nerve so that they arise to the brain of the subject. E.g. sensors on a hand protheses that will send the signal to the brain so the subject can feel if the object is soft.

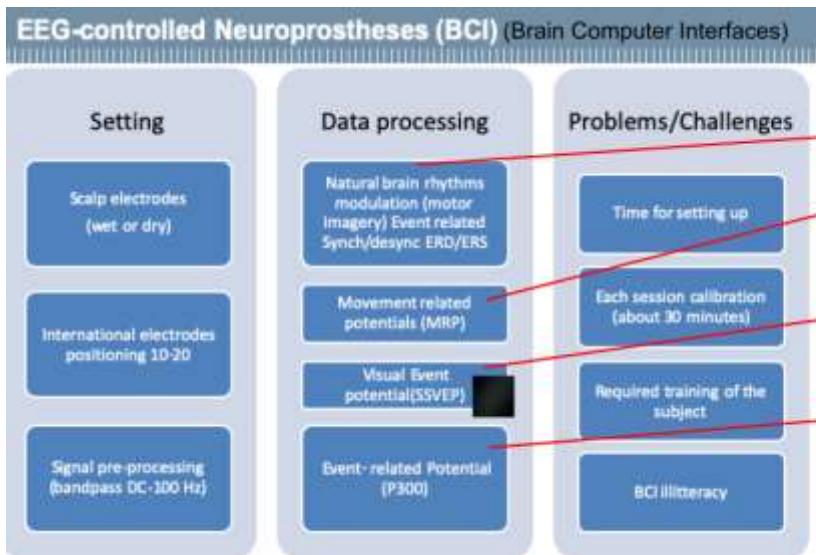


Where can we put sensors in order to collect the intention of where to go?



There are many areas contributing to motor control:
brain → spine → nerve → muscle (where we can record EMG).

The more pertinent choice of recording depends on the objective of the intervention!



- we give the stimulus
- imagination of the motion
- inputs at different frequencies are given
- and the patient has to yes/no, left/right
- big peak when we're looking at a stimulus that we expect.

Multi Electrodes Arrays controlled robots (BMI)

Setting	Data processing	Problems/challenges
Cortical arrays of electrodes (4x4mm)	Spike decoding observation-based seven dimensional neural decoder of firing rate	Surgery Each session calibration (about 15 minutes)
One single implantation in M1 (hand area, Hochberg; two for Collinger)	model that linearly related neural firing rate to movement velocity	Required training of the subject (three times per week for 13 weeks; each session was about 4 h, Collinger et al)
Signal pre-processing (spike detection, spike sorting , spike classification)	orthomagnitude attenuated the brain-command component perpendicular to the ideal seven-dimensional trajectory	Illiteracy? few tested people so far Is the simplest, cheapest and most usable solution?

ENG-controlled robots

Setting	Data processing	Problems/Challenges
Peripheral nerves activity	Triggering of Drop foot stimulations	Surgery
Cuff electrodes /transversal Electrodes	Control of hand neuroprostheses (Tombini et al); efferent fibers MOTOR NP	Required training of the subject Illiteracy? few tested people so far

EMG-controlled robots

Setting	Data processing	Problems
Signal generated by muscular contraction (Mostly) Surface electrodes Band 10-500Hz	Triggering of Impedance control robots Triggering of FES by other muscles Triggering of FES by the same target muscle (Blanking circuit) Myocontrolled NP (Blanking circuit + extraction of volitional control)	Electrodes positioning, electrode-skin contact instability and calibration at each session (about 15 min) Crosstalk from adjacent muscles Same muscle control only if a weak but functional activation is still present In the case of other muscles control the resulting task is rather unnatural

Conclusions

Restoration /rehabilitation

Sensors to connect intention to action: neuroprostheses

- Motor NP/Robots
 - EEG controlled NP
 - MEA controlled NP
 - ENG controlled NP
 - EMG controlled NP

TAKE HOME MESSAGES

The main resource is always brain neuroplasticity

The optimal sensor and NP solution depends on the target of the application and the disability

Brain-computer interface (BCI) neurotechnology has the potential to reduce disability associated with paralysis by translating neural activity into control of assistive devices.