in growth promotion) is not essential to postpartum lactation. Since lactation was also normal in the midget mother of two midget children, it appears that placental lactogen (*9*) is under genetic control separate from pituitary growth hormone or is not necessary for lactation.

Mutations resulting in isolated deficiency of pituitary gonadotropin (*10*), adrenocorticotropin (*11*) or thyrotropin (*12*) probably also occur in man.

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Input Resistance, Electrical Excitability, and Size of Ventral Horn Cells in Cat Spinal Cord

Abstract. *Experiments on cat lumbosacral alpha motoneurones showed that, in comparison with cells possessing rapidly conducting axons, the cells with slowly conducting axons have the higher input resistance, that they need weaker stimulating currents to reach the threshold for repetitive firing, and that they need a relatively larger increment in current strength for a given increase in firing rate. Measurements of the number and diameters of dendritic trunks gave larger values for the larger cell bodies. The discussion deals with the interrelation between cell geometry, electrical properties, and the reflex action of alpha motoneurones.*

The alpha motoneurones innervating the hind limb of the cat show, for each motor pool, a continuous variation in the diameter (*1*) and conduction velocity (*2*) of their axons. In previous investigations, the cells with small and slowly conducting axons have often been referred to as "tonic" (or "slow"), and those with large and rapidly conducting axons as "phasic" (or "fast") motoneurones (*2*, *3*). In response to most synaptic inputs, tonic and phasic cells differ markedly with respect to the size of the postsynaptic potentials (inhibitory and excitatory) and to the "reflex threshold" for repetitive firing. In most instances the postsynaptic effects are more pronounced in tonic than in phasic cells (*3–7*), but the reverse situation also occurs (*4*, *8*). With synapses at similar locations, the current generated by a given conductance change (inhibitory or excitatory) would be expected to alter the soma membrane potential more in a cell with a high than in one with a low "input resistance" (*9*), that is, the d-c resistance offered by the cell to the flow of current between an electrode inside the soma and one outside the cell. Thus, less current (for example, a smaller number of active synapses) would presumably be needed for eliciting a repetitive discharge in a cell with a high than with a low input resistance. Therefore, the input resistances of motoneurones with slowly and rapidly conducting axons must be known if the causes for their different reflex behaviors are to be understood (*7*).

The experiments were performed on cats (2.0 to 3.4 kg) anesthetized with pentobarbitone (Nembutal). Single-barreled microelectrodes filled with a solution of 3*M* KCl or 2*M* potassium-citrate were employed for intracellular recording. The dorsal roots caudal to L4 were cut, and various hind-limb nerves were used for antidromic stimulation of the motoneurones. Conduction velocity was calculated from measurements of antidromic latency and conduction distance (*2*). The rectal temperature was generally 37° to 38°C.

For the resistance measurements, rectangular current pulses of variable strength and polarity were injected through the intracellular electrode. Current strength was continuously recorded. A Wheatstone bridge was used to balance out potential changes produced in the microelectrode and the biological structures in series with it (*10*, *11*). When the bridge was balanced, the resistance of the microelectrode and the structures in series with it could be read off from the potentiometer of the bridge to within about ± 0.1 Mohm. The input resistance of a motoneurone was determined in one or more of the following ways: (i) by balancing the Wheatstone bridge with the electrode tip inside and just outside the neurone ("direct" method), (ii) by measuring, with the same bridge setting, the potentials produced by currents with the electrode tip inside and just outside the neurone ("direct" method) (*10*, *12*), (iii) by measuring the effect of currents on the amplitude of anti- or orthodromic spikes ("spike" method) (*11*, *12*), and (iv) by measuring the effect of currents on the spike threshold to an excitatory post-synaptic potential ("threshold" method) (*11*, *12*). Methods (ii), (iii), and (iv) have been earlier used and discussed (*10–13*). With many electrodes the "direct" methods could not be used, mainly because the resistance of these electrodes changed on movement through the tissue (*11*). In order to possess a steady state for determining the input resistance (*13*), none of the measurements were performed before at least 0.5 second after the onset of current. Records showing signs of electrode polarization were discarded.

