Synaptic Morphology and Differences in Sensitivity

Abstract. *A relation between synaptic morphology and physiology was observed in an in vitro preparation of a sense organ (the ampulla of Lorenzini), in which activity was monitored from the primary afferent neurons before electron microscopic examination of the afferent synapses. The depth of the postsynaptic trough decreased as prefixation sensitivity of the sense organ decreased. This relation and other ultrastructural differences suggest that physiological properties of synapses are influenced by morphological features. Thus, synapses might be morphologically dynamic to modulate synaptic efficacy in relatively long-term phenomena.*

The relation between structure and function is of particular interest in the nervous system because morphological plasticity could account for long-term changes in behavior such as learning, memory, sensitization, and habituation. We have observed that the morphology of afferent synapses in the sense cells of the ampullae of Lorenzini is related to the sensitivity of the organ to stimulation in vitro. Furthermore, the sensitivity and rate of spontaneous activity fluctuated during the recording periods. These results suggest that these synapses could be structurally and functionally plastic and that these properties may be interrelated to modulate the efficacy of transmitter release.

The ampullae of Lorenzini are tubular structures as long as several centimeters in some animals, which open through several hundred pores on the head of sharks, rays, and chimaeras (*1*). The proximal alveolus-shaped ending of each organ contains several thousand electrosensory cells that synapse with axons of afferent neurons in the anterior lateral line nerve. These sense cells respond to slight differences in electric potential, which are conducted through the canal, to modulate the release of transmitter from ribbon synapses with the six to ten afferent fibers leaving each organ (*2*). The afferent nerves are normally tonically active, such that a cathodal stimulus at the opening of the canal is excitatory. There are no known efferent synapses or other integrating connections in the sensory epithelium. The normal stimuli for these sensory receptors are the numerous weak electric fields associated with various bioelectric and electrophysical processes in seawater. These animals use the sensory modality to detect other organisms such as food and conspecifics, and possibly for orientation by sensing the geophysically induced electric field generated by motion through the earth's magnetic field (*3*).

Individual organs from the thornback ray *Platyrhinoides triseriata* were dissected free with a portion of the afferent nerve and canal intact and placed in a Plexiglas recording chamber similar to that used in the current-clamp studies by Clusin and Bennett (*4*). The ampullar bulb and the opening of the canal were placed in separate wells containing elasmobranch Ringer solution. Silver–silver chloride electrodes in each well were used to pass a weak constant current through the canal to excite the electrosensory cells in the ampullar bulb. The rate of spontaneous activity and responses of the organ to stimulation were monitored from axons of the afferent neurons (*5*). Threshold sensitivities were determined from single or multiunit responses of the afferent neurons to 1-Hz square-wave stimulation of various intensities.

The rate of spontaneous activity and threshold sensitivity varied considerably among different units. In addition, individual organs often fluctuated in the degree of sensitivity and rate of spontaneous activity during longer recording periods (of up to 1½ hours) (Fig. 1B). Increasing, decreasing, stable, and oscillatory changes in these physiological characteristics occurred. The dynamics of this fluctuation differed among units and could not be controlled experimentally through stimulus regimes that modulate sensitivity in many other sensory systems. Thus, these changes in sensitivity seem related to some process other than accommodation, adaptation, or simple degradation in vitro (*6*).

Synapses (n = 262) from 30 organs were examined after electrophysiological recording and fixation in vitro (*7*). Samples were coded during all stages of processing and analysis to control for observational bias, and they were processed for transmission electron microscopic examination (*8*).

Figure 1A shows the morphology of the synapse, which contains a prominent presynaptic ribbon with numerous synaptic vesicles adhering to it (*9*). The presynaptic membrane protrudes into the postsynaptic membrane, forming a synaptic trough of variable depth. Densi-

