What is the Origin of the Hyper-excitability of cortical and spinal motor neurons in ALS?

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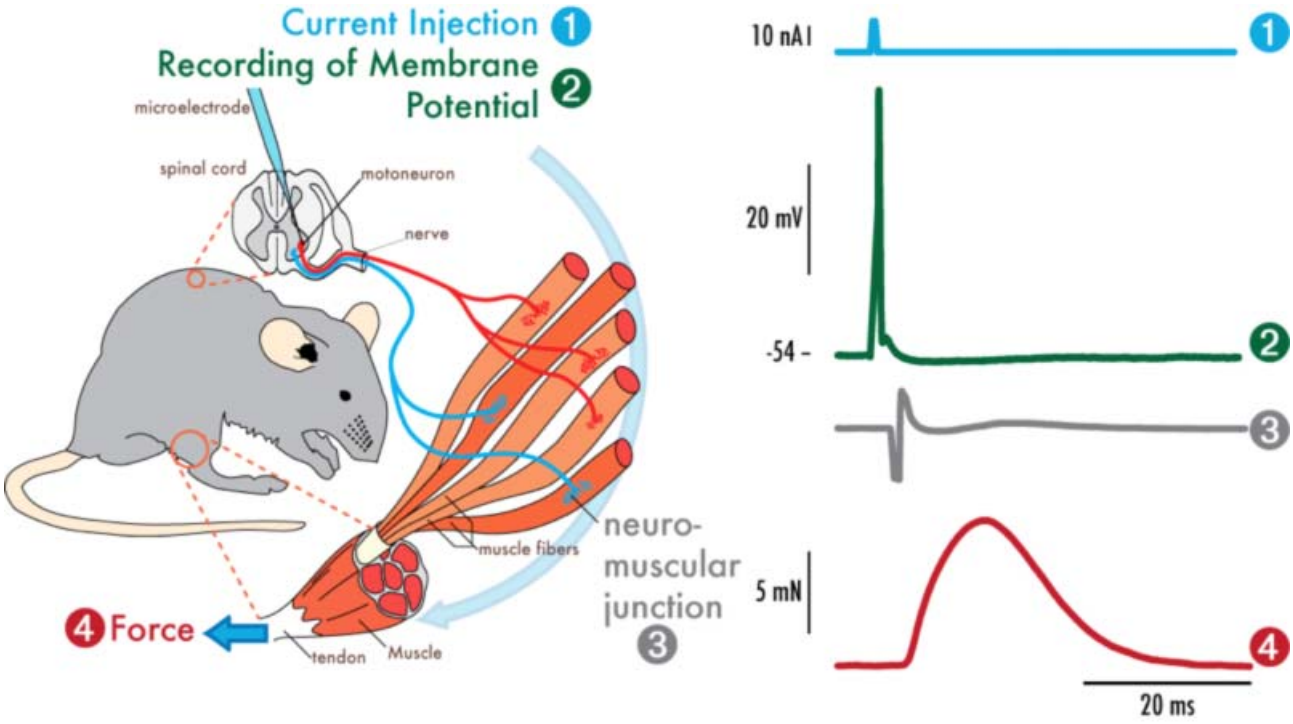
One current hypothesis is that a hyperexcitable state of the motoneuron could induce excitotoxic damages in ALS (Lewinski and Keller, TINS 2005). Studies of excitability in neonatal SOD1 motoneurons in vitro and in cell cultures have produced conflicting results (Kuo et al. J Physiol 2005; Bories et al. Eur J Neurosci 2007; Quinlan et al., J Physiol 2011). Voltage sensitive Na and Ca currents are upregulated at a very early stage, within 5-10 days post birth. Yet it is not clear if this upregulation places the cells in a hyperexcitable state because electrical conductance also increases, which tends to reduce excitability.

The key question of this project was to determine which of these two trends would dominate as the animal matures and symptoms develop. To answer this question, we systematically investigated the subthreshold and firing properties of spinal motoneurons of SOD1 mice at ages ranging from P30 to P80 using the in situ preparation that we have developed (Manuel et al. J Neurosci 2009 and see figure). In adult animals, we found that the input conductance was 35% higher in SOD1 than in WT MNs. In many MNs, the excitability parameters (recruitment current, gain) were unchanged despite the increased input conductance. This suggests a homeostatic regulation of the excitability. However, the excitability declined in other motoneurons since they were no longer able to elicit a sustained discharge in response to a stationary input (but they were still able to fire one spike in response to a transient input).

Our work shows that motoneurons are not intrinsically hyperexcitable in SOD1. Instead the intrinsic excitability progressively decreases in the motoneuron pool. However, excitotoxicity could still arise from an unbalance between excitatory and synaptic inputs. We are investigating this question in the two main targets of the disease (spinal motoneurons, corticospinal neurons) and at two different ages (2nd postnatal week and adults).



New method to study the electrical properties of adult mouse motoneurons

We are studying the excitability of motoneurons by injecting current through the microelectrode (1) and by recording the electrical response of the motoneuron (2). Furthermore, we are recording the electrical activity (EMG) of the muscle fibers innervated by the motoneuron under investigation (3) which allows testing if the connection between the motoneuron and its muscle fibers is strong or starting to fail. Finally, the physiological type of the motoneuron can be identified based on the force profile of the muscle fibers (4).

Credits: Marin Manuel

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The selective death of motor neurons in the cerebral cortex and the spinal cord during ALS remains poorly understood. In mutant mice with a mutation responsible for the disease, some early alterations of neuronal properties have been observed at a very early stage of the disease, long before the symptom onset. We will attempt to distinguish the part of these changes due to the network synaptic activity from the part that is due to the modification of the neuronal intrinsic properties. Furthermore, in the spinal cord of young animals these early alterations have been shown to stem on modification of the morphology of the neurons. We will analyze the morphology of neurons recorded in the motor cortex and in the spinal cord of adult mutant animals (just before the first symptoms of the disease), to assess whether their morphology is still impaired at this later stage.

With this study we want to understand the alterations of the excitability observed in upper and lower motor neurons, with the aim to individuate new strategies for the ALS treatment.

Scientific background and aims of the project

The precise molecular pathways leading to motor neuron injury and cell death in ALS remain not understood. Several hypotheses have been framed to account for the neurodegeneration, including deregulation of Ca^{2+} homeostasis (Choi, 1988; Tortarolo et al., 2006; Guatteo et al., 2007), mitochondrial disturbances and neurofilament accumulations (Cleveland and Rothstein

2001, Rao and Weiss 2004; von Lewinski and Keller 2005; Grosskreutz et al., 2007), altered functionality of ionic channels inducing neuronal hyperexcitability (Kuo et al., 2004, 2005; Zona et al., 2006; Pieri et al., 2009), and glutamate excitotoxicity (Trotti et al., 19999; Heath and Shaw, 2002; Rao and Weiss, 2004). Although the involvement of each of these factors has been well established, their temporal and spatial interplay remains elusive. It is generally thought that excitotoxicity arises from excessive Ca^{2+} entry into motoneurons and is exacerbed by the poor motoneuronal Ca^{2+} buffering capacity. Such a Ca^{2+} entry can originate from the excitatory synaptic events through the activation of both NMDA receptors and Ca^{2+}-permeable AMPA/kainate receptors lacking the GluR2 subunit. Moreover, the extracellular glutamate level is increased in ALS mice, presumably because of a reduced glial glutamate uptake, which can be caused by oxydative damage to the glutamate transporter EAAT2 (Milanese et al. 2010, Rattray and Bendotti 2006, Van Den Bosch 2006). On the basis of the finding that intrinsic excitability is increased in cortical motor neurons (Carunchio et al., 2010), it is reasonable to predict that hyperexcitable neuronal networks in mutant mice would generate an abnormal increase of neurotransmitter release (excitatory and inhibitory) in both cortical cells and their spinal targets : the motoneurons. Such impairments of the balance of the frequency of excitatory and inhibitory synaptic events impinging on the neurons could either be reinforced or counterbalanced by postsynaptic effects such as those due to receptors modifications (Guatteo et al. 2007, Carunchio et al. 2008) or the early modifications of the excitability of the spinal motoneurons (Amendola and Durand 2008, Pambo-Pambo et al. 2009, Quinlan et al. 2011). Finally, hyperexcitability of the neuron itself may increase the number of emitted spikes. Since Ca2+ also enters the cell during action potential generation, membrane hyperexcitability might also contribute to the excitotoxic process.