The input resistance was higher in the cells with slowly conducting axons (tonic motoneurones) than in those with rapidly conducting axons (phasic motoneurones). Figure 1 shows the relation between the input resistance of a motoneurone and the reciprocal square of the conduction velocity of its axon. With this latter function of conduction velocity the relation was approximately linear, and the correlation coefficient

was + 0.92 (50 cells, $P < .001$). The range of variation in input resistance was 0.5 to 8.1 Mohm. Values (Fig. 1) of input resistance determined by the "direct" methods and the "spike" method varied over the same range, and they were related to conduction velocity in a similar manner. The average spike size was 83 ± 10 mv (standard deviation, 50 cells). The membrane potential was monitored during the resistance measurements in 33 of the cells, and averaged 72 ± 5 mv (S.D.). Input resistance in these cells did not correlate with spike amplitude or membrane potential. The relation between input resistance and axonal conduction velocity was not in any obvious way different for motoneurones belonging to different hind limb nerves (soleus, gastrocnemius, popliteal, common peroneal, and hamstring).

Values of input resistance obtained with the "threshold" method were also related in a statistically significant manner to the axonal conduction velocity. However, with this method the values were more scattered than those shown in Fig. 1, probably because of changes in firing level that may occur during the passage of long-lasting currents (*14*).

In intracellular work, tonic motoneurones have often been distinguished from the phasic ones by the longer duration of their after-hyperpolarization (*2, 6*). The input resistance was better correlated with the conduction velocity than with the duration of after-hyperpolarization. However, the latter correlation was also highly significant ($r = + 0.79$, 50 cells; $P < 0.001$). Cells with an after-hyperpolarization shorter than 70 msec had an average input resistance of 0.9 ± 0.5 Mohm (S.D., 14 cells), whereas neurones with an after-hyperpolarization exceeding 140 msec had one of 5.2 ± 2.2 Mohm (S.D., 6 cells).

The previously published values for the input resistance of motoneurones (0.5 to 2.5 Mohm, *11* and *12*) correspond in the present work to those obtained from cells with relatively rapidly conducting (that is, large diameter) axons (Fig. 1). The predominance of such cells in earlier studies was probably due to the fact that good intracellular recordings are most easily obtained from the cells with the largest axons, which are likely to be those with the largest cell bodies.

A relation, similar to that of Fig. 1, between the input resistance and conduction velocity has been found to hold for the pyramidal cells in the cat's cerebral cortex (*15*).

Eighteen of the cells (Fig. 1) were stimulated to steady repetitive firing by long-lasting injected currents (*16, 17*). In Fig. 2*A* the threshold current strength for steady firing (*16*) is plotted against input resistance. As expected, the threshold for steady firing varies inversely with the input resistance. The threshold for steady firing for these cells did not correlate with the membrane potential or spike size. The data of Fig 2*A* indicate that, for motoneurones with comparable membrane potentials, the input resistance is a significant factor determining the current strength needed (synaptic as well as injected) for eliciting a steady repetitive discharge. In Fig. 2*A* the values are too few and the spread is too large to demonstrate clearly the shape of the curve relating threshold current strength to input resistance. It should be noted that for cells requiring the same threshold depolarization, the threshold current strength should be linearly related to the reciprocal of input resistance rather than to the input resistance itself.

For some of the motoneurones (Fig. 2*A*, filled circles) the slope was determined for the linear relation between steady discharge frequency and current strength within the lower, "primary," range of firing rates (*16, 17*). In Fig. 2*B* the increment of current needed for increasing steady firing rate by 1 impulse per second is plotted against neurone resistance. Figure 2*B* somewhat unexpectedly indicates that more current is needed per unit increase in steady firing rate in cells with a high input resistance than is necessary in cells with a low input resistance. Thus, in spite of their greater input resistance, the firing rate of tonic cells is relatively little affected by variations of

