A comparison of homonymous and heteronymous connectivity in the spinal monosynaptic reflex arc of the cat

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Summary. Multi-unit spike triggered averaging was used to determine functional connectivity between spindle afferent fibers from the medial gastrocnemius muscle and the motoneurons innervating the medial (homonymous connections) and the lateral gastrocnemius-soleus muscle (heteronymous connections). As many as 288 possible connections between 24 motoneurons and 12 afferent fibers were studied in single, acute experiments. The influences of morphological and topographical factors, as well as of motoneuron species on functional connectivity were analysed. The probability that a motoneuron would receive functional connections from a given population of afferent fibers was related to its size and its proximity to the spinal entry level of the afferent fibers. The faster the axonal conduction velocity of the motoneuron (i.e. the larger the motoneuron) and the closer its location to the entry zone of the afferent fibers, the higher was its probability of receiving functional connections. The greater the conduction velocity (i.e. diameter) of a stretch receptor afferent fiber, the higher was its probability of making functional connections with motoneurons. These relationships were qualitatively similar for homonymous and heteronymous connections. 58% (233/399) of the Ia and group II afferents (combined) had functional connections with homonymous motoneurons, 32% (75/234) with heteronymous motoneurons. However, homonymous and heteronymous motoneurons of similar sizes were equally likely to receive functional connections when located at the same craniocaudal level. Differences in the locations and mean sizes of homonymous and heteronymous motoneurons however, cannot account completely for the observed overall differences in homonymous and heteronymous connectivity.

Key words: Spinal cord – Motoneurons – Spindle Ia afferents – Spindle group II afferents – Connectivity – Species recognition – Topographic connectivity

Introduction

Liddell and Sherrington (1924) noted that a myotatic reflex in a decerebrate cat remains confined to the particular muscle stretched. Not even close synergists are involved in this stretch reflex. Similarly Lloyd showed that electrical stimulation of a muscle nerve evoked a reflex discharge which was confined to motoneurons innervating the same muscle (Lloyd 1943). Later Eccles et al. (1957) showed that the amplitude of aggregate postsynaptic potentials is larger in homonymous than in heteronymous motoneurons. Mendell and Henneman (1971) found by means of the spike triggered averaging technique that single Ia-fibers from the medial gastrocnemius muscle established functional connections with virtually all homonymous motoneurons whereas the same afferent fibers made connections with only about 65% of the heteronymous motoneurons (lateral gastrocnemius) motoneurons. Interestingly they also noted that heteronymous motoneurons were equally likely to receive connections as homonymous motoneurons if the cells were located at the same craniocaudal level in the spinal cord.

In the course of this brief historical survey it is seen that the concept of exclusive specificity of the stretch reflex in Sherrington’s work gives way to that of quantitative differences in the experiments of Lloyd and of Eccles. The more the focus of interest shifts from the system level to the cellular level, the more the specificity of connections appears to weaken until it disappears altogether in the observations on individual neurons made by Henneman.
Since the motoneurons supplying different muscles (e.g., medial and lateral gastrocnemius) are intermixed to a great extent in the motor columns of the spinal cord (Romanes 1951; Burke et al. 1977), the muscle spindle afferent fibers must follow specific rules in order to establish the necessary orderly connectivity which gives rise to the localized stretch reflex. Chemical molecules on the cell surface have been proposed as a recognition mechanism between the afferent fibers and the motoneurons (Eccles 1964). Wyman (1973) and Wyman et al. (1974) were the first to challenge the theory of species recognition in the spinal cord. They suggested that topographic factors may suffice to account for the specificity in the connectivity. They based their arguments on genetic considerations and analogies with sensory systems. The first direct experimental evidence that topographic factors play an important role in routing sensory fibers to motoneurons was presented by Lüscher et al. (1980, cf. also, Lucas and Binder 1984). Later Clamann et al. (1985) identified three morphological variables (such as sizes of motoneurons and afferent fibers and their topographic relation) that influence functional connectivity on a cell-to-cell level. This report expands the analysis of the structural and topographical influences on connectivity found in the spinal homonymous reflex arc to the heteronymous connections. The results clearly indicate that structural and topographical factors are major determinants for the development of connections in the spinal cord. At least for the close synergists studied here, species-related cellular recognition processes cannot explain the overall differences in homonymous and heteronymous connectivity. Some of the results have been presented in abstract form (Vardar and Lüscher 1986; Lüscher and Vardar 1987).

Methods

The experiments to be described here were designed to study rules governing the connectivity between spindle afferents and motoneurons. An adequate comparison between homonymous and heteronymous connectivity requires the investigation of a large number of synaptic connections in the same experiment. This was accomplished with methods developed in this laboratory and described in detail in previous papers (Lüscher et al. 1983a; Clamann et al. 1985). A summary of the procedure is presented here.

Surgical procedures. The experiments were performed on 15 cats of either sex weighing between 1.5 and 3.0 kg. The animals were anesthetized with sodium pentobarbital, 40 mg/kg i.p. and supplemented as needed. Spinal segments L5 to S1 were exposed by laminectomy. The popliteal fossa of the left hindlimb was maintained at 37 ± 0.5 °C by means of infrared heating lamps. The Achilles tendon was freed and separated from its insertion. Stretch was applied to the muscle with a rack and pinion attached by a strong thread to the muscle tendon.

Five dorsal root filament was separated in continuity from L7 and S1 dorsal roots by microsurgical techniques and placed on recording electrodes. The filaments had been dissected until each contained two to six spindle afferent fibers from the m.g.-muscle. The remaining filaments had been dissected until each contained two to six spindle afferent fibers from the m.g.-muscle. The remaining filaments in L7 and S1 dorsal roots were cut. Thus the only afferent fibers entering the spinal cord via L7 and S1 dorsal roots were from the m.g.-muscle and passed through the fine filaments on the recording electrodes.

The integrity of the afferent fibers was tested in the following way. A silver ball electrode was used to record from the dorsal surface of the spinal cord cranial to the entry points of the dorsal root filaments by means of spike triggered-averaging (see below). The presence of a signal correlated with the activity of a single afferent was taken as evidence that the afferent was intact and conducting action potentials as far as the dorsal columns.

Data collection. m.g. and l.g.s. motoneurons, identified by stimulating them antidromically from the muscle nerves, were impaled with glass pipette micro-electrodes filled with 3 M KCl. Final tip resistances ranged between 8 and 20 MΩ. Microelectrodes were mounted on a Burleigh Inchworm piezoelectric microdrive. Potentials were measured with an electrometer (WPI). Cells with resting potentials less than −60 mV were not accepted for study. In order to rank the sizes of the motoneurons, the conduction velocities of their axons were determined (Cullheim 1978; Kernell and Zwaagastra 1981). Other motoneuronal parameters, such as input resistance, afterhyperpolarization, motor-units type or contractile strength could be used as a measure of motoneuron size as well. However, it has been demonstrated that recruitment order is highly correlated with axonal conduction velocity (Bawa et al. 1984). In addition, conduction velocity of the motor axon can be determined very precisely without introducing further complications in the already difficult experiments. We therefore choose the axonal conduction velocity as a measure of motoneuron size. In doing that we regard the motoneuron pool as an ensemble of cells covering a continuous spectrum of different sizes.

Synaptic noise from a motoneuron was recorded on one channel of a precision 7-channel f.m. tape recorder (Honeywell 101) at 30 inches per second (10 kHz upper frequency limit); the stretch evoked activity of spindle afferents recorded from 5 dorsal root filaments and that recorded from the muscle nerve were recorded concurrently on 6 other channels. Usually all the Ia and group II fibers on the recording electrodes remained functional for several hours, while intracellular recordings were made from as many m.g. and l.g.s. motoneurons as possible. A diagram of the experimental plan is illustrated in Fig. 1A.

Data analysis. A modification of the method of spike-triggered averaging (Mendell and Henneman 1971) was used to generate an averaged e.p.s.p. waveform produced by each of 6-12 spindle afferents in one filament. The multi-unit spike train sequences recorded from each of the five dorsal root filaments were decomposed, using a window discriminator (Lüscher et al. 1983a) with two time-voltage "windows", whose dimensions and