Table 2. Kinetic parameters for water pool in cattle. For the cow used in trial 1, the body water pool was 73.4 percent of body weight; for that used in trial 2, it was 72.4 percent.

Trial	Half-life (days)	Water turnover (pool fraction/day)
	Group I	
1	3.3	0.21
2	3.4	0.204
3	3.5	0.198
4	3.8	0.082
5	3.7	0.187
6	3.0	0.231
7	3.9	0.178
8	3.7	0.187
	Mean 3.54 ± 0.105	
	Group II	
9	3.4	0.204
10	4.1	0.169
11	2.8	0.247
12	3.8	0.182
13	3.6	0.192
14	2.9	0.239
15	3.2	0.217
	Mean 3.4 ± 0.179	
	Group III	
16	2.8	0.247
17	3.5	0.198

conditions do not effect the half-life of body water. Rather, it appears that their effect was very small and, with our limited data, cannot be determined. More extensive investigations are in progress to evaluate the individual effects of these factors on water turnover.

The data collected in trial 1 (or 2) can be used to calculate the daily water flux through the cow. For example, in trial 1, the body water pool for cow 1186 was 73.4 percent of 763 kg, or 560 kg of water, and her turnover was 0.21 of the pool per day. The water flux, based on these figures, would be 118 kg/day (560 kg × 0.21). This cow was producing 23 kg of milk per day, which would contain 19.8 kg of water, or about 17 percent of the total flux. Although water losses via feces and urine were not measured in these trials, data reported by Adolph (*4*) can be used to estimate that daily losses of 24 kg occurred (0.04 and 0.09 percent of body weight per hour for urine and feces, respectively). If this cow stopped lactating, so that her water flux decreased to 98 kg/day while her water pool remained 560 kg, her water turnover would become 0.18 of the pool per day and the half-life would increase to 4.0 days. This value for half-life is on the high side of those obtained with nonlactating cattle but still falls within the maximum observed with a steer (4.1 days). Since the average half-life for nonlactating cattle was not significantly different from that for cows with various lactation levels (9 to 24 kg of milk per day) it seems likely that other adjustments generally occur which minimize the change in half-life with changes in water flux. One possibility would be a compensatory decrease in water loss via urine and feces as the lactation level rises; however, this would be of limited magnitude, since, of the total amount of water lost in this way, most is needed for removal of undigested and nonmetabolized waste from the body. Another, and perhaps more reasonable, adjustment would be a parallel change in total pool size—the pool increasing as the water flux increases with higher levels of lactation. A parallel change of pool size and water flux would minimize any change in half-life. The greater percentage of body water in dairy cattle compared to beef cattle (*3*) would support this explanation. Also, the fact that the half-life of body water was nearly the same during ketosis (trial 4) when the cow was undergoing marked decrease in body content (22 percent weight loss in 3 weeks), as when the cow had recovered and was slowly gaining weight (trial 5), indicates that adjustments involving pool size and turnover must occur rather rapidly.

An increase in pool size associated with greater water flux could have important physiological significance. In lactating animals it would provide a greater reservoir of soluble metabolites for biosynthesis of milk. It would also provide greater water reserve for hot weather conditions when losses due to respiration and body cooling increase. The larger pools with their greater capacities would resist, for a longer time than smaller pools, the adverse physiological effects of depletion associated with high fluxes through these pools.

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5 March 1964

"Electrical Transmission" at an Excitatory Synapse in a Vertebrate Brain

Abstract. *A type of excitatory synaptic transmission which is novel for the vertebrate brain has been found in the medulla of the goldfish. Certain synaptic terminals (the club endings of Bartelmez) appear to stimulate the Mauthner neuron (M-cell) by means of the passive spread of their action currents across the synaptic membrane. After stimulating the ipsilateral eighth cranial nerve, an excitatory postsynaptic potential (EPSP) appears in the M-cell with a latency which is very brief (about 0.1 msec) and which probably represents a negligible synaptic delay. This response is attributed to the club endings: there were steep gradients of potential along the lateral dendrite of the M-cell during activity and the early EPSP was maximal in the distal part of the dendrite where the club endings predominate. Potential changes in the M-cell spread (passively) backwards into certain eighth-nerve fibers (probably club endings) indicating the presence of special low-resistance connections between them and the M-cell.*

The Mauthner cells (M-cells) are a pair of giant neurons found in the medulla of most fish. They are large enough to permit intracellular recordings to be obtained from their axons, cell bodies, and dendrites; their rich synaptic input is morphologically specialized, certain distinctive groups of nerve endings being localized to particular regions of the cell (Fig. 1).