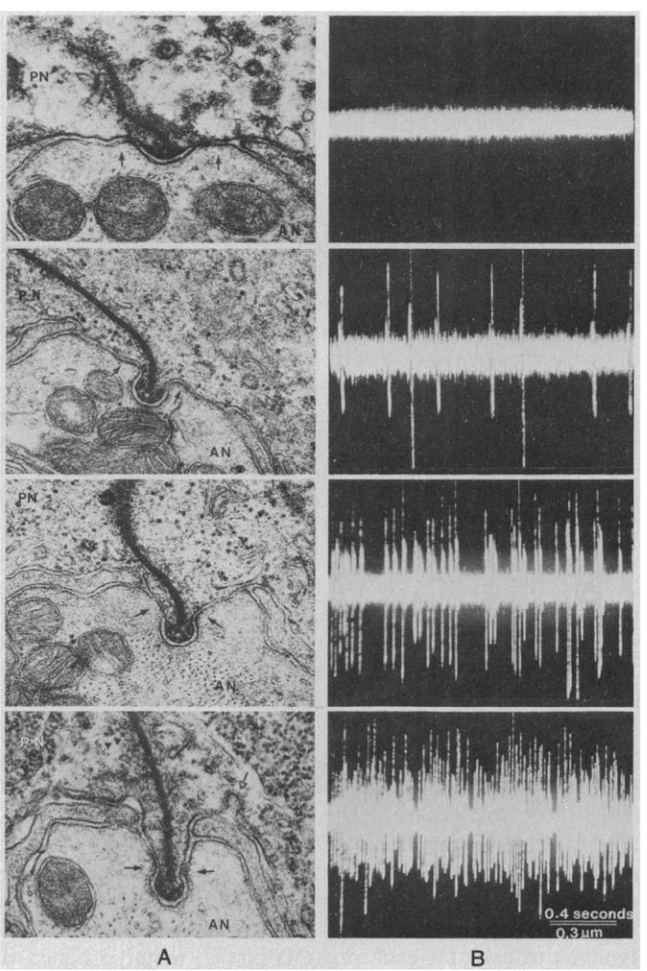

Fig. 1. The morphology of the ribbon synapses in the ampullae of Lorenzini correlates with the spontaneous activity and sensitivity of the organ measured at the time of fixation. (A) The sense cells (*PN*) contain synapses with a presynaptic ribbon with numerous synaptic vesicles adhering to it (double arrowheads), which protrudes into the postsynaptic membrane of the afferent neuron (*AN*) to form a trough of variable depth. The active zone is marked by solid arrows at the postsynaptic membrane. A coated vesicle is indicated by an open arrow at the presynaptic membrane. (B) Record of one preparation, showing the changes in spontaneous activity observed in the primary afferent neurons during 100 minutes of recording. Bar, 0.4 seconds and 0.3 µm.

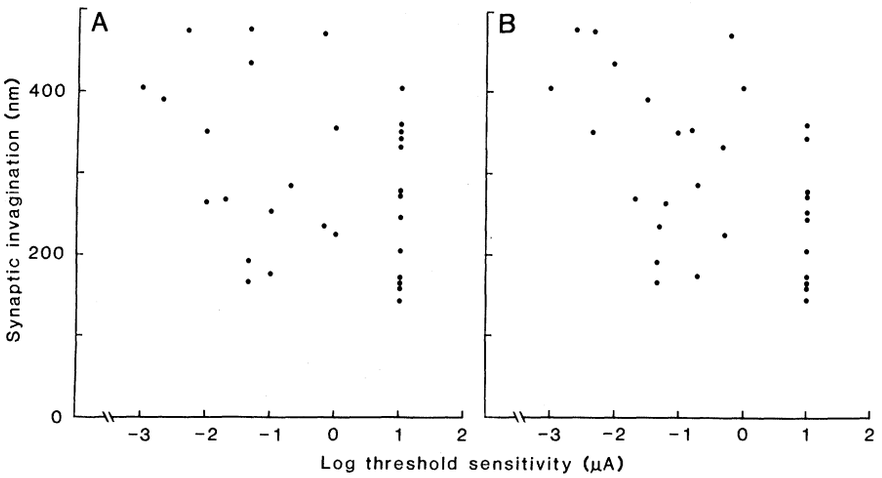

Fig. 2. The depth of the synaptic invagination plotted as a function of the $\log_{10}$ of threshold sensitivity. (A) The relationship is not significant when the morphology is correlated with measurements of sensitivity made at the start of the recording period ($\tau = -0.252$, $n = 30$). (B) A significant relationship is apparent when the morphology and sensitivity are measured at the time of fixation ($\tau = -0.357$, $P < 0.01$, $n = 30$). Each point represents the mean depth of synapses in a single organ. Units that did not respond to stimulation are arbitrarily plotted at 10 μA, which was 10 times the maximum stimulus used in these experiments. The scatter is greater for the less sensitive units (>0.5 μA), presumably because factors other than the depth of the synapse diminished measurements of the sensitivity of these organs.

fications of the postsynaptic membrane proximal to the end of the ribbon mark the active zone, which is believed to be the site of vesicular exocytosis (*10*). Support cells surround the afferent neuron and extend interposing processes between each sense cell and the afferent neuron. Coated vesicles are frequently observed in formation on the presynaptic membrane at the borders of the synaptic evagination.

The shape of this synaptic complex varied considerably in the width and depth of the synaptic evagination and in the relative positions of the active zones and the support cell processes (*11*) (Fig. 1A). The depth of the synaptic evagination was chosen as an objective measure of these morphological differences, and measurements were made from 20 by 25 cm prints at ×82,500.

As a preliminary analysis, the data on the depth of the synaptic troughs were arbitrarily subdivided into four categories on the basis of threshold before fixation (10^{-3} to 10^{-2}, 10^{-2} to 10^{-1}, 10^{-1} to 1, and >1 μA) and subjected to a nested two-way analysis of variance. These results demonstrate homogeneity in the depth of synaptic troughs among synapses from a given organ and statistically significant differences between organs of the four threshold categories (*12*). Thus, the cause of the variation must be a phenomenon affecting the organ rather than individual sense cells.

Part of this variation is clarified by Fig. 2B, in which the mean depth of synaptic troughs for each organ is plotted against prefixation threshold, and which shows a statistically significant trend toward shallower synaptic troughs at higher thresholds (Kendall rank correlation coefficient, which is insensitive to the arbitrary categorization of all data for threshold values greater than 1 μA). Those organs that were completely insensitive to stimulation and often lacked spontaneous activity tended to have synapses that were only minimally evaginated.

This relation between morphology and sensitivity was significant even though the sensitivity of many organs had changed during the recording periods (*13*). However, the relation was less evident if morphological and physiological measurements were not made simultaneously. For example, there was no significant correlation between the threshold sensitivity measured at the start of the recording periods and the synaptic morphology of organs fixed at the end of in vitro recording, suggesting that morphology and physiology may vary concurