more than half the normal width of both these structures (Fig. 3, A and B). By light and electron microscope examination it was documented that, along their course within the spinal cord or medulla, many of these fibers were ensheathed by Schwann cells that had migrated into the CNS tissues; other axons were surrounded by glial processes. Many of the penetrating fibers terminated in close proximity to CNS neurons, but it is not known if they formed synapses because connectivity was not investigated.

The results of these studies indicate that some of the axons within the PNS "bridges" originate from neurons in the spinal cord and brainstem. Under the conditions of these experiments such axons have been shown to be capable of a growth that exceeds 30 mm, a distance that could be equal to or greater than the length of axons from some of these neurons in the intact rat.

This new experimental model has several advantages for studies of regeneration in the living animal. (i) By selectively positioning the graft, it is possible to direct the course of axons from and into specific regions of the CNS. (ii) The origin, length, and termination of axons within the graft can be documented. (iii) The long extraspinal course of these grafts should facilitate the electrophysiologic investigation of axons within the "bridges." (iv) Because these animals are not paralyzed and retain bowel and bladder control, in contrast to the case with animals grafted after complete transection (*4*), their care and survival is greatly facilitated. (v) If it is eventually demonstrated that axons from CNS neurons establish functional connections with cells in the target regions to which they have been directed, it may be possible to devise experimental strategies for selected populations of axons to bypass damaged CNS tissue and connect with specific groups of neurons at a distance.

Whether the central axons in the bridging grafts originate by regrowth of damaged CNS fibers or by sprouting from uninjured neurons in the proximity of the graft endings, or both, could not be decided in this study. Regardless of the mechanisms involved, the remarkable elongation of axons in these animals suggests that PNS tissues exert a striking facilitation of the growth of axons from central neurons after CNS injury. Even though the cells of origin varied in size and were distributed widely within the CNS areas neighboring the site of entry of the graft, it remains to be determined whether they constitute a special neuronal population or whether their responses are examples of a more general potential for regeneration. Our experiments also demonstrate that regenerating axons only penetrate the damaged CNS for short distances. It is possible that elongation fails in the CNS because the central neural environment lacks the growth-promoting properties of the PNS or because there are changes in the injured CNS that inhibit fiber growth (*8*). If the conclusion is corroborated that interactions between axons and their immediate environment play a determinant role in the success or failure of regeneration, the study of the molecular basis of these interdependencies may lead to better experimental approaches to promote CNS regeneration.

Table 1. Boldface numbers represent the numbers of spinal and medullary neurons labeled in each animal after the application of HRP to the caudal end of the graft, approximately 30 mm away from the medulla (group A) or to the rostral end of the graft at the same distance from the spinal cord (group B). Nonboldface numbers designate cells labeled by HRP applied to the shorter, 5-mm long, remaining stump of the bridging nerve. Group C represents findings in control rats in which the regenerated grafts were crushed approximately 30 minutes before HRP application.

Labeled neurons		Weeks after grafting
Spinal cord	Medulla	
	Group A	
53	**162**	22
48	**13**	24
493	**24**	25
112	**62**	30
	Group B	
247	159	26
69	9	27
0	21	27
	Group C	
4	0	25
0	0	25
0	0	25

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Spinal Motoneuron Recruitment in Man: Rank Deordering with Direction but Not with Speed of Voluntary Movement

Abstract. *Single motor units in human interosseous muscle are recruited in order from small to large in slow or brisk voluntary abduction of the index finger. When the same muscle acts as a synergist as opposed to a prime mover, about 8 percent of the unit pairs consistently reversed their recruitment order. Motor commands appear to be patterned in terms of movements rather than muscles and to involve different connectivities to the motoneuron pool of a muscle executing movements in different directions.*