In previous studies of goldfish M-cells (*2*) very early intracellular changes in potentials were sometimes observed after a stimulus was applied to the ipsilateral eighth cranial nerve. The study reported herein was undertaken to examine these short-latency responses more closely.

The experimental methods were similar to those previously described (*2*), but a more radical dissection was made which exposed the utricle and parts of the eighth nerve. The fine silver wires used for stimulating were insulated except at the tips and could be manipulated independently. Intracellular recordings were made with glass micropipettes filled with either 0.6*M* K_2SO_4 or 3*M* KCl.

The responses with short latencies could be evoked in the M-cell by stimuli of relatively low intensities when the

stimulating cathode was placed near the entry into the medulla of the posterior branch of the eighth nerve (which innervates the sacculus and lagena). These responses were present in intracellular recordings from the M-cell lateral dendrite, cell body, and axon (Fig. 2). Their amplitude was finely graded according to the intensity of the stimulus, indicating that many eighth-nerve axons participated in their production. At a certain intensity of the stimulus these responses exceeded threshold and evoked a conducted impulse in the M-cell (Fig. 2, *c*, *d*). They were very effectively diminished by inhibitory inputs to the M-cell. In short, they had the characteristics of conventional excitatory postsynaptic potentials (EPSP's) remarkable only for their time scale. The latency from the beginning of the eighth-nerve stimulus to the start of the EPSP was very brief (usually 0.10 to 0.15 msec). Their overall duration was only 1.5 to 2 msec, the rising phase being complete in 0.2 to 0.3 msec. Other examples of rapid EPSP's of very short latency are known (*3*) and these occur at certain "electrical" synapses which can be distinguished from "chemical" synapses by the presence of specialized low-resistance connections

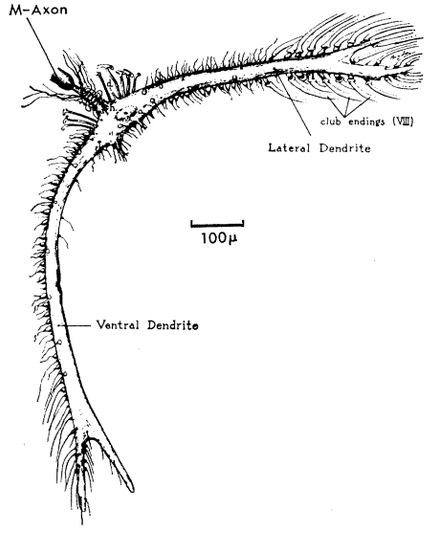

Fig. 1. A reconstruction of a Mauthner cell; taken from Bodian (*1*). Note the myelinated club endings on the distal part of the lateral dendrite; although not shown in the figure, some small boutons are also present in this region and a few club endings are found on other parts of the cell, as well. The scale, which is only approximate, is not that given with the original drawing (*1*) but is more appropriate to the larger cells used in the present experiments.

which provide effective electrical coupling between the pre- and postsynaptic cells. These preliminary observations thus raised the possibility of "electrically" transmitting excitatory synapses on the M-cell. At "electrical" synapses the presynaptic structure must be large enough to supply the required current to the postsynaptic cell. In the M-cell, the myelinated club endings seemed the most likely candidates for mediating this type of transmission, because of their large size. At about this time Robertson *et al.* (*4*) were independently studying the M-cell with the electron microscope and observed a peculiarity at the club-ending synapses which pointed to the same hypothesis. However, before the anatomical and physiological observations could be said to support one another it was necessary to determine whether the early EPSP does, in fact, arise at the club-ending synapses.

