tions were evoked by regenerating axons from the donor ganglion which formed new synaptic junctions with this muscle.

A further indication of reinnervation of the extensor tibia muscle is that normal resting potentials of approximately 60 mv can be consistently recorded under the conditions just described. The return of normal resting potential levels in previously denervated *Periplaneta* muscle is associated with reinnervation (*3*). The recorded junctional activity differs from the normal by the frequent occurrence of hyperpolarizing potentials. Hyperpolarizing potentials have not been reported for normal muscle of *Periplaneta*, although they have been observed in normal muscle of *Blaberus* and some Orthoptera (*5*). We have previously seen these hyperpolarizing potentials in reinnervated muscle of *Periplaneta* (*2*) and take this as additional evidence that the extensor tibia of the host leg has been reinnervated by axons from the implanted ganglion.

The transplanted ganglia were fixed in place in the host leg 30 to 70 days after implantation, sectioned transversely, and stained with pyronine-malachite green (*6*). The histological appearance of the individual nerve cell bodies in the transplanted ganglion was normal. Many cells had eccentric nuclei, an indication of a high level of synthetic activity typical of regenerating neurons (*7*). The spatial distribution of the nerve cell bodies in the transplanted ganglion was somewhat altered thus making difficult comparison of individual cell bodies with the motor neuron maps we have prepared from normal ganglia (*2*). These results show that the regenerating axons grow from the nerve stumps of the ganglion transplant, but they leave in doubt the identification of the specific neurons that have reinnervated the extensor tibia muscle of the host.

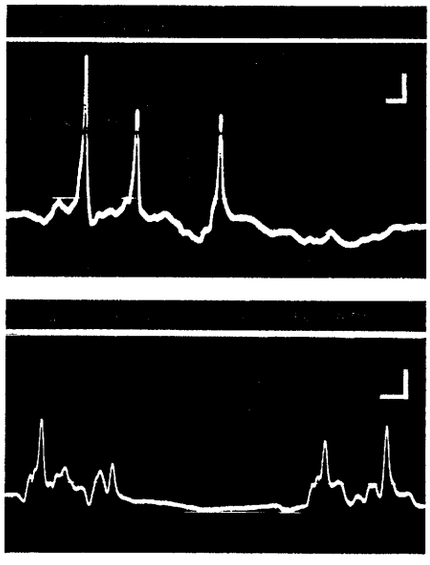

Fig. 2. Intracellular records of hyperpolarizing and depolarizing synaptic activity in the extensor tibia muscle driven by spontaneous activity of a ganglion implanted in the coxa. All normal innervation to this muscle has degenerated. The larger fast-rising depolarizations are correlated with a twitch extension of the tibia. The lower record shows periodic bursts of electrical activity associated with clonic extensions of the tibia. Summation of the small junctional potentials frequently occurs. The upper line in each record is the zero potential level. Scales: 10 msec, 10 mv.

Intracellular recordings were taken from flexors of the tibia in host legs containing an implanted ganglion. These legs were showing the spontaneous clonic tibial extensions after the normal motor innervation to the leg had been cut. In the five preparations examined, no electrical activity could be recorded from the tibial flexors under these conditions. Only muscles that were previously denervated during the implantation operation had electrical activity that could be correlated with regenerated fibers from the implanted ganglion. This finding indicates that only denervated muscles will accept regenerating fibers from the implanted ganglion and establish functional neuromuscular junctions with them.

The transplanted ganglion apparently has periods of intrinsic activity when isolated in the coxa of a host animal. These periods are indicated by the bursts of electrical activity and evoked movements in host muscles innervated by the transplant. We have seen some evidence of reflex activity from the transplanted ganglion indicating that some regenerating sensory fibers may have been captured by the transplant, as indicated by others (*8*). We can specify which muscles the transplant will innervate by selective denervation of the host muscle because only denervated muscles will form connections with the regeneration axons. The transplanted ganglion provides an "in vivo" tissue culture preparation isolated from all other central nervous influences. It offers the intriguing possibility of constructing an integrated excitable system where the connections can be specified and related to overt behavioral acts. Such a system seems ideally suitable for answering some of the questions about the factors that control connections between excitable cells and the relation of these connective patterns to behavior.

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Nerve Regeneration: Correlation of Electrical, Histological, and Behavioral Events

Abstract. *Within 5 days after the leg nerves of a cockroach are injured, miniature end-plate potentials have disappeared, and the muscle is unresponsive to electrical stimulation. The soma of the injured neuron has a dense perinuclear ring of RNA. By 40 days after the injury, locomotor activity has returned, and the miniature end-plate potentials and evoked electrical responses have reappeared in the muscle. The RNA ring has disappeared, and the nucleus of the regenerating neuron has shifted to an eccentric position.*

Integrated behavior arises from groups of excitable cells related to one another by specific patterns of connections. A suitable preparation for the investigation of the factors controlling the connections between excitable units and for relating the patterns of connection to behavior should satisfy at least three conditions: (i) the individual units within the system must be recognizable from one animal to another and must be available for analysis at several different levels of organization, (ii) the connections between units must be open

to experimental modification, and (iii) the units should be involved in a discrete bit of overt behavior such that cellular changes can be linked to specific behavioral alterations.

The thoracic ganglia and associated limbs of the cockroach *Periplaneta americana* provide a preparation satisfying these criteria. Individual ganglia are involved in a variety of interesting behavior ranging from the control of locomotion to learning (*1*). The cell bodies of motor neurons in the metathoracic ganglion have been identified with regard to the peripheral nerve trunk containing their axons (*2*) and the leg muscle they innervate (*3*). In the adult, the motor axons of this ganglion will regenerate when injured, and they appear to reestablish neuromuscular connections (*4*). The soma of motor neurons whose axons are injured show changes in cytoplasmic RNA correlated with injury and subsequent regeneration of the axon and its neuromuscular junctions (*5*). Our purpose in this study was to correlate critical metabolic, structural, electrical, and behavioral events that occur during degeneration and subsequent regeneration of specific neuromuscular elements in the cockroach *Periplaneta americana*.

In 70 adult males we exposed metathoracic peripheral nerves 3 through 6 that serve the leg musculature by laying back a flap of cuticle overlying the ganglion. The nerve trunks were cut or crushed on one side, and the cuticular wound was then sealed with a wax having a low melting point. We examined the animals at intervals from 0.5 to 150 days after the injury.

We judged the behavioral state of the affected leg visually and with analysis of motion pictures of the gait in walking and running animals. We also examined leg behavior of animals suspended from a wax block. When the animal is held free of the substrate, its legs thrash violently. If a cork sphere is presented to the animal, he grasps it securely with his pretarsal claws. Cutting or crushing nerve 5, which innervates the pretarsus depressor muscle as well as other limb musculature, causes the leg to be held fixed in an elevated position. The pretarsus is raised and is unable to grasp the cork ball. Within 30 to 70 days after the leg nerve is injured, the affected limb of a suspended animal may show some abortive movement. A partial return of movement in the affected limb occurs in approximately two-thirds of the animals with crushed nerves and in one-third of the animals with cut nerves. In many cases, this progresses to full functional recovery in which the pretarsus can once again firmly grasp the cork sphere.

We then examined some of the histological, electrical, and metabolic factors correlated with these behavioral changes. We prepared the leg muscles for intracellular electrical recording by removing the overlying cuticle and covering the muscles with cockroach saline (*6*). We used glass capillary micropipettes filled with 3*M* KCl and coupled to conventional direct-current recording apparatus.

Intracellular records from normal coxal muscles of the metathoracic leg show spontaneous miniature end-plate potentials (MEPP's). They occur at a frequency of 1 to 15 per second and range from 0.5 to 1 mv in amplitude (Fig. 1B). "Fast" responses similar to action potentials can be reflexly evoked in the muscle by mechanical stimulation of the anal cerci (Fig. 1B). The cell bodies of motor neurons in the

