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Cortical Mapping of Hindlimb Muscle Circuitry in the Mouse

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Introduction

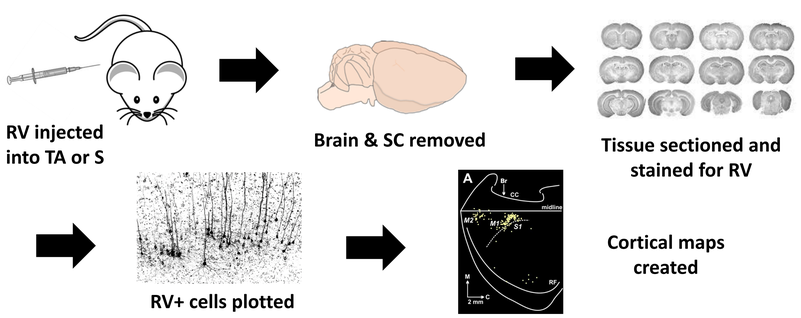
Motor neurons, the cells responsible for the control of voluntary muscle movement, degenerate and die in amyotrophic lateral sclerosis (ALS). This progressive and fatal condition affects more than 20,000 people in the U.S., with 5,000 new cases diagnosed each year[1]. The motor neurons located in the spinal cord, brainstem and brain are all affected; however, it is unknown where the disease-initiating event(s) occurs and how the neural circuitry mediates disease spread. Furthermore, it is not known whether the denervation pattern for fast-twitch muscles differs from that of slow-twitch muscles.

Current studies of ALS make frequent use of the G93A SOD1 mouse model, which shares pathological features with human ALS. Before we can begin to catalog innervation changes as a result of ALS in this well-established mouse model, **we must establish a basis for comparison via a neural atlas for normal mouse**. Such maps will allow for investigation into how and when synaptic connections are affected as ALS progresses and to determine at which locations the network is most vulnerable.

Methods

Rabies virus (RV), an established retrograde transneuronal viral tracer, was used to map the neural circuitry. Both tibialis anterior (fast-twitch) and soleus (slow-twitch) muscles were investigated in control mice on the C57Bl/6 background. At the designated survival time, the animal was sacrificed and the brain and spinal cord were removed and stained for rabies-positive cellular proteins.

Brain sections were examined and plotted using bright-field microscopy. Laboratory software allowed for the aligning of these sections and the creation of an unfolded cortical map. These maps display the location of labeled neurons in a two-dimensional representation of the cortex and were developed for both tibialis anterior (TA) and soleus (S) muscles.



Rabies Virus Transport

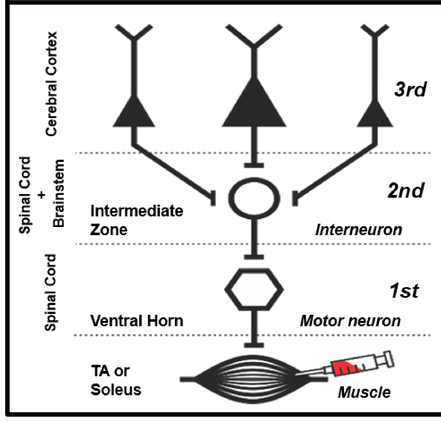


Figure 1. After injection into muscle, rabies virus crosses the neuromuscular junction to be taken up by the motor neurons responsible for direct innervation (first order). The virus then replicates and continues to move trans-synaptically to neurons directly (second order) and then indirectly (third order) connected to the motor neurons[2].

Cortical Map

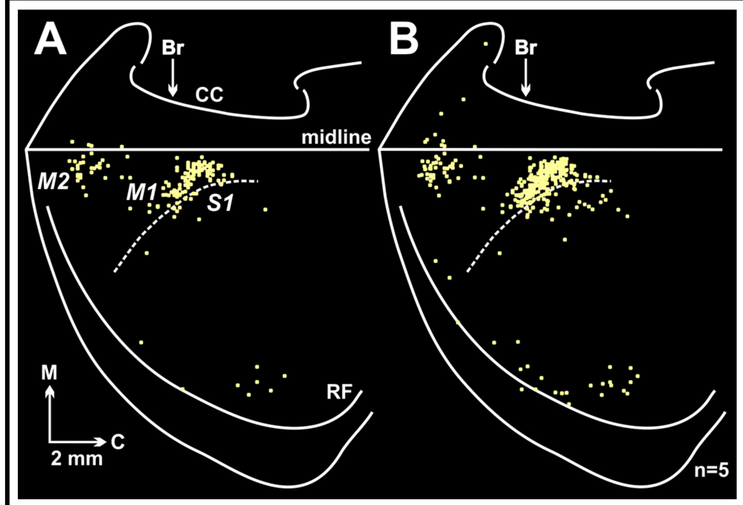


Figure 2. An example cortical map illustrating motor cortex communication with the kidney in the rat, presented from D. Levinthal and P. Strick[3]. **A**. Flattened cortical map of cortical neurons in Layer V labeled after retrograde transneuronal transport of RV from the kidney. The medial wall of the hemisphere has been reflected upward and joined to the lateral surface at the midline. The dashed line indicates the border between granular (S1) and agranular (M1) cortex in the region of the forelimb and hindlimb representations. **B.** Composite map (n = 5) of cortical neurons in Layer V. Each square represents a single labeled neuron. Bregma (Br), caudal (C), corpus callosum (CC), medial (M), midline of the hemisphere (midline), primary motor cortex (M1), secondary motor cortex (M2), rhinal fissure (RF), and primary somatosensory cortex (S1) are labeled.

Discussion

We identified rabies-positive staining in spinal cord motor neurons, spinal cord and brainstem interneurons, and layer V cortical neurons. This staining represents three distinct stages of innervation in the mouse: first order, second order, and third order (see Figure 1). Required survival times for each level of transport were determined by evaluating multiple survival times and then increasing or decreasing the survival time as needed. Preliminary cortical maps illustrating innervation of the hindlimb muscles are consistent with electrophysiology studies of the mouse. In further comparisons of tibialis anterior and soleus muscle cortical maps, we expect representation of soleus innervation to be more medial than tibialis anterior.

Ongoing Work

We have successfully established a protocol for cortical mapping of the mouse hindlimb muscles, and we are working towards a comprehensive atlas. We will do so by developing cortical maps for both TA and S at additional survival times, allowing for direct comparison between the innervation of fast-twitch and slow-twitch hindlimb muscles. Additionally, by evaluating the same survival times in the ALS mouse model, we will be able to identify the timing and origin of transport deficits, aided by a basis for comparison via our established atlas of normal mouse circuitry.

References

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