The voluntary motor commands to a pool of spinal motoneurons can be analyzed in intact humans by recording the action potentials of single motor units with fine metal electrodes inserted through the skin (*1*). In graded voluntary contractions of a muscle, motor units are recruited in a stereotyped order at reproducible levels of muscle force (*2*). This is usually referred to as Henneman's size principle (*3*) because the recruitment sequence is correlated with several graded properties such as the size of the motoneuron and the diameter of its motor axon (*4*). The orderly recruitment of motor units that prevails when the muscle is used as a prime mover undergoes significant changes when the same muscle contracts as a synergist in another movement. In contrast to the concept of a fixed recruitment order, it has been occasionally reported that human subjects, when provided with visual or auditory feedback from their active motor units, can learn to voluntarily activate or suppress any arbitrarily chosen motor unit

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within the range of the recording electrode (*5, 6*). We report here the results of a study of the recruitment of single motor units in human interosseous muscle.

The first dorsal interosseous muscle of the hand was studied because it has two degrees of freedom. It is the only muscle that produces abduction of the index finger away from the axis of the hand, and it is also involved in flexion of the index finger as a synergist to the long flexor muscle (Fig. 1a). In 14 experiments with three normal adult volunteers, highly selective bipolar tungsten electrodes (*7*) were inserted in appropriate muscle sites to record large extracellular action potentials of one to four single motor units. The palm and the last three fingers and the thumb were fastened to rigid Plexiglas holders. The contraction forces in abduction or flexion were recorded under isometric conditions with ELF-1000-250 Flatline load cell transducers sturdily fixed against the lateral side or the front of the first knuckle of the index finger.

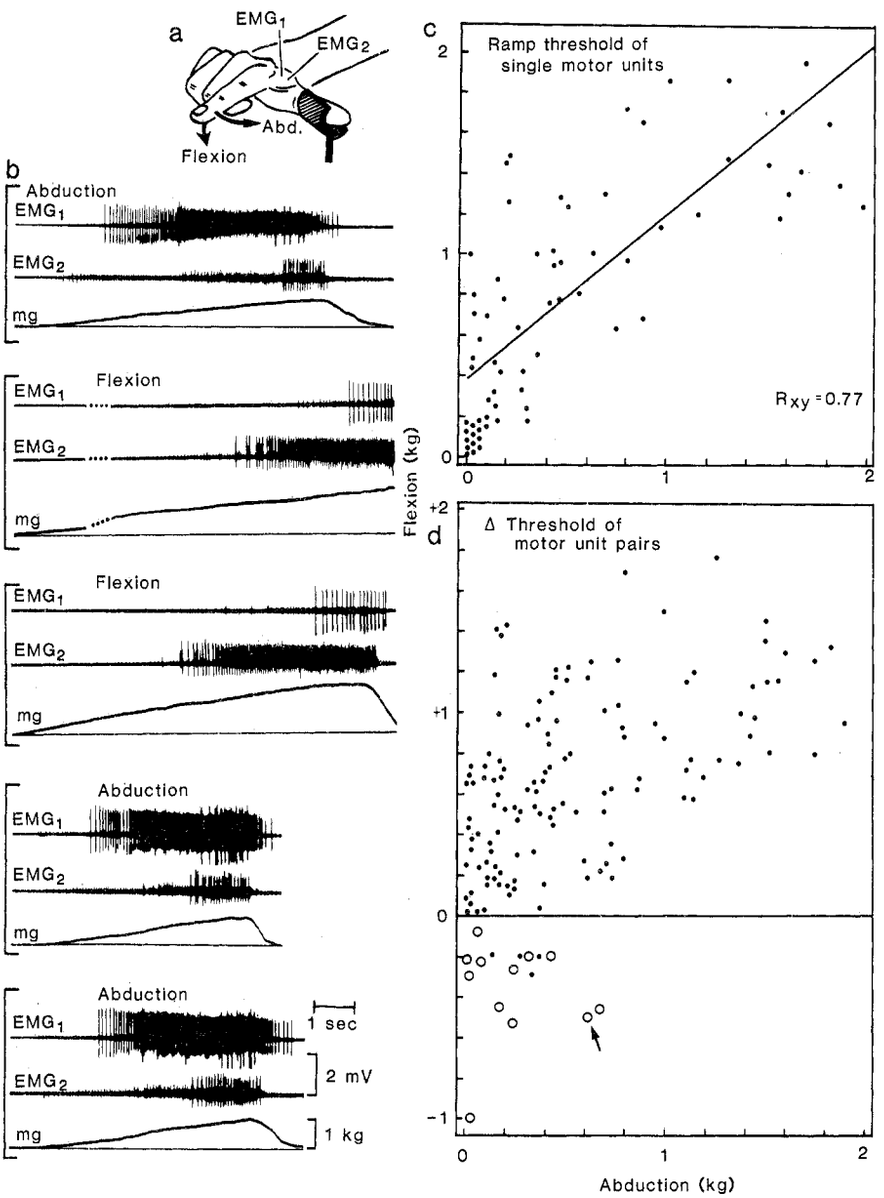

Fig. 1. Consistent reversal of recruitment order of two single motor units with different movement directions. (a) The potentials were recorded from two sites (EMG_1 and EMG_2) 10 mm apart in the first dorsal interosseous muscle of a normal adult man. (b) Simultaneously recorded cathode ray oscillograms of motor unit potentials (two upper traces) and of the isometric myogram of the muscle (lower trace). The records are from five successive trials (top to bottom) involving slow ramp voluntary contractions producing index finger abduction (trials 1, 4, and 5) or flexion (trials 2 and 3). (c) Scatter diagram of the recruitment thresholds of 70 single interosseous motor units comparing their recruitment threshold in finger abduction or flexion. The calculated linear regression is $y = 0.38 + 0.81\,x$ ($R_{xy} = .77$). (d) Scatter diagram of differences in recruitment thresholds for 142 pairs of interosseous motor units simultaneously recorded during finger abduction or flexion. Negative values on the ordinate correspond to unit pairs that were recruited in reverse order during finger flexion. For the 12 unit pairs indicated by a circle, the threshold difference exceeded 2 standard deviations in either direction. The arrow points to the unit displayed in (b).

Figure 1b shows the consistent deordering of two single motor units recorded 10 mm apart in the interosseous muscle of one man during slow progressive voluntary contractions, with isometric force increasing at about 0.17 kg/sec (third trace of each trial). Five successive trials are shown in the order in which they were recorded. The large motor unit potential from muscle site EMG_1 was recruited at a voluntary force of 0.2 to 0.23 kg for abduction of the index finger, but only at 1.49 kg for flexion of the same finger. Another motor unit simultaneously recorded from site EMG_2 had a higher threshold of 0.77 to 0.85 kg in abduction, but was recruited at 1.04 or 0.84 kg in flexion. The tests document reproducible shifts in the relative thresholds of the two motor units when the interosseous muscle functions as a prime mover (abduction) or as a synergist (flexion). By adjusting the voluntary force at a level between the two thresholds, the subject could selectively activate the large motor unit spike of EMG_1 in abduction or that of EMG_2 in flexion, showing that the phenomenon was under complete control.

The voluntary force thresholds for any single motor unit were estimated in at least five separate trials for abduction or flexion contractions and had a wide scatter (Fig. 1c). The calculated linear regression ($R_{xy} = 0.77$) suggests that many motor units with a low abduction threshold (< 0.4 kg) presented a higher threshold in flexion (see below). Pairs of motor units recorded in the same trials from one or two muscle sites were then considered in order to evaluate the actual incidence of rank deordering. For the 142 pairs studied, the differences of mean abduction thresholds ranged from 0.02 to 1.9 kg (Fig. 1d). If the unit with the higher abduction threshold also had the higher flexion threshold, the threshold difference in flexion was positive; this was the case for 126 pairs. However, 16 pairs presented a negative threshold difference in flexion. For 12 of these the threshold difference exceeded 2 standard deviations for both directions (in at least five trials). The recruitment reversals in these 12 pairs frequently exceeded 0.2 kg and were occasionally greater than 0.5 kg. To our knowledge, fully reproducible

rank deordering of motor unit pairs with such large threshold differences was not previously reported for undisturbed voluntary contractions.

