

In Mammalian Muscle, Axonal Wiring Takes Surprising Paths

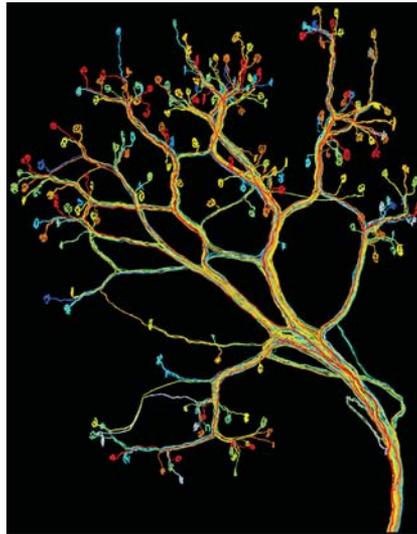
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From at least the dawn of the electronic age, the oddballs among us have been described as being “wired differently.” If that’s so, then a new study suggests we are probably all oddballs. By mapping the paths and branch points of individual axons reaching out to a single muscle, Ju Lu, Jeff Lichtman, and colleagues show that even for one of the smallest muscles in a mouse, the wiring diagram differs between two individuals, and even between the left and right sides of the same mouse.

A complete wiring diagram—where axons go, how they branch, and where they link to muscle—has been developed for roundworms, but never before for a mammal. Performing the same feat in the mouse has presented an enormous technical challenge because of the larger size and complexity of the nervous system. The authors chose to study the interscutularis muscle, which connects the base of the ear to the middle of the skull, because it was small, accessible, and innervated by relatively few neurons.

Using mice whose cells all bore a fluorescent tag, and a confocal microscope with a computer-controlled, motorized stage, the authors took thousands of individual photographs and reconstructed them to trace individual axons as they stretched across the skull, entered the muscle, branched repeatedly, and ultimately formed synapses with individual muscle fibers. Even with computers humming at full speed, tracing a single axon along its 7-millimeter average length took about 14 hours, and each muscle contained roughly 14 axons.

But averages hide the details and the differences among individual muscles that were the very point of the study. In the worm, the routes taken and connections made by axons are highly stereotyped, indicating a high degree of genetic control. But in the mouse, the actual number of axons



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The mouse interscutularis muscle connectome. The complete set of innervating motor axons, imaged with confocal microscopy, gives the anatomical underpinning of the graded tensions elicited by motor neurons according to Henneman’s size principle. (Image Credit: Ju Lu, Harvard University)

innervating a single muscle ranged from 13 to 16, differing between mice and even between the left and right sides of the same mouse. Axonal length, including all the branches, varied much more widely, from 1.5 millimeters to 13.3 millimeters, and the number of synapses formed by a single axon ranged from a low of one (for an axon that didn’t branch at all) to a high of 37. The branching pattern of axons that innervate a similar number of muscle fibers from different muscles also differed significantly, suggesting that unlike in the worm, branching in the mammal is not a deterministic process; instead it is governed by some general developmental rules, while the details of final connection are shaped by contingencies, as excess synapses are pruned.

A single axon, together with the muscle fibers it stimulates, is

called a motor unit, and previous measurements of the force generated by fiber contraction (“twitch tension”) have revealed that there is a characteristic range of such twitch tensions in each muscle. In their study, the authors showed that the distribution of motor unit sizes they identified correlates with the range of twitch tensions, providing an anatomical basis for what had previously been only a physiological observation.

A long-held principle, dating back to the famed neuroanatomist Cajal, dictates that the total wiring length in a branched axon should be minimized, since the energy costs for conduction increase with length. Not so in the mouse, the authors found, where almost every axon was longer than optimal, and could have been shortened had the branches occurred elsewhere or the axon followed a different available nerve path through the muscle. They calculated the extraneous length at 25% of the total. However, they note, there may be little substantive penalty for this extra tissue, since the energy cost of muscle contraction is far higher than that of axonal conduction.

This study is only the beginning of mapping “connectomes” for mammalian neural circuits. While the general principles seen in the interscutularis muscle may apply elsewhere, there is every reason to suspect that the details will differ. The connectome of even a single other muscle will offer further insight into the balance of chance and necessity in muscle wiring. In principle, similar techniques could be used to tease out connection patterns in the brain, although that challenge would dwarf the Herculean efforts required in this study.

Lu J, Tapia JC, White OL, Lichtman JW (2009) The interscutularis muscle connectome. doi:10.1371/journal.pbio.1000032