Intracellular recordings were made at a number of positions along the M-cell in order to discover if the amplitude of the early EPSP was maximal at the region of the club-endings. The results were definite. Whenever a successful entry was made in the distal half of the lateral dendrite (more than about 300 μ lateral to the axon hillock) very large early EPSP's could be recorded (up to 50 mv); more proximally in the dendrite or in the cell body the early response to the same stimulus was always smaller. This is illustrated in the upper row of Fig. 3 which shows early EPSP's recorded from three different sites in the same M-cell. The zero position corresponds to the axon-hillock region, the other two recordings were from the lateral dendrite 175 μ and 350 μ from the first. The stimulus applied to the eighth nerve was submaximal and was kept constant. The early EPSP was largest at the most lateral recording site. The lower row of records in Fig. 3 were made at the same three positions and show the analogous but converse phenomenon for the antidromic action potential. The latter does not actively propagate along the dendrite (*2*) but is generated at the axon and axon hillock (position *0*). In accordance with this different site of origin, the action potential decreases in height along the dendrite in the direction opposite to that in which the EPSP decreases. Experiments of this type show that the early EPSP's originate in that part of the M-cell where the club endings pre-

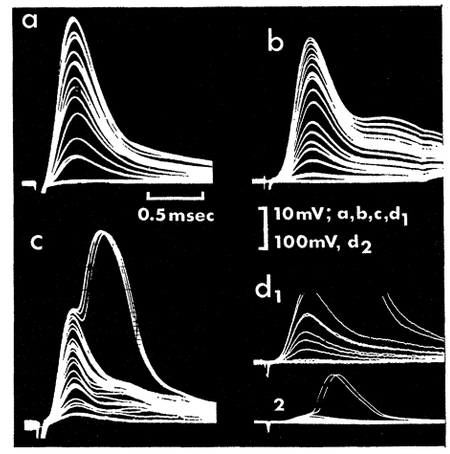

Fig. 2. Intracellular recordings from the M-cell showing the short-latency responses to stimulation of the ipsilateral eighth-nerve. Each record shows a multiple exposure; between successive traces the stimulus intensity was increased. Each set of records is taken from a different experiment. The microelectrodes were inserted at the following sites: (*a*), in the lateral dendrite, 450 μ from the axon hillock; (*b*), lateral dendrite 250 μ from the axon hillock; (*c*), in the perikaryon about 75 μ from the axon hillock; and (*d*), in the myelinated axon about 300 μ posterior to the axon hillock. The two sets of traces in (*d*) show the same responses at different amplifications.

dominate. Although some very small boutons are also present there, it seems unlikely that the latter give rise to the very large early EPSP's and the observations thus strongly suggest that these responses are generated by the club endings.

Evidence has also been obtained for the presence of low-resistance connections between the M-cell and certain axons of the eighth nerve, presumably club endings. Attempts were made to record intracellularly from eighth-nerve fibers in the general vicinity of the M-cell dendrite and cell body. There was wide variation in the success of penetration (resting potentials, about 20 to 60 mv; action potentials, 10 to 50 mv); nevertheless, a consistent set of observations emerged. Twenty-two units were identified as eighth-nerve axons by their short-latency (0.1 to 0.4 msec) and fatigue-resistant (followed 100 stimuli per second for many minutes) action potentials evoked by stimulating the eighth nerve. They fall into two distinct groups. In fourteen of the units (group 1) the delay from the beginning of the shock to the start of the impulse was in the range 0.08 to 0.12 msec (for example, Fig. 4a_1), whereas in the re-

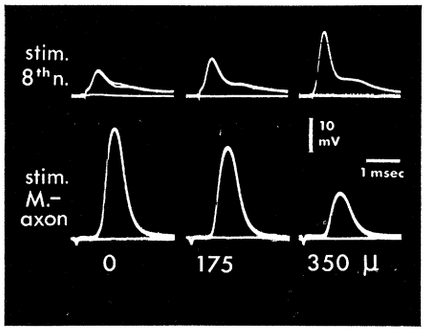

Fig. 3. Intracellular recordings from three sites on the same M-cell. Zero position is the axon hillock; the other two sites were in the lateral dendrite at the distances indicated. At each site the response was recorded to a given ipsilateral eighth-nerve stimulus (upper traces) and to a spinal-cord stimulus which gave rise to an antidromic action potential propagating up the M-axon to the axon hillock (lower traces). Note that the two responses both decayed along the dendrite, but in opposite directions corresponding to the different sites at which they are generated.

