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TRANSDUCTION AND ENCODING MECHANISMS IN MUSCLE SPINDLE

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1. *Controversy on Spindle potential*

Katz[1] has recorded a slow depolarizing potential from the sensory nerve terminal of the frog IVth toe muscle during stretch, and has termed this the 'spindle potential'. Since the amplitude of the spindle potential changed almost in parallel with the muscle tension[1] and also with the rate of afferent discharges,[2,3] the spindle potential was considered to be equivalent to the receptor potential in other receptors by Ottoson and Shepherd.[4]

There are found, however, some situations in which the rate of afferent discharges is not always in parallel with the amplitude of the spindle potential (we would employ this term to distinguish it from a genuine receptor potential in the muscle spindle). When muscle spindle is stretched in Ringer solutions with abnormal ionic compositions or osmolalities, no spindle potential is observed or a reversed (hyperpolarizing) spindle potential is found while afferent discharges occur with the same pattern as in the normal Ringer solution.[5,6] It has been also demonstrated that the amplitude of the spindle potential is dependent upon the intensity of injury currents produced in extrafusal muscle fibers.[7] Consequently, Ito[8] has suggested that a part of the spindle potential may consist of an artificial potential.

The supposition on the artificial potential was criticized by Ottoson.[9] He believes that the artificial potential is undetectable in his preparation which is isolated completely from the extrafusal muscle fibers, in contrast to semi-isolated preparation used by Ito.[8] Ottoson[9] also points out a discrepancy in recording method. In experiments made by Ottoson[10] the axon was lifted up with an Ag-AgCl electrode into liquid paraffin layer which was floating above the Ringer solution containing the spindle receptor. In a paraffin gap method employed by Ito,[8] the axon was passed through a liquid paraffin embankment separating the spindle's pool of Ringer solution from the other Ringer solution. Differences in potential between the two pools, resulting from perfusing the spindle receptor and the axon respectively, were recorded through two Ringer-agar bridges immersed in the pools; each bridge was in turn connected to a calomel eletrode which led to a differential, high input-impedance amplifier.

Recently, Ito and Yokoyama[11] have compared the responses of the completely isolated spindles, which are identical with that made by Ottoson,[10] by means of the paraffin-gap method and the Ottoson's method. Figure 1 A and B show the results of Ottson's method. The relationship between the amplitude of the spindle potentials and the rate of afferent discharges during dynamic phases in stretches at different velocities was indistinct, and also an unexplained deflection occurred after relaxation of muscle spindle. The relationship between the amplitude and the discharge rate recorded by the paraffin-gap method was also non-linear as shown in Fig. 1 C, D and E.

The following two possibilities are considered for elucidating the non-linear relationship. (1) An external longitudinal resistance along the axon enclosed with paraffin may be

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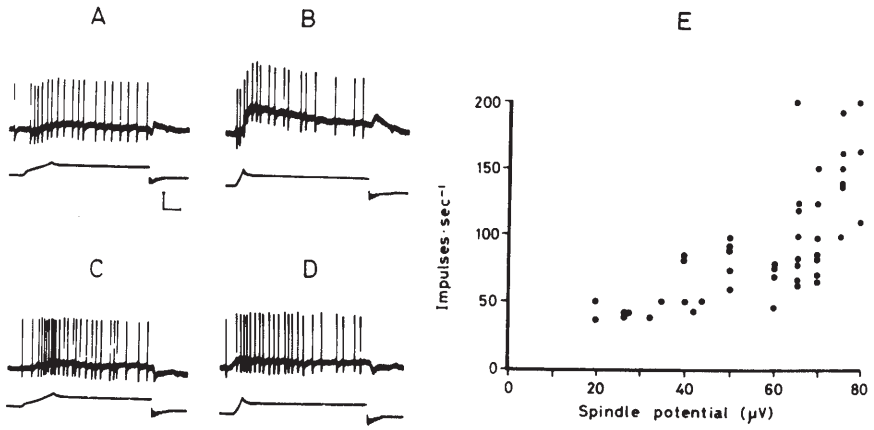

Fig. 1. Spindle potential and afferent discharges recorded from an isolated muscle spindle. A and B: Ottoson's method[10], in which an isolated axon was lifted up with an Ag-AgCl electrode into liquid paraffin over Ringer solution. C and D: paraffin gap method.[8] The records in B and D represent the terminal responses during stretch at a velocity of times faster than those in A and C. Upper traces: terminal responses for 0.5 mV calibration. Lower traces: tension for 0.3 g calibration. Time: 0.2 sec. E: Relationship between the amplitude of spindle potential (abscissa) and the rate of afferent discharges (ordinate) recorded by paraffin gap method. Each point represents the maximum amplitude and rate at the completion of stretches at various velocities from different initial muscle lengths.

changed in association with change in the length and thickness of a thin layer of Ringer solution intervening between the axon and the surrounding liquid paraffin during stretch of the muscle spindle. If an injury current flows constantly from the nerve terminal to the stump of the axon through the external longitudinal resistance, a change in potential difference across the resistance may be detectable during stretch. Although the discharge rate may be increased in parallel with enhancement of the amount of stretch, the amplitude of the spindle potential may be a function of the length and thickness of the thin layer of Ringer solution. (2) The spindle potential may be a secondary response elicited at the terminal nodes or branching nodes along the myelinated axon branches in the spindle capsule, in contrast with a genuine receptor potential which may be evoked at the terminals of the non-myelinated filaments. Though the amplitude of the genuine receptor potential is in parallel with the amplitude of stretch, the amplitude of the secondary potential may depend on a transfer function from the genuine receptor potential to it.

Hunt and Ottoson[12] employed Ottoson's method to record the terminal responses of the primary and secondary endings in the cat muscle spindle. After they established the parallel relation between the rate of discharges and the amplitude of the receptor potential, the frequency characteristics in transduce mechanism were analysed from the wave forms of the receptor potential in response to sinusoidal stretch.[13] They studied the effects of alteration of the ionic compositions of perfusing fluid on the receptor potential of primary endings in isolated cat spindles, and obtained the following conclusion[14]: the receptor potential is due to an increased conductance to Na^+ mainly and to Ca^{2+} in part and a hyperpolarization evoked after release from the stretch may be induced by an increase in the g_K/g_{Na} ratio. A similar conclusion is also obtained in a

study by means of voltage- and current-clamp techniques in crayfish stretch receptor cell by Brown *et al.*[15] These studies are in a line with Ottoson's theory that the receptor potential in the frog muscle spindle is due to an increase in conductances of Na^+ and some other ions.

2. *Structural and functional aspects of muscle spindles*

Individual motor units in mammalian muscles possess at least a muscle spindle.[16] The intrafusal bundle consists of 4 - 12 muscle fibers in which those of small diameter and short length (nuclear chain fibers) predominate over those that are thicker and longer (nuclear bag fibers). The total length of the bundle, as attained by the longest fibers, usually falls between 7 and 10 mm, and part of this length, generally the middle third, is ensheathed by a lamellated capsule.[17] As both poles of the bundle contact by a connective tissue on the surface of extrafusal muscle fibers,[18] the muscle spindle may play a role as a length detector of the attached region in the muscle rather than as that of the whole length of the motor unit. Primary endings terminate spirally on the sensory regions of nuclear bag and nuclear chain muscle fibers, but secondary endings make their terminals predominantly on the nuclear chain fibers.[17,19] Beta motor fibers, in which dynamic and static types are distinguished functionally and morphologically, innervate both the extra- and intra-fusal muscle fibers, while gamma motor fibers which are differentiated only functionally into dynamic and static innervate exclusively intrafusal muscle fibers.[20] In order to keep at constant the sensitivity of the spindle receptor during contractions of extra-fusal muscle fibers, the γ and β motor nerve fibers are often coactivated with α motor axons during rough and large movements (α - γ linkage[21]). During skilled and slow movements, however, dynamic and static γ or β fibers are activated independently without association with the α motor fibers.[22]

In amphibian skeletal muscles spindle receptors are independent of motor units. As all intrafusal muscle fibers continue from tendon to tendon (Ito, unpublished data), the muscle spindle may play a role as a detector for the whole length of muscle. A kind of afferent nerve fiber terminates on the sensory region along 2 - 7 intrafusal muscle fibers in which twitch and/or slow muscle fibers are contained. The different kinds of intrafusal and some extrafusal muscle fibers are innervated with the branches of different kinds of motor fibers respectively.[23] Matthews and Westbury[24] studied both the twitch and tonic motor innervation in the frog muscle spindle. Stimulation of the large motor fibers increased the overall response of the spindle afferent to ramp stretch while stimulation of the small motor axon increased specifically the sensitivity to the dynamic component of the stretch. These effects compared well with those respectively of static and dynamic fusimotor neurones in the cat. More detailed studies by Brown[25,26] confirmed and extended these observations. It is concluded that the amphibian muscle spindles may act always in α - γ linkage. In other words, amphibia may not have skilled movements but may provide only rough and large movements.