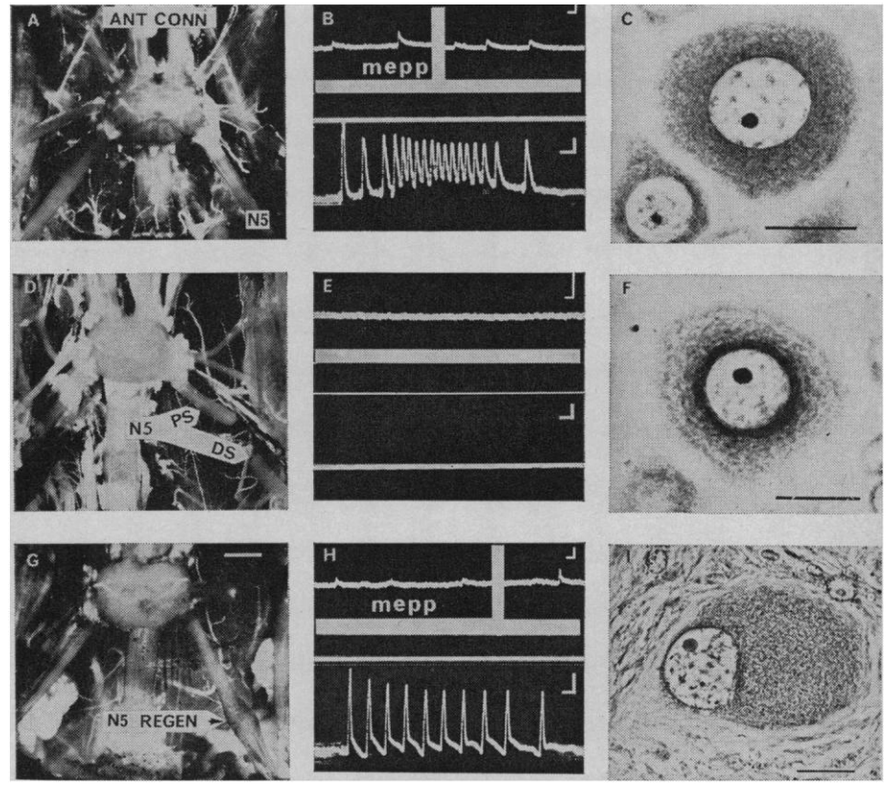

Fig. 1. (A) Ventral view of normal metathoracic ganglion of the cockroach. The left nerve trunk 5 (*N5*) and the anterior connectives (*ant. conn.*) are indicated. The superficial tracheole trunks in all ganglia in these pictures were removed to expose the nerve trunks. Scale (Fig. 1G) on all pictures of ganglia is 0.5 mm. (B) Upper record, spontaneous miniature end-plate potentials (MEPP) from normal coxal muscle. Lower record, intracellular recording of "fast" activity in normal coxal muscle 181C evoked by reflex stimulation. Scales in all electrical figures: upper record 1 mv, 10 msec; lower record 10 mv, 10 msec. (C) Normal motor neuron soma from metathoracic ganglion showing the uniform distribution of cytoplasmic RNA. The stain used in all cells shown is pyronine-malachite green; scale is 20 μ. (D) Ventral view of experimental ganglion 1 day after section of N5. The proximal stump (*PS*) and distal stump (*DS*) have sprung apart. (E) Intracellular recording from muscles, as in Fig. 1B, 5 days after denervation. Note lack of spontaneous MEPP's in upper record. Lower record shows lack of evoked muscle response when distal stump is stimulated electrically. (F) Motor neuron soma 5 days after its axon was cut. Note the densely stained pyronine ring in the perinuclear cytoplasm indicating a concentration of RNA in this region. (G) Ventral view of metathoracic ganglion 45 days after the left N5 was cut. Gap between the nerve stumps has been bridged by regenerating axons (*N5 regen*). (H) Intracellular records from reinnervated coxal muscle 181B at 140 days after nerve section. Note near normal MEPP's in upper record and reflexly evoked "fast" activity in lower record. (I) Motor nerve cell body 24 days after section of its axon when axon regeneration is well along. Note that the perinuclear RNA ring has disappeared and the nucleus has shifted to an eccentric position.

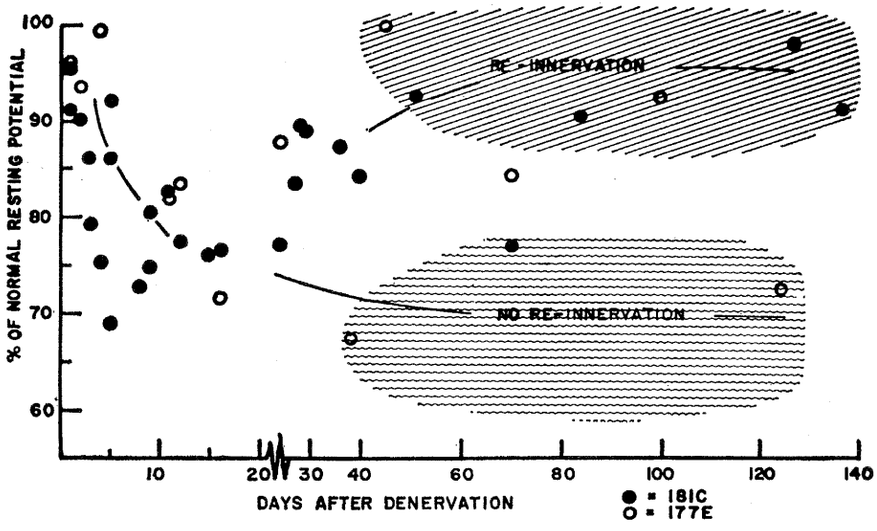

Fig. 2. Changes in the resting potentials of coxal muscles 181C and 177E. Resting potentials are given as a percent of the contralateral normal muscles of the same animal. Each point represents the average of 20 to 40 penetrations of the muscle from one animal. Averages of corresponding normal muscles from opposite legs are within a few percent of each other. The hatched areas indicate behavioral and electrophysiological evidence that the muscle has been reinnervated or that it has not been reinnervated.

normal ganglion show a fine granular distribution of cytoplasmic RNA when stained with pyronine-malachite green (*7*) (Fig. 1C). The nucleus of the normal motor neuron is typically located in the center of the soma.