Only slow contractions have been considered so far, and the question arises whether any further deordering might occur in brisk ballistic contractions. For contractions in either direction, the ballistic force threshold of single interosseous motor units is consistently smaller than the slow ramp threshold. The two thresholds are linearly correlated over the range of 2 kg, with R_{xy} = 0.97 for index finger abduction (*8*) and 0.95 for finger flexion. When any motor unit pair is deordered in flexion, this is recorded irrespective of the speed of the voluntary contraction. For example, three simultaneously recorded motor units that were recruited in the sequence 1, 2, 3 in abduction changed their order to 1, 3, 2 in flexion, whether the contraction was slow or ballistic.

The proposal that spikes of different motor units of a muscle could be used as distinct switches for operating mechanical prosthetic devices (*5, 6*) would imply a degree of selective volitional access to single motoneurons that is not borne out by our data. Although voluntary control of the motor unit of lowest threshold at the recorded muscle site is possible, Henneman's principle (*3*) is incompatible with the idea that subjects could selectively activate a single motor unit of higher threshold while keeping the lower threshold units silent. We observed orderly recruitment over a wide range of thresholds (0.02 to 2.0 kg), but found consistent rank reversals in only 8 percent of the unit pairs for contractions of different physiological function (Fig. 1). The effect reported by Basmajian (*6*) may thus have occurred only in multifunctional muscles under certain conditions.

Motoneurons to small hand muscles receive a rich direct corticospinal innervation (*9*), and it is of interest to know how the connectivities in the motoneuron pool allow for the observed deordering of unit pairs with threshold reversals of several hundred grams. This problem has been addressed by identifying which motor unit of the reversing pairs (the smaller, the larger, or both) exhibits unusual shifts in recruitment threshold. In an intact human the size of a motor unit can be roughly estimated from the peak force of its twitch that can be extracted by spike-triggered averaging (*10, 11*). Each of the 32 single interosseous motor units so studied contributed mechanical force to movements in either direction, and the twitch extracted during either abduction or flexion of the index finger had the same contraction time and roughly proportional peak force. When the interosseous acted in the finger abduction, the motor units with the larger twitch force were consistently recruited after the smaller ones, and there was a linear relation between twitch force and recruitment threshold (R_{xy} = 0.93) (*11*). This has been explained by saying that the smaller motoneurons innervating the smaller motor units have a higher input resistance, so they present larger excitatory postsynaptic potentials and are more strongly activated when receiving the same number of active excitatory synapses (*4, 12*). However, in finger flexion the relation between twitch force and threshold is less satisfactory (R_{xy} = 0.62), the main reason being that smaller motor units tend to have unusually high recruitment thresholds. For example, the 11 interosseous units with a twitch force below 1.2 g are recruited at forces below 0.15 kg in abduction, whereas their thresholds can be up to 0.5 kg in flexion. Interosseous units with a twitch force larger than 7 g were never recruited at less than 1.2 kg in either direction, and their threshold cannot be said to decrease significantly in finger flexion.