In the way of studies on the transduction and encoding mechanisms of muscle spindles, it is not necessary to consider the motor system; so, the mechanism may be more easily analysed in the more simple amphibian spindles than in those of mammals.

It has in general been thought that crustacean stretch receptors are useful for intracellular record of the receptor potentials,[27] because the receptor cell body is attached onto an intrafusal muscle fiber.[28] Even in the receptors, however, the site of transduction is suppose

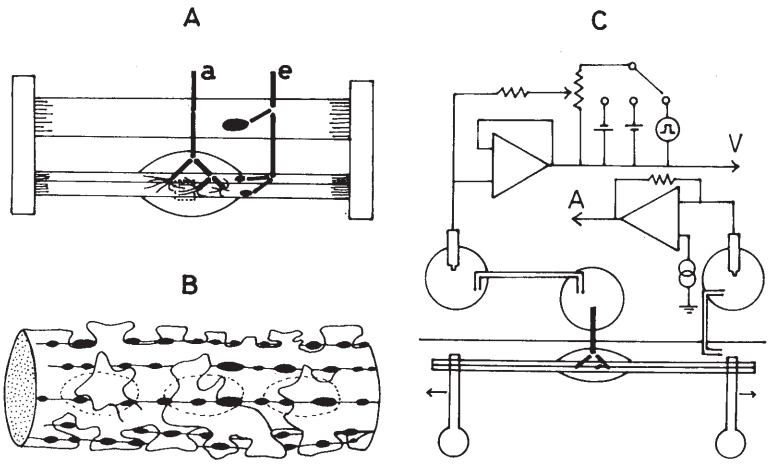

Fig. 2. Schematic diagrams of the morphology of frog muscle spindle (A and B) and the arrangement for recording the sensory terminal responses and for applying the polarizing currents, across an air-gap on which a myelinated segment of the axon was laid (C). B: Beaded chains of non-myelinated filaments on the sensory region of an intrafusal muscle fiber, as an enlargement of dotted frame in A (courtesy of Dr. Y. Uehara, personal communication).

to be located on the tip of the dendrites which are so thin and so long as to be impossible to study under voltage- or current-clamp conditions by means of micro-electrodes inserted into the soma.

We used simple type muscle spindles isolated from the frog sartorius muscles in our experiments. As is illustrated in Fig. 2 A, the axon is divided into two myelinated branches at a node in capsule; one of the branches further subdividing (Bs) and the other not (Bn) (cf. Ito *et al.*[29]). Individual terminals of the myelinated branches emit several non-myelinated filaments, each of which consists of a beaded chain of 100 - 200 μm in length.[30] The bead of 2 μm in diameter are connected with a fine thread of 0.2 μm in diameter (fig. 2 B). Those non-myelinated filaments are packed with a kind of mucopolysaccharide in an innercapsule.[31,32] Ito *et al.*[29] compared the terminal responses before and after one of the myelinated branches was cut by irradiation of the micro-beam of an argon-ion laser or one of the branch terminals was inactivated by irradiation of a far-ultraviolet light. We found that the afferent impulses initiate at the terminal of Bn. The inability to produce impulse at the terminals of Bs was supposed due to a cathodal depression which may be owing to addition of depolarization induced by an interaction between the terminals of subdivided branches, because of short length (30 - 50 μm) of the myelinated branches in Bs.[29] This supposition is supported by an experiment showing that the membrane potentials in the Bs terminals are significantly lower than those in the Bn. This is seen by means of a cyanine dye which changes fluorescent intensity depending upon the potential differences across the membrane bound with the dye.[33] Recently, Grossman *et al.*[34] have found that failure of propagation of action potentials by high frequency stimulation does not occur at the same time for each branch of the axon: conduction into the larger branch is blocked more easily. They suggest that the conduction failure into the daughter axons may arise from changes in intracellular ion concentration of Na^+ and Ca^{2+}, and also from extracellular accumulation of K^+.