When a nerve trunk to a muscle is cut (Fig. 1D), the spontaneous MEPP's decline in amplitude within 2 days after the injury. At 3 days, there is an obvious decrease in their frequency, and by 5 days after nerve section they disappear entirely from the involved muscle (Fig. 1E). The reflexly evoked muscle responses are completely eliminated immediately after nerve section. Electrical stimulation of the distal stump or direct stimulation of the muscle surface can evoke typical junctional activity and electrogenic responses in the muscle membrane immediately after nerve section. Within 2 days, the evoked electrical response in the muscle fibers has declined in amplitude. At this time the muscle membrane may show a tendency to fire twice in response to one stimulus. By 5 days after nerve section no electrical activity can be evoked in the muscle either by direct stimulation of the muscle surface or by stimulation of the distal nerve stump. The resting potential of the affected muscle fibers declines in parallel with the loss of miniature and evoked potentials. By 5 days after nerve injury, the resting potential of the affected muscle fibers has declined to a value 20 to 30 percent below the normal (Fig. 2).

In the central nerve cell body whose axon has been cut, a dense accumulation of RNA forms in the perinuclear cytoplasm within 2 days after the cell's injury (*5*) (Fig. 1F) and becomes most dense by 5 days after the operation; the ring is generally gone at 10 days. While the ring is present, there is little if any growth of regenerating axons from the proximal stump, although there may be some abortive sprouting of axon terminals. The nucleus of the cell with the RNA ring still remains in a central position (Fig. 1F).

Within 2 weeks after a nerve trunk is cut, axon sprouts can be seen emerging from the proximal nerve stump, as reported by other workers (*4, 8*). If the two stumps of the cut nerve trunk have remained well aligned, the regenerating axons of the proximal stump may grow directly into the distal stump and form a swollen bridge of neural and connective tissue (Fig. 1G). In other preparations the regenerating fibers may follow a tortuous route before joining the distal stump. Spontaneous MEPP's may reappear as early as 30 days after axon injury in muscles which showed denervation changes. This is accompanied by the morphological and behavioral evidence of reinnervation described above. These muscles may also show evoked electrical activity caused either reflexly or by electrical stimulation of the regenerated nerve trunk (Fig. 1H). Generally these early responses are not of normal amplitude and frequency. It may take up to 100 days after injury for the electrical responses to approximate the normal levels. This is probably correlated with full maturation of the regenerating axons and neuromuscular junctions. With the reappearance of spontaneous and evoked electrical activity in the muscle fiber, the resting potential once again increases toward the normal level (Fig. 2). Hyperpolarizing electrical potentials are often recorded in reinnervated muscle fibers. These responses have not been previously reported in *Periplaneta americana*, although they have been described in normal muscles of *Blaberus* and some Orthoptera (*9*).

The nucleus of the regenerating neuron shifts to an eccentric position within the soma when the regenerating fibers reach the distal stump. This shift may occur as early as 11 days after nerve section, but it is generally most pronounced at approximately 24 days. The nucleus usually shifts to the region of the axon hillock (Fig. 1I). When it does the perinuclear ring of RNA has already disappeared (Fig. 1, F and I). The eccentricity of the nucleus persists for as long as 100 days after the injury while the regenerating axons mature and the newly regenerated neuromuscular junctions reach their full functional capacity.

If the muscle is not reinnervated, there is fat-body invasion and atrophy of the muscle fibers. It appears that the neuron has a trophic effect on the muscle, since reinnervation prevents structural deterioration of the muscle fibers and tends to restore the reduced membrane resting potential to its normal value. Functional connection with the muscle is also necessary for the maintenance of the neuron. If no connection with the muscle occurs by 150 days after axon injury, the motor nerve cell body appears reduced in size and distorted in histological section. Thus, there is a reciprocal trophic relationship between neuron and muscle, as has been demonstrated in the vertebrates (*10*).