It is of interest to know whether such features are shared by the long flexor muscle, which is the prime mover for finger flexion and does not contract during voluntary abduction of the index finger. A good correlation was found between twitch force and flexion threshold of single motor units in this muscle (R_{xy} = 0.92). Therefore the poor correlation for the interosseous muscle in finger flexion must be related to the interosseous acting as a synergist rather than as the prime mover. The feature of interest in rank deordering with movement direction is therefore the increase of the recruitment threshold of some of the smaller motor units, whose motoneuron must have a higher input impedance (*4*). It seems most likely that the mechanism involves connectivities throughout the motoneuron pool such that the flexion commands engage a smaller number of excitatory synapses on some smaller motoneurons of the synergist interosseous pool (Fig. 2). This suggests that synaptic terminals are consistently distributed throughout the motoneuron pool of the prime mover, but not that of the synergist. In this respect it is of interest that the larger motoneurons of the interosseous (with lower input resistance and higher synaptic threshold) did not appear to be more easily recruited. We conclude that the motor commands are patterned in terms of movements rather than of muscles, since their final path is not common, but involves different connectivities in motoneuron pools for movements in different directions. However, movements in a particular direction, thus fulfilling a given physiological function, activate the motoneuron pool of the prime mover in a consistent order independent of the speed of the movement.

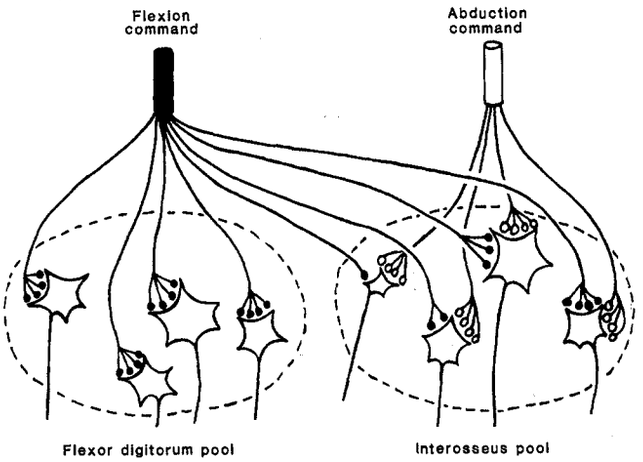

Fig. 2. Suggested connectivities of voluntary motor commands in motoneuron pools. Interneurons, propriospinal relays, and other details of spinal circuitry are omitted. Active excitatory synapses are evenly distributed throughout the motoneuron pool of the prime mover in flexion or abduction. Only four synaptic terminals per motoneuron are depicted. By contrast, the active excitatory synapses for flexion commands are less numerous on some of the smaller motoneurons of the synergic interosseous pool. There is no evidence for selective volitional access to single motoneurons.

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7. The electrodes were made by inserting two diamel-coated Nichrome wires (40 μm in diameter: tip separation, 20 to 100 μm) into a short hypodermic steel needle. Careful positioning in the muscle optimized the isolation of motor unit potentials of discriminable wave form and amplitude that were clearly isolated from background activities, even in strong or brisk contractions. In order to minimize mechanical disturbances, a 15-cm loop of the flexible nichrome wires connected the electrode to the high-input-impedance differential amplifier.
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10. For extracting the twitch of a motor unit, the analog signal of the interosseous muscle myogram was averaged by a Nicolet model 1074 digital computer (words of 9 bits; bin width, 200 μsec) during steady voluntary contractions just exceeding the threshold of the chosen unit (fatigue effects were avoided). The spike of the motor unit initiated the averaging cycle through an electronic window trigger, except when the same unit fired in the interval from 90 msec before to 90 msec after any selected spike. This conditional rejection prevented distortion of the spike-averaged twitch by mechanical superposition of contractions of the same unit (*11*). Activities of other motor units sometimes influenced the relaxation profile of the twitch, but this was minimized by averaging as many as 512 samples.
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Fetal Alcohol Syndrome: Embryogenesis in a Mouse Model

Abstract. *When two small doses of ethanol were administered to pregnant mice during the gastrulation stage of embryogenesis, the embryos developed craniofacial malformations closely resembling those seen in the human fetal alcohol syndrome. Striking histological changes appeared in the developing brain (neuroectoderm) within 24 hours of exposure. Decreased development of the neural plate and its derivatives apparently accounts for the craniofacial malformations. The critical exposure period is equivalent to the third week in human pregnancy.*

Maternal consumption of ethanol severely affects the mental ability and facial appearance of at least one in 750 infants born in the United States (*1*). Ethanol consumption is associated not only with abnormal live births but also with an increased incidence of stillbirths and a tenfold increase in perinatal mortality (*2*).

In 1968 Lemoine *et al.* (*3*) described some characteristic defects in the offspring of alcoholics. However, it was not until 1973 that the pattern of malformations was more completely described and the term "fetal alcohol syndrome" (FAS) coined (*4*). There followed considerable interest in the teratogenicity of ethanol. Although clinical, behavioral, and epidemiological studies in humans and experimental studies in laboratory animals have shown that ethanol adversely affects development, critical exposure periods and underlying developmental alterations have not been clearly identified.

Major features of FAS are intrauterine and postnatal growth retardation, microcephaly, central nervous system dysfunctions (including mental retardation and hyperactivity), and craniofacial dysmorphology. Although many disorders feature mental and growth deficiency, Clarren and Smith (*5*) state that "it is the facial similarities among the children with the syndrome that unite them into a discernible entity" (Fig. 1, A and B). Typical of the facial characteristics are a narrow forehead, a flat midface, narrow palpebral fissures (eyelid openings), a short nose, a long upper lip with a narrow vermilion border, and a diminished or absent infranasal depression (philtrum).

Most experimental (*6*) and clinical studies have focused on the consequences of long-term exposure to ethanol. However, studies of mice by Webster and co-workers (*7, 8*) indicate that brief exposure on gestational day 7 or 8 is sufficient to produce severe facial malformations. Our study was designed to examine further the effects of brief ethanol exposure and the mechanisms underlying ethanol-induced abnormal development.

Procedures utilized by Webster and co-workers (*7, 8*) were modified in accordance with the suggestion (*9*) that two small doses administered intraperitoneally 4 hours apart on gestational day 7 might be more effective than a single larger dose. Female C57BL/6J mice were examined for vaginal plugs after being placed with males for 1 hour between 9:00 and 10:00 a.m. The day of plug detection was designated gestational day 0. The females received intraperitoneal doses of 25 percent ethanol (0.015 ml per gram of body weight) at 7 days 0 hours (10:00 a.m.) and again at 7 days 4 hours. Vehicle-treated controls were injected according to the same regimen.

Blood ethanol concentrations, as determined by a very accurate gas chromatographic technique (*10*), peaked at 193 to 215 mg per 100 ml of blood 20 to 25 minutes after each injection and then fell to about 30 mg within 4 hours. The behavioral alterations seen during the first 1½ hours after each injection were similar to the lethargy and ataxia observed in humans with comparable blood ethanol levels. Food and water consumption were normal the first night after ethanol administration.

We examined 72 live fetuses from ten litters for malformations 7 days after the injections (14 days after fertilization). The incidence of embryonic resorption in these litters was 18 percent, compared to 10 percent in the control litters. Females treated with the same doses only a few hours later had a much higher incidence of resorptions. These early embryonic deaths may be related to interference with heart development.

The most easily identifiable malformations involved the eyes. Thirty of the live fetuses had eye malformations, including coloboma of the iris, microphthalmia, and apparent anophthalmia (Fig. 1C). The right eye was affected more frequently and more severely. As in human FAS (Fig. 1, A and B), primary growth deficiency of the eye was reflected in shortened palpebral fissures. Short palpebral fissures are one of the most important diagnostic signs in FAS (*5*). Structural alterations of the eye and microphthalmia have also been seen in clinical cases (*11*).

The C57BL/6J mouse has a genetic predisposition for the types of eye malformations induced by ethanol exposure; a 12 percent incidence of microphthalmia, anophthalmia, and other eye defects was observed in our controls. Thus the ethanol-treated mice manifested a multifactorial threshold phenomenon. However, ocular anomalies have also been noted in other strains of mice chronically exposed to ethanol—including strains that do not have a high incidence of spontaneous ocular malformations (*12*).

Nine of the 30 live fetuses with eye

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