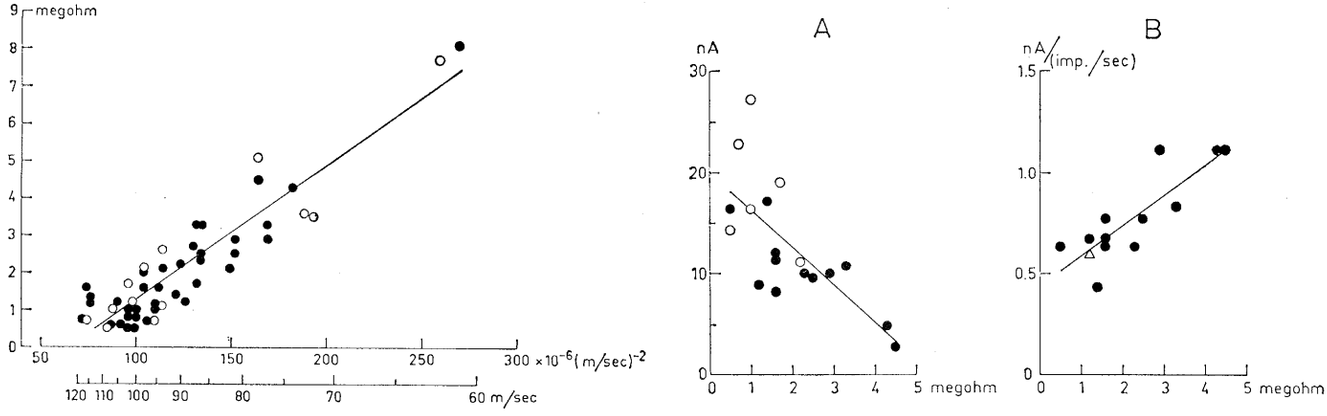

Fig. 1 (left). Relation between input resistance and conduction velocity in 50 motoneurones. Abscissa: Lower scale, conduction velocity m/sec; upper scale, the square of the reciprocal of conduction velocity $(m/sec)^{-2}$. Ordinate: Input resistance in megohms. The input resistance was measured by the "direct" methods (open circles), and the "spike" method (filled circles), respectively. The regression line was calculated by the method of least squares. Fig. 2 (right). *A*, The threshold for steady firing (1 na = 10^{-9} amp) plotted against input resistance (Mohm) for 18 motoneurones. The correlation coefficient was −0.72 ($P < .001$). Spike size averaged 89 ± 9 mv (S.D., 18 cells), and membrane potential 71 ± 4 mv (S.D., 14 cells). *B*, The increment of current strength needed per unit increase of firing rate (na per impulse per second) plotted against input resistance (Mohm) for 13 motoneurones. The correlation coefficient was + 0.84 ($P < .001$). Spike size averaged 89 ± 10 mv (S.D., 13 cells) and membrane potential 72 ± 3 mv (S.D., 12 cells). Cells represented both in *A* and *B* are indicated by filled circles. Regression lines calculated by the method of least squares.

current strength. Possibly, factors taking part in the production of the long after-hyperpolarization of tonic cells may counteract increases of firing rate.

In a larger number of motoneurones in good condition it was found that, on an average, the rheobase, the threshold current strength (in impulses per second the slope relating steady firing rate to current strength (in impulses per second per nanoampere) were all higher for cells in which the after-hyperpolarization was short rather than long.

The relation between input resistance and axonal conduction velocity (Fig. 1) could be explained on a morphological basis if motoneurones with small and slowly conducting axons regularly had smaller cell bodies and a less well developed dendritic apparatus than the neurones with large and rapidly conducting axons. Previous anatomical evidence indicates that the cell body actually is smaller for a neurone with a small axon than for one with a large axon (*18*). In my study ventral horn cells were examined to determine if neurones with small cell bodies have smaller and fewer dendritic trunks than neurones with large cell bodies.

The morphological studies were performed on spinal cords from adult cats. The cord was stained by the Golgi-Cox method (*19*) and cut in sections 200 to 250 μ thick. Cells from the ventral horn of the segments L5-S1 were measured in enlarged photomicrographs. There were no obvious differences between cells belonging to different cord segments. The diameter of the dendritic trunks was measured just distal to the initial tapering. The surface area of the soma was estimated as π times the product of the major and minor diameters (*20*). The average cell body diameter ranged between 25 and 90 μ. For comparison it should be mentioned that the largest alpha motoneurones are known to be among the largest cells of the ventral horn, and they may have a soma diameter of 70 μ or more (*21, 22*). The soma diameter of the smallest alpha motoneurones is less well known. Previous workers have considered it to be of the order of 25 to 30 μ (*21*).

Figure 3 shows that there is an approximately direct proportionality between the total cross-sectional area of all the visible dendritic trunks and the surface area of the soma ($r = +0.91$, 30 cells, $P < .001$). Neurones with small cell bodies had, on the average, both fewer and thinner dendritic trunks than the neurones with large bodies. Thus, the total dendritic surface area (*22*), as well as the combined dendritic input conductance, would presumably be much larger for neurones with large cell bodies than for those with small cell bodies.

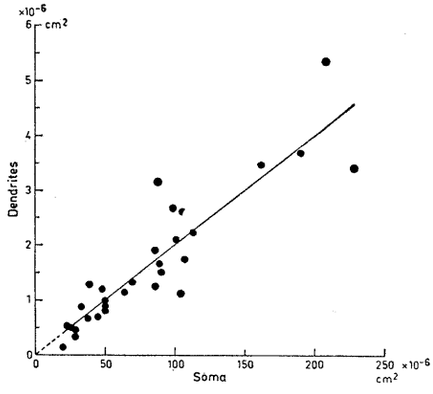

Fig. 3. The total cross-sectional area of all visible dendritic trunks (dendrites, cm²) plotted against somatic surface area (soma, cm²) for 30 ventral horn cells. The cross-sectional area was for each dendritic trunk obtained by multiplying the square of the trunk diameter by $\pi/4$. The regression line was calculated by the method of least squares.

The ratio of dendrite to soma conductance is most probably related, but not necessarily directly proportional to, the value of $\Sigma\ d^{3/2}/S$, where d is the diameter of the dendritic trunk and S the soma surface area (*20*; also *12, 23, 24*). In the present material the $\Sigma\ d^{3/2}/S$ values for small and large cells overlapped extensively. For 15 cells with a soma surface area exceeding 80×10^{-6} cm² (Fig. 3), the value of $\Sigma\ d^{3/2}/S$ was 0.99 ± 0.23 cm$^{-1/2}$ (S.D.), and for the 15 smaller cells it averaged 1.15 ± 0.30 cm$^{-1/2}$ (S.D.) (see *21*). The difference is small, and not statistically significant ($P > .1$). The results indicate that the dendrite to soma conductance ratio would be of the same order of magnitude for small and large alpha motoneurones. The input resistance of an alpha motoneurone would then essentially be proportional to the reciprocal of its somatic surface area (*20*), which in its turn is related to the size of the axon (*18*).

Previous studies have indicated that large alpha motoneurones have a specific membrane resistivity of 600 ohm cm² and a ratio of dendrite to soma conductance of 2.3 (*12, 23*). With these values, and with a soma diameter of 25 to 30 μ (*21*), the smallest alpha motoneurones would be expected to have an input resistance of the order of 6.4 to 9.3 Mohm. This is in rather good agreement with the experimental data (Fig. 1). On the whole, the experimental findings (Fig. 1) seem to be well accounted for by the probable variation in the geometrical dimensions of the motoneurones.

As noted earlier, information concerning the input resistance (Fig. 1) and the "electrical excitability" (Fig. 2) of motoneurones should help to explain some of the previous results on reflexes. With many synaptic inputs the postsynaptic potentials are larger, and the "reflex threshold" for repetitive firing is lower in tonic than in phasic motoneurones (*3–7*). At least with some of these synaptic inputs, the discharge rate appears to undergo great changes more easily in phasic (such as quadriceps) than in tonic (such as soleus) motoneurones (*4, 25*). The results (Figs. 1 and 2) suggest that tonic and phasic motoneurones would show such differences in their reflex behavior even if both types of cell were activated by the same number of similarly located synapses (*7*). With some inputs, however, the postsynaptic potentials are larger and the "reflex threshold" is lower in phasic than in tonic cells (*4, 8*). With such synaptic inputs the active synapses should be much more numerous or more effectively located for phasic than for tonic cells in order to compensate for the low input resistance of the former (Fig. 1). Phasic cells are likely to have a much larger total surface area than tonic cells (Fig. 3), and they would therefore be expected to receive a much larger total number of synaptic endings (*26*).

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Heterosis: Complementation by Mitochondria

Abstract. *Many (but not all) hybrids between two genetic pure lines show heterosis; that is the hybrids grow more rapidly or larger than the parental pure lines. The mechanism is not known. Two corn crosses were studied. In one hybrid known to exhibit heterosis, the mitochondria and an artificial 1:1 mixture of parent mitochondria showed heterosis with respect to oxidation and phosphorylation. In the other cross, neither the hybrid plant, its mitochondria, nor the mixture of parent mitochondria showed heterosis.*

Heterosis has been defined as the superiority of a hybrid over its parents in any measurable attribute. Generally, in studies of heterosis, hybrid superiority has been measured by rate of growth and total growth. Heterosis is a product of hybridization, but hybridization need not necessarily result in heterosis. Heterotic growth may be explained by definitive physiological studies of the enzyme and hormonal activities which elicit the initial manifestation of hybrid vigor during the early development of the organism (*1*). Hybrid maize seedlings when compared with their parents germinate earlier, grow faster, and exhibit a higher metabolic activity (*2*). Hanson and co-workers have pointed out that heterosis exhibited by hybrid maize seedlings may be judged by mitochondrial activity (*3*).