maining eight (group 2), the values were 0.2 to 0.4 msec (Fig. 4b_1). There were no consistent differences in the recorded amplitudes of resting and action potentials between the two groups but the rise time and duration of the action potentials were consistently shorter in group 1. The most interesting difference, however, was seen when an action potential was set up in the M-cell by antidromic stimulation. A concomitant positive-going potential change (range 1.7 to 4.3 mv) was recorded in each of the fourteen units of group 1 (Fig. 4a_2; c, upper trace), but was absent in the eight units of group 2 (Fig. 4b_2). The potential change disappeared along with the resting and action potentials as the electrode was withdrawn from the fiber. It had the same latency and threshold as the M-cell action potential (lower trace, Fig. 4c). This transmission of a potential change from M-cell to eighth-nerve fibers, without delay and in the opposite direction from normal, indicates that the spread is passive and takes place across low-resistance connections between the two structures. The short delay, between the eighth-nerve stimulus and the action potential recorded in the group 1 fibers, suggests a high conduction velocity and thus a large diameter; this again points to the club endings which are terminations of the largest myelinated axons in the eighth nerve. However, direct demonstration of this by marking the position of recording electrodes in group 1 fibers, has yet to be made. The identity of the group 2 fibers is not known, nor is there reason to suppose that they are a homogeneous group.

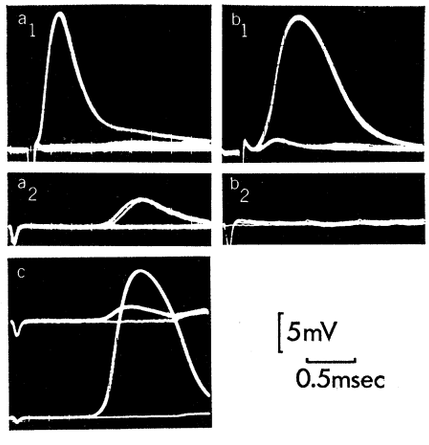

Fig. 4. Intracellular recordings from eighth-nerve fibers. (a), A fiber of group 1; (b), a group 2 fiber. (a_1, b_1) Stimulation of the eighth nerve gave rise to action potentials. (a_2, b_2) Stimulation of the spinal cord set up antidromic action potentials in the M-cell; some of the potential spread into the group 1 fiber (a_2) but not into the group 2 fiber (b_2). (c) Simultaneous recordings from a group 1 axon (upper trace) and the soma of the ipsilateral M-cell (lower trace), following a shock to the M-axon in the spinal cord. Both potential changes had the same threshold. The sloping deflections at the ends of the traces in c are monitoring pulses.

Much evidence has recently accumulated for a morphological correlate of electrical coupling between cells. In eight situations in which physiological evidence has indicated the presence of low-resistance intercellular connections (*3*, *5–7*), electron microscope studies (*6*, *8*) have demonstrated a special relationship between the surface membranes of the electrically coupled cells. Regions are seen in which the extracellular cleft between the cells is greatly reduced or, more frequently, obliterated. Various terms have been used to refer to these special regions, such as *zonula occludens,* tight junction, external compound membrane, and nexus. The list includes several different tissues: nervous system, smooth muscle, cardiac muscle, and epithelium; however, the connections are in each instance between cells of the same type.

The appearance of the regions of membrane contiguity is not identical in all the cases studied, although differences in technique almost certainly account for some of the variability. Furthermore, "tight junctions" may have additional or even different functions in some cells, for example, that of forming a barrier between different extracellular spaces (*9*) or possibly allowing for intercellular transfer of metabolites (*7*). Nevertheless, the correlation between low-resistance intercellular connections and the presence of "tight junctions" is very striking. At the very least, the presence of such structures should be considered to raise the possibility of electrical coupling. "Tight junctions" in the M-cell have been clearly demonstrated (*4*) and these are confined to the club-ending synapses. Thus, the physiological evidence, together with the anatomical observations, provide a very strong case for "electrical transmission" at these synapses. We can now recognize at least four and very probably five different synaptic mechanisms in the M-cell: (i) "electrical" inhibition (*10*); (ii) "chemical" inhibition (*10*); (iii) presynaptic inhibition (*11*); (iv) "electrical" excitation; and (v) "chemical" excitation (not yet conclusively demonstrated). The functional significance of these different mechanisms will be discussed in a longer report to be published elsewhere.

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