SELECTIVE INNERVATION OF AXOLOTL LIMB MUSCLES

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I am interested in the way developing neurons find and connect with appropriate target cells. The specificity of connections between nerve cells is particularly striking when you consider that there are in excess of 10^{11} potential target neurons in most vertebrate nervous systems, yet individual neurons make contacts with only a few of these and in a remarkably consistent manner. Because it is exceedingly difficult to study the mechanisms responsible for the specificity of neuronal connections in the brain itself, most work addressing this problem has been done in various parts of the peripheral nervous system, with the notable exception of the retino-tectal system. Studies on the development of motor nerves in chick embryos (Landmesser, 1984) have shown that one important aspect of the formation of specific connections is the ability of growing axons to respond to cues within their immediate environment that help them navigate towards their target. This is generally referred to as axon guidance. Another factor contributing to specificity is the ability of axons to discriminate between individual cells within a particular region and the preferential formation of synapses with only the appropriate ones. This component of specificity, called selective synapse formation, is well illustrated by the connections of Ia sensory axons on homonymous motoneurons and the patterns of innervation and reinnervation of sympathetic ganglion cells (see Purves and Lichtman, 1984). Finally, a third mechanism might actually improve the specificity of connections after they have been made: the regression of axon collaterals during neonatal development has now been seen in several parts of the central nervous system (Stanfield, 1984). The fine-tuning of already established connections has also been observed in vertebrate skeletal muscle, and this has been shown to be the result of the physical withdrawal of functional nerve-muscle synapses (Brown and Booth, 1983).

I have been studying the second of these mechanisms of neuronal specificity, selective synapse formation. In particular I am interested in whether muscles from different positions in the body can be distinguished from one another by developing or regenerating axons. My interest in this question harks back to work I began while I was a student in Australia, concerning the competition between axolotl motor nerves for control of a particular muscle (Wigston, 1980). My working hypothesis is that muscles (and other neuronal targets too) acquire some kind of recognition labels during development related either to their position at that time or the segmental origin of their precursors. I first examined a very simple muscle system, the mammalian intercostal muscles. The beauty of this system is that each intercostal muscle is anatomically and functionally similar, but each muscle develops in a different segment. My approach (Wigston and Sanes, 1982, 1985) was to take pieces of intercostal muscle from donor rats and put them in the position of the superior cervical ganglion of a host, where they were functionally reinnervated by the cholinergic