The electrical changes in the denervated muscle of the cockroach are similar to those described in the only other insect studied, the locust (*11*). However, in the locust, these events occur much later after the axon is cut. The resting potentials of denervated vertebrate muscle fibers show much variability. In some preparations the resting potential is reduced, and in others it is not (*12*).

The events associated with regenera-

tion of the motor axon are remarkably similar in the cockroach and the vertebrates. In both the vertebrates (*13*) and the cockroach, the shift of the nucleus to an eccentric position is associated with a high degree of protein production needed to regenerate an injured axon. The primary difference between cockroach neurons and those of vertebrates seems to reside in the organization of cytoplasmic RNA. In the vertebrate neuron the cytoplasmic RNA is grouped in large granular masses termed Nissl bodies (*13*). These consist of stacks of granular endoplasmic reticulum (*14*). It is apparently the RNA of the attached ribosomes in these structures that stains with the classical Nissl procedures. The normal vertebrate neuron is constantly producing protein at a high rate (*15*). When an extraordinary demand for protein synthesis, such as the need for regeneration, is placed on the vertebrate neuron the Nissl bodies break down (chromatolysis) causing the cytoplasmic RNA to become finely dispersed. In this condition the cell has shifted to a "superactive" level of protein production (*15*). The cockroach neuron normally has cytoplasmic RNA in a finely dispersed state; there are no prominent Nissl bodies (*5, 16*). When a high demand for protein synthesis is placed upon a cockroach cell, it forms a perinuclear aggregate of RNA which resembles in part the vertebrate Nissl body. These aggregates in the cockroach neuron then break down to a finely dispersed state once again, and at this time the cell shows obvious evidence of protein production by regenerating a new axon. This breakdown can be considered similar to vertebrate chromatolysis. This implies that the secondary dispersed state of RNA in the cockroach neuron, stemming from the breakdown of the perinuclear ring, differs in some critical manner from the normal, dispersed state of RNA in these cells. Perhaps the ribosomes require structural alignment on the cisternae of the endoplasmic reticulum in order to combine with messenger RNA needed to produce new protein for axon regeneration. In insects such as the locusts which do not show axonal regeneration (*11*), our studies indicate that injured neurons do not form a perinuclear ring of RNA.

In the cockroach, changes within specified cells can be related to alterations in the connections between these cells. The similarity with respect to neural regeneration between the cockroach and the vertebrates indicates that information from the insect preparation may lead to some general conclusions about the factors which determine connections between cells in excitable systems.

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Evoked Cortical Potentials: Relation to Visual Field and Handedness

Abstract. *The amplitude of evoked responses of occipital cortex in man depends on the visual field in which the stimulus appears. Greater responses occurred repeatedly for two of three subjects, both left-handed, when the stimulus appeared in the left field than in the right. Subsequent tests of 13 right- and 13 left-handed males indicated that the magnitude of the response of the right lobe, relative to that of the left, was greater for left-handed individuals. We conclude that the difference in amplitude between the two lobes is related to handedness.*

We recently demonstrated that reaction time and evoked potential (EP) latency and amplitude vary concomitantly with variations in site of retinal stimulation. Reaction time parallels changes in EP latency and varies inversely with EP amplitude (*1*). These results provide evidence for the existence of a relationship between reaction time and EP's, in agreement with other studies (*2*).

In that study our observations were limited to the temporal retina of the right eye. Since stimulation of the nasal retina yields shorter reaction times (*3*), we subsequently attempted to demonstrate the generality of the relation between reaction time and EP's by showing that the cortical responses to nasal retina stimulation are of larger amplitude and shorter latency than to temporal retina stimulation. However, EP's obtained from the right occipital lobe due to nasal stimulation of the right eye were consistently smaller than those obtained from temporal stimulation of that eye (*4*).

Since the nasal retina of the right eye projects to the left lobe and the temporal retina to the right lobe, it is possible that the nasal-temporal effects being sought were masked by hemispheric differences. That such differences may exist also is suggested by photic driving studies indicating that one hemisphere tends to be more susceptible to driving than the other (*5*). One purpose of the present study, therefore, was to determine whether differences actually do occur in EP's obtained from the two lobes when flashes are presented in the right and left visual fields. A second purpose was to determine whether such differences, if they do occur, are related to handedness.

To investigate the first question, three of the authors served as subjects in two experimental sessions each. In each session, 10 retinal sites located 10° apart along the horizontal meridian were stimulated, red light being used in one of the sessions, blue in the other. Both eyes were stimulated simultaneously. There were five trials in a given session. Each trial was separated by a 15-minute rest interval during which the subject was required to leave the experimental room. In a given trial a