Purification and Culture of Spinal Motor Neurons

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Motor neurons are responsible for voluntary movement. Lower motor neurons are characterized by large soma, the potential to form very long axons, and wide-ranging dendritic arborization. They receive direction from various neuronal cell types and induce movement of skeletal muscle fibers through acetylcholine release at the neuromuscular junction. Each lower motor neuron can communicate with 10 to several hundred muscle fibers at firing rates modulated by the balance of ongoing neurotransmitter signaling. Disease and trauma that affect lower motor neurons can cause paralysis and, in some cases, death. Studies using primary cultures of these cells have ongoing potential to facilitate a deeper understanding of their biology and function.

MOTOR NEURON CHARACTERISTICS

Motor neurons arise from the ventral portion of the neural tube and are among the first cells born in the spinal cord (Altman and Bayer 1984). A large proportion of these cells undergo apoptosis during postnatal development (Yamamoto and Henderson 1999). Lower motor neurons are located in the ventral horn (anterior horn in human), throughout the spinal cord, and in brain-stem nuclei. They induce voluntary movement via acetylcholine release at the neuromuscular junction on skeletal muscle fibers. Each muscle fiber is supplied by a single axon terminal, and a single motor neuron can form synapses with as few as ten or as many as several hundred muscle fibers. Direction for voluntary movement is received from neurons in supraspinal regions (predominantly the primary motor cortex), interneurons, sensory neurons, and even other lower motor neurons. The sum of ongoing inhibitory (i.e., GABA) and excitatory (i.e., glutamate) signals determines the motor neuron’s firing rate and resultant muscle-fiber contraction (Carp and Wolpaw 2001).

The soma of motor neurons is typically two to three times larger than that of most other neurons. In addition to their large soma size, lower motor neurons can have an axon that extends very long distances. A motor axon arising from the lumbar spinal cord with its target muscle in the foot can reach a length of up to 1 m in humans. Paradoxically, the majority of the cell surface area is in its uniquely wide-ranging dendritic arborization (Carp and Wolpaw 2001).

DISEASE AND TRAUMA OF LOWER MOTOR NEURONS

Dysfunction or degeneration of lower motor neurons can result in debilitating and even lethal paralysis. Amyotrophic lateral sclerosis, Kennedy’s disease, progressive muscular atrophy, and spinal
muscular atrophy are examples of hereditary and/or idiopathic diseases involving the degeneration of lower motor neurons. Myasthenia gravis is an example of an autoimmune disease involving autoantibodies directed most commonly against acetylcholine receptors, which disrupts neurotransmission at the neuromuscular junction. Trauma to motor neurons induced by spinal cord injury, radiculopathy, or peripheral nerve injury can be transient or permanent depending on the severity and location of tissue crush or transection.

SPINAL MOTOR NEURONS IN CULTURE

Culturing of lower motor neurons is an important, albeit simplified, system for studying fundamental cellular and molecular neurobiological questions that may shed light on neurological diseases involving motor neurons. Cultures may be studied pure or nearly so, or mixed with other cells (distinctly purified or mixed), depending on the experimental paradigm. Such cultures have been used to study glial–neuronal interactions (Ullian et al. 2004), synaptogenesis (Ullian et al. 2004), axonal transport (Stommel et al. 2007), mitochondrial movement (Stommel et al. 2007), neurotrophin function (Hanson et al. 1998), and neuronal degeneration/apoptosis/necrosis (Hanson et al. 1998) by a handful of laboratories.

The accompanying protocol, Purification and Culture of Spinal Motor Neurons from Rat Embryos (Graber and Harris 2013), was derived from a methodology first reported by Camu and Henderson (1992) and later modified only slightly by Ben Barres’ and our laboratories. It takes advantage of several distinct properties of rat lower motor neurons to isolate them away from their neighboring cells. First, an ideal stage in development after motor neurons are born (embryonic day 14 during rat gestation), but prior to extensive axonal extension or developmental apoptosis, is exploited (Yamamoto and Henderson 1999; Sendtner et al. 2000). Lower motor neurons cannot be viably isolated using this method after birth. After dissociating embryonic spinal cord tissue, which contains lower motor neurons among many other cell types, cells are separated based on cell density because motor neurons are uniquely large. Finally, this collected cell population is further purified based on selective immunopanning for motor neurons, which express the low-affinity nerve growth factor receptor often referred to as p75 (Yan and Johnson 1988). The near-pure lower motor neuron cultures are plated and seeded in defined conditions optimal for survival. These cultured motor neurons are rounded when initially plated to the growth substrate, and then project an elaborate array of axons and dendrites within five days.

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