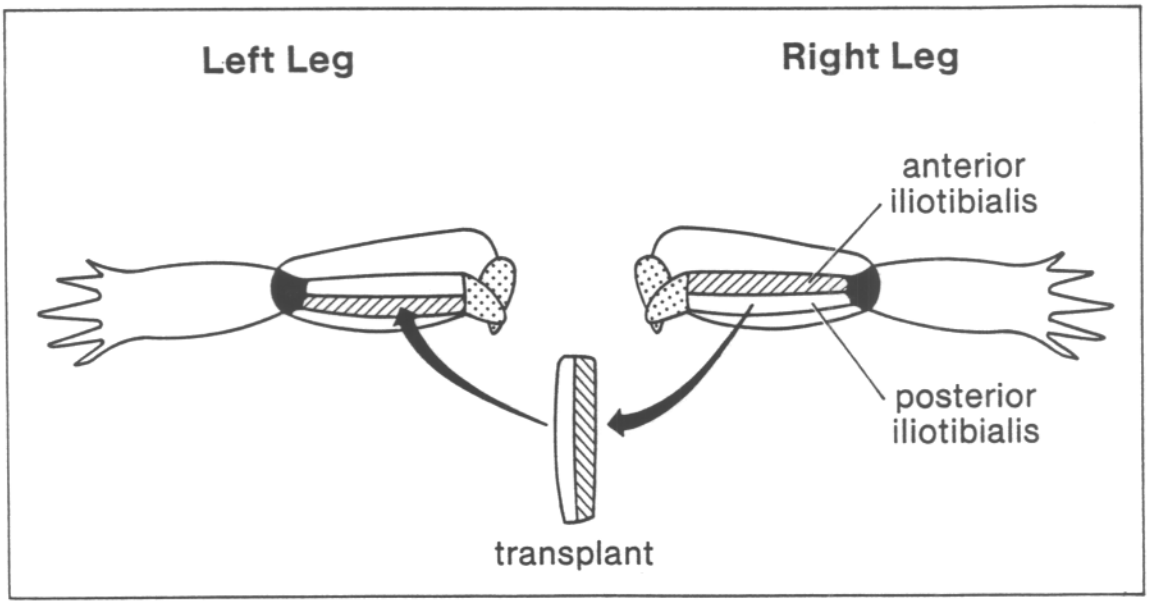

Fig. 1. Diagram of the extensor muscle surface of left and right axolotl hindlimbs. The four proximal extensor muscles are outlined in each limb: pubo-ischio femoralis internus (the most anterior muscle), anterior iliotibialis (cross-hatched), posterior iliotibialis, and iliofibularis (the most posterior muscle). For transplantation experiments, anterior and posterior iliotibialis muscles were removed together and implanted in the opposite limb in reversed configuration. The experiments were performed using adult axolotls (total length 12-20 cm).

preganglionic axons that normally innervate the superior cervical ganglion. These axons will readily innervate skeletal muscle although of course they never do this during normal development. When I compared the pattern of reinnervation of intercostal muscle pieces taken from rostral segments (for example T2 or T3) with the innervation pattern of intercostal muscles taken from more caudal levels (T4, T5, T8), I found that they were quite different. I observed a tendency of preganglionic axons emerging from the most rostral thoracic ventral roots (T1, T2, T3) to innervate intercostal muscles transplanted from more rostral segments of the ribcage better than muscles transplanted from more caudal levels. Although years of research had suggested that reinnervation of mammalian muscles was non-selective (see Brushart and Mesulam, 1980), it seemed that regenerating mammalian autonomic axons were able to detect differences between mammalian intercostal muscles from different segments. In a lower vertebrate (pardon me, folks) such as the axolotl, where motor nerve regeneration restores normal limb function, I expected that I might see even better selectivity between different muscles, and so improve my chances of elucidating its basis.

The system I have chosen for this study is the proximal extensor muscles of the adult axolotl hindlimb (see Fig. 1). By recording intracellularly from muscle fibers in isolated limbs while I stimulate each of the four spinal nerves that innervate the limb, I have determined the segmental origin of the innervation of the four proximal extensors (Fig. 2a). Each muscle has a unique segmental innervation

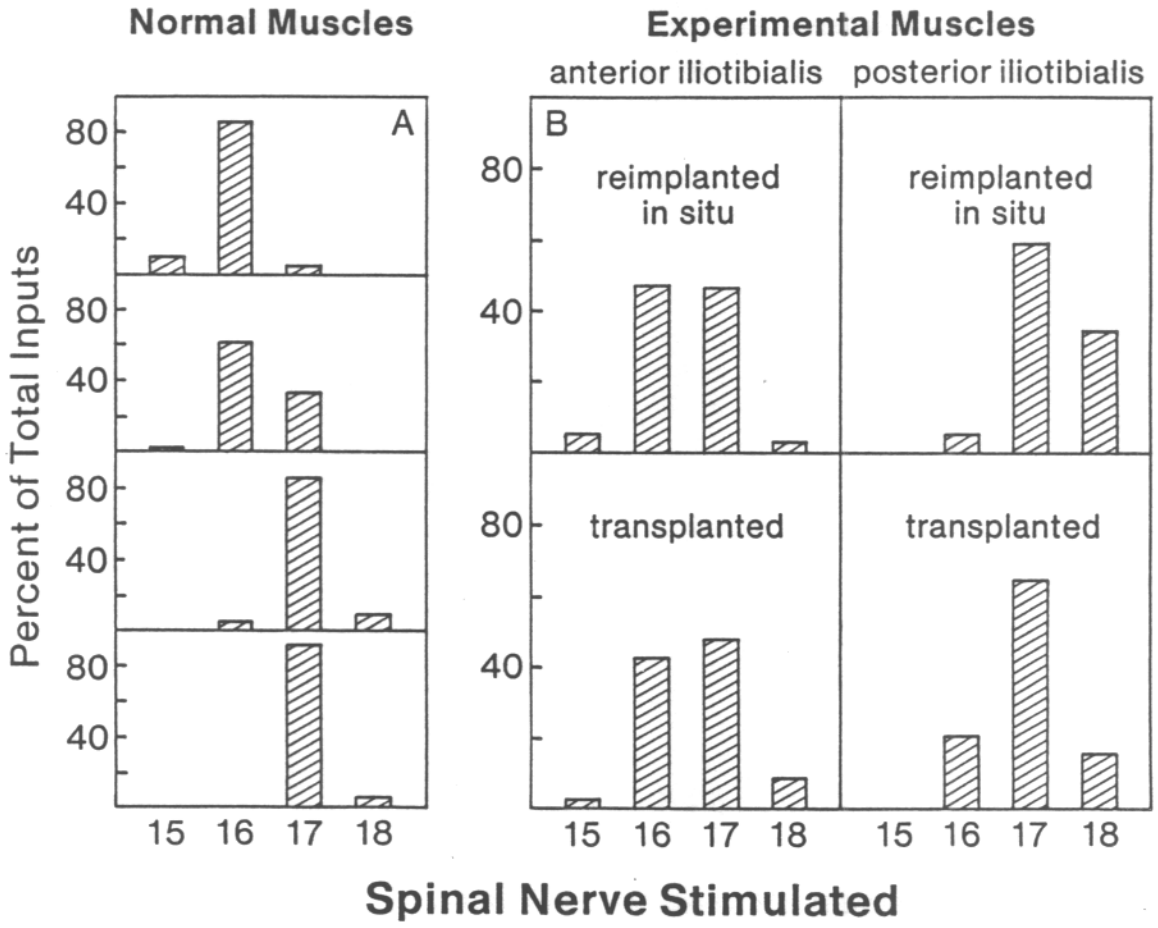

Fig. 2. Electrophysiological determination of the segmental origin of extensor muscle innervation. A: Normal muscles. Top panel shows the relative proportion of all synaptic inputs recorded intracellularly in pubo-ischio femoralis internus muscle fibers, that arose from each spinal nerve innervating the limb as a whole. Second from top, segmental innervation of anterior iliotibialis muscle fibers. Second from bottom, posterior iliotibialis. Bottom, the segmental innervation of iliofibularis. B: Experimental muscles. For both anterior (left) and posterior (right) iliotibialis muscles note the similarity between the segmental innervation pattern of those transplanted to a novel site (bottom panels) and their appropriate controls (top panels) that were removed but reimplanted in their old site.

profile with the exception of iliofibularis, whose innervation resembles that of posterior iliotibialis.

To look for muscle recognition cues, instead of denervating the limb and studying how the motor nerves regenerate their connections as others have done, I have attempted to eliminate the necessity for regenerating axons to find their targets before they can express their preferences. I do this by removing both anterior and posterior iliotibialis muscles together from one leg and putting them in the place of the same pair of muscles in the opposite leg (Fig. 1). In this way the anterior and posterior muscles are reversed in the anterior-posterior axis. Each operated animal then has four muscles that have been

shifted in the a-p axis. When I study the pattern of reinnervation of these muscles with intracellular recording several weeks later I find that the muscles are reinnervated in a pattern consistent with their origin or identity rather than their new position (Fig. 2b). This supports my belief that different muscles have some unique feature, perhaps molecular markers of some sort, that regenerating axons can recognize and take into account during synapse formation (see also Holder et al., 1983).

In the future I would like to find out when in development these target recognition cues arise, and what they represent. For instance, from my results so far I cannot rule out the possibility that they are somehow laid down by the set of axons that initially innervate each muscle, and so might not be involved in determining the original pattern of innervation. An interpretation I find attractive is that each muscle acquires an identity that is related to the segmental origin of its myoblasts. Most muscles are generated by myoblasts from more than one somite; however, the relative proportion of myoblasts originating from each contributing somite may be unique for each muscle (Beresford, 1983). The adult innervation pattern then may simply represent a matching of the segmental source of a muscle's myoblasts and its motoneurons. I am considering embarking on a series of somite transplantation experiments in embryos employing the triploid marker used in Susant Bryant's laboratory to determine the somitic origin of the hindlimb extensor muscles. I would then look for a correlation between the origin of a muscle's myoblasts and its motor innervation.

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PLEA FOR HELP

I often have trouble locating axolotls of the size I prefer for these experiments ($\geq$10 cm). If anyone ever has any to spare and would send them to me, I would be most grateful. I can reimburse any shipping costs. My phone number is (404) 329-7497.

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