Ito *et al.*[35] developed a new technique to record the responses of sensory terminals by an air-gap method. The sensory axon which innervates the isolated frog muscle spindle is also isolated for 1 - 2 mm near the spindle capsule; the motor axon is removed. Just outside the capsule the myelinated segment of the sensory axon was laid across an air-gap of 0.5 - 0.7 mm in distance between two pools of Ringer solution made on two glass plates (Fig. 2 C). The axon is cut within 1 mm proximal from the air-gap and is perfused by 117 mM KCl. Potential differences between the two pools are recorded through two Ringer-agar bridges immersed in the pools; each bridge is in turn connected to a calomel electrode which leads to a differential high input-impedance amplifier. Different strengths of constant current are applied onto the spindle axon terminal through the air-gap. The transgap current is monitored by a high input-impedance amplifier. The transgap resistance and resting potential range between 20 and 40 MΩ and between 10 and 20 mV respectively. These values are approximately 1/5th - 1/6th of those recorded from trunk axon by an air-gap method.[36] This could be owing to a leaky circuit in the recording system or in the nerve terminal, which may be due to the shorter internodal distance (400 - 600 μm) than in the trunk axon (1 mm).

The spindle is suspended between two stainless steel rods (diameter 1 mm), connected to the tip of the levers of a differential electromagnetic puller, which stretches the muscle symmetrically towards both poles at constant velocities (0.5 - 20 mm/sec). The procedure produces indiscernible movement of the axon during stretch, consequently artifical potential deflections should be minimized. During a ramp-and-hold stretch from the *in situ* length to its 110 % length, dynamic and static increases in the rate of spike discharges are observed (Fig. 3 A). After the afferent discharges are blocked by application of

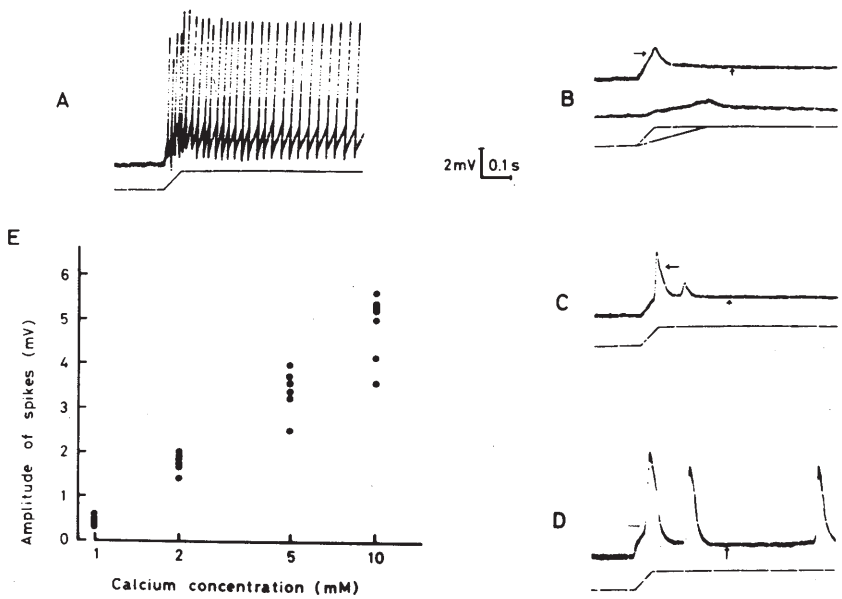

Fig. 3. Calcium spikes recorded from a muscle spindle terminal by air-gap method during superfusion with TTX-Ringer containing 1 (B), 5 (C) and 10 mM Ca^{2+} (D), in comparison with sodium spikes in normal Ringer (A). The axon response is shown in upper traces while the driving voltage for the stretcher is shown in lower traces. The amplitude of the calcium spikes depends upon the concentration of Ca^{2+} (6 examples in E).

10^{-7} g/ml tetrodotoxin (TTX), spindle potential is revealed alone during stretch (Fig. 3 B).

3. *Calcium spike*

Ito and Komatsu[37] found that calcium spikes, whose amplitude depends upon the external calcium concentration, are observed at the afferent nerve terminal of the frog muscle spindle during stretch in Ringer solution with TTX. Figure 3 shows calcium spikes recorded from a muscle spindle terminal by air-gap method during perfusion with TTX-Ringer solution containing 1 (B), 5 (C) and 10 mM Ca^{2+} (D), in comparison with sodium spikes in normal Ringer solution (A). The duration of the individual calcium spikes, which occurs in an all-or-none manner, varied between 12 and 60 msec from preparation to preparation, which was 10 to 50 times longer than that of sodium spikes. The amplitude of the calcium spikes depends upon the concentration of external calcium, as shown in Fig. 3 E. A 5 mV increase in the amplitude for a 10-fold elevation of $[Ca^{2+}]_0$ is approximately 1/5th - 1/6th of the value expected for a calcium electrode. This shift is probably due to the effects of a leaky circuit in the recording system or in the nerve terminal as described above. Considering the attenuation of the transgap potential and resistance, the dependence of the calcium spikes on $[Ca^{2+}]_0$ seems to be very close to that expected from calcium electrode behaviour.

The calcium spikes are reversibly blocked by addition of 5 mM $CoCl_2$ or $MnCl_2$ or of 15 mM $MgCl_2$, which have been known to block calcium channels.[38,39] With the addition of the experimental results showing that Sr^{2+} and Ba^{2+} are able to substitute for Ca^{2+} as current carriers in calcium spikes in muscle spindle as in many other nerve and muscle cells, it is concluded that calcium spikes are generated in the frog muscle spindle terminal.

Accumulation of a number of mitochondria in the beads along the non-myelinated filaments was found by Katz[30] and Karlsson *et al.*[32] They supposed that the intracellular calcium concentration in the filaments was kept low by the function of the mitochondria, resulting a large difference in the concentration across the membrane. When a calcium spike is evoked across the membrane, a large amplitude of the spike is expected. This is profitable as an amplifier device in transmission from transducing process to encoding. Katz and Miledi[40] have demonstrated that an efferent impulse propagating along the motor nerve fiber is provided by sodium inflow while a calcium spike at the nerve terminal plays an important role (as an amplifier) for release of transmitter from it.

There are 150 to 200 Rohon-Beard cells in the spinal cord of *Xenopus* embryos. Anatomical and physiological studies suggest that they are primary sensory neurons.[41,42] The majority of them develop during gastrulation[43,44] and disappear early in larval life.[42] The cells are initially inexcitable.[45] When the action potential can be elicited, at early stages it depends on the inward movement of calcium ions and is several hundred milliseconds in duration.[46] Later in development a sodium component of the action potential appears, and the calcium component ultimately disappears.[47] During the development mitochondria and Golgi apparatus become progressively more localized to the center of the cells. The mitochondria contain dense intra-mitochondrial granules which are known in other cells to contain concentrations of divalent cations.[48] The dense intra-mitochondrial granules, an indication of calcium accumulation in mitochindria, decrease in parallel with the loss of the Ca^{2+} component of the inward current of the action potential in Rohon-Beard neurons.[44] Since Ca ions act as charge carriers during excitation even in dorsal root ganglion cells of the adult mouse,[49] it seems likely that a part of the Rohon-Beard neurons

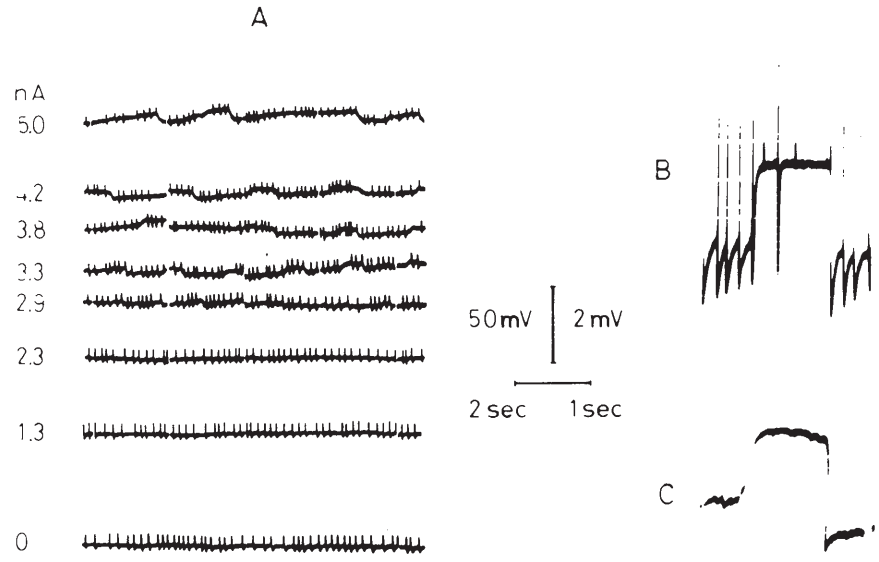

Fig. 4. Rectangular fluctuations in transgap potential during application of depolarizing currents on isolated muscle spindle. A, responses of a spindle at different strengths of depolarization which can be read as upward displacement from the base line for 0 current record (left side numbers), using calibration of 50 mV. B and C, single cycles of the rectangular fluctuations recorded from another spindle before (B) and after (C) treatment of Na^+-free Ringer solution. Small spikes in B are abortive spike.

may innervate the muscle spindle.

4. *Oscillation of potential at the encoding membrane*

Ito and Komatsu[50] have observed rectangular oscillations of the transgap potential during application of 1 - 5 nA depolarizing currents (Fig. 4 A). At the threshold strength of depolarizing current, the oscillation of 0.5 - 1.5 mV in amplitude occurs at 0.5 - 1.6 Hz in frequency. Increasing the strength, decreasing the frequency and increasing the amplitude. The oscillation disappears with depolarizing currents of 6 nA or more.

The decrease in the transgap potential without any changes in current represents a decay in transgap resistance, being calculated to be approximately 1 MΩ in this case. This implies an increase in membrane conductance during the lower level of the potential at the sensory nerve terminal.

As the rectangular oscillation can be elicited in TTX treatment or in Na^+ - free (choline) Ringer solution (Fig. 4 C), it is independent of changes in Na^+ permeability. The rapid potential decay in the oscillation often synchronized with the after-hyperpolarization following individual afferent impulses (Fig. 4 B). Since the after-hyperpolarization has been known to be due to increase in potassium permeability, it seems likely that the low potential level in the oscillation may be induced by the increase in potassium permeability. Potassium channel blockers, as 4-aminopyridine, cesium chloride and tetraethylammonium chloride (TEA), block reversibly the oscillation.

Similar oscillations in potential have been observed in the β-cell membrane of Langerhans

in mouse pancreas.[52,53] Atwater *et al.*[54,55] suggested that Ca^{2+} influx during the spike potential induced by application of glucose to the membrane might stimulate K-permeability and thus limit further Ca^{2+} influx. This $[Ca^{2+}]_i$-activated P_k is proposed to be present in many kinds of cells,[56] while potential-dependent P_k is also found in many other cells.[57] It is of interesting that the $[Ca^{2+}]_i$-activated P_k is provided in spontaneously activated cells as Helix bursting pace-maker neuron[58,59] or cardiac Purkinje cell.[60] Since calcium spikes were recorded from the sensory terminal of the frog muscle spindle by Ito and Komatsu,[37] the existence of the $[Ca^{2+}]_i$-activated P_k in the spindle terminal membrane was anticipated. This possibility was verified by Ito *et al.*[49] who demonstrated that the rectangular oscillations in the spindle nerve terminal could be blocked reversibly by quinine, which has been known to be a selective blocker of the $[Ca^{2+}]_i$-activated P_k in red blood cell[61] and in pancreatic β-cell.[54]

5. *Genuine receptor potential*

We propose a model that the ratio of the density of Ca channels against that of Na channels increases from the nodes of the myelinated branches to the terminals of the non-myelinated filaments. Unlike the squid giant axon membrane in which calcium ion may inflow through sodium channels,[62,63] the calcium and sodium ions may pass through separate channels in the spindle nerve terminal, which is in conformity with the crustacean X organ cells[64] or in the other neurons.[65] It seems possible that all of the calcium channels may act to mechanical transduction of the membrane and also to electrical excitation, as has been assumed to be so in premature cells such as the Rohon-Beard neuron. However, it, is not impossible that all of calcium channels may cause only electrically active, if the following assumptions are made.

(1) A kind of mucopolysaccharide (MPS) in the inner-capsule[31,32,66] may play a role in the transduction mechanism, because the rate of afferent discharges can be easily altered by chemical modification of the MPS (Fujitsuka, in preparation). This would be like the piezo-electric effect of the MPS hypothesized to provide a mechano-electric transduction in the cupulla of the inner-ear hair.[67,68,69] (2) By analogy to auditory reception in the vertebrate inner-ear or on photo-reception in a compound eye,[70] intrafusal muscle fibers in the frog muscle spindle may be presumed as an accessory cell in the mechano-electric transduction system. When the resistance across the contact region (a ball-and-socket region)[30] between the surface of the intrafusal muscle fibers and the non-myelinated filaments is decreased during stretch of the spindle, the filaments may be stimulated electrically by a current from a source of the intrafusal muscle fibers. The resting potential of the fialment has to be lower than that of the intrafusal muscle fiber. Evidence in support of this analysis is the electrical coupling between the intrafusal muscle fiber and the sensory nerve terminal found by Ito *et al.*[71]

et al.[71]

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