No operational descriptions of heterosis have yet been advanced. We now report an operational basis for seedling heterosis with regard to mitochondrial behavior.

Mitochondria were isolated from scutella (diploid cotyledons) or embryonic axes of 2- to 5-day-old maize inbred (Wf9, C103, Ohio 43, Ohio 45) and hybrid (Wf9/C103, Ohio 43/Ohio 45) seedlings grown at 27°C on moist paper toweling. Isolation procedures (4°C) have been described (*4*). Mitochondrial activity was measured at 27°C in a Warburg respirometer by manometric techniques (*5*). The reaction mixture was composed of 40 μmole of α-ketoglutarate, 1.5 mg of adenosine monophosphate, 0.5 mg of thiamine pyrophosphate, 0.33 mg of diphosphopyridine nucleotide, 0.1 mg of cytochrome *c*, 0.1 mg of coenzyme A, 1.5 mg of hexokinase, 0.11 μmole of glucose, 250 μmole of sucrose, 2.5 μmole of $MgSO_4$, and 25 μmole of potassium phosphate in a final volume of 2.5 ml, *p*H 6.8. In general, reactions were started within 90 minutes after the excised tissues were homogenized. Phosphorylation was determined over a 20-minute period (*6*). All measurements are expressed on the basis of mitochondrial nitrogen (*7*).

It is evident (Fig. 1) that mitochondria of the hybrid Wf9/C103 exhibit definite superiority with respect to oxidation. This superiority is manifested regardless of the tissue from which the mitochondria were isolated. The difference in activity (hybrid mitochondria compared with parental mitochondria) is statistically significant (Table 1).

The results with mitochondria from another hybrid and its parents are shown in Fig. 2. In this instance the hybrid is not different from its parents with regard to oxygen uptake. Of utmost importance in the problem of heterosis with regard to oxidative activity is that hybrid Wf9/C103 (Fig. 1) is an extremely heterotic hybrid, heterosis being expressed not only by germination rate and postgermination development, but by height and yield of grain at maturity. Hybrid Ohio 43/Ohio 45, on the other hand, is almost completely nonheterotic, even at maturity, and frequently is indistinguishable from it parents (*2*). The nonheterotic behavior is exemplified by data in Fig. 2, and the statistical analysis in Table 1 shows that this hybrid is not different from its parents.

Mitochondria from the heterotic hybrid have a lower ratio of phosphorus to oxygen (μmole Pi esterified per μatom of O) than did the parental mitochondria but, significantly, phosphorylation and oxidation were markedly greater in the hybrid mitochondria than in the parent mitochondria. While hybrid Wf9/C103 does not exhibit marked superiority in phosphorylation to both parents, we have clear indication of superior phosphorylation by mitochondria from heterotic hybrids (*8*). The oxidative phosphorylation by mitochondria from the nonheterotic hybrid Ohio 43/Ohio 45 again did not differ from that of its parents.

Thus heterosis is reflected in activities of mitochondria prepared from a heterotic hybrid. Similar behavior was observed in mitochondrial preparations from other heterotic hybrids. Hybrids of diverse genetic background exhibited

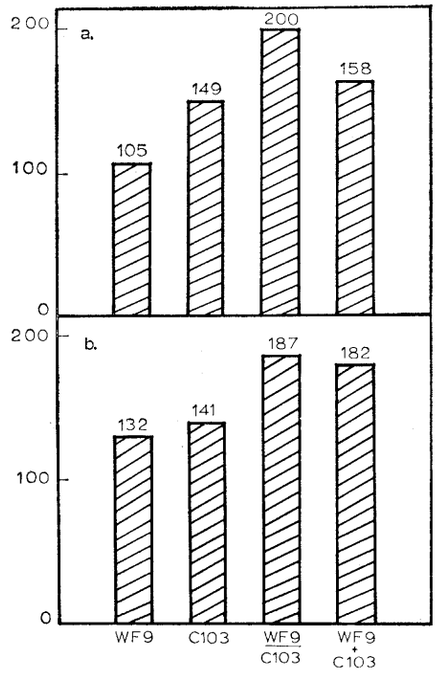

Fig. 1. Oxygen uptake (ordinate, microliters of oxygen per milligram of nitrogen per hour) by mitochondria from maize seedlings. *a*, Mitochondria from 2½-day-old scutella; *b*, mitochondria from 4½-day-old embryonic axes. Wf9 and C103 are inbred strains; Wf9/C103 is hybrid; Wf9 + C103 is a mixture of equal portions of mitochondrial suspension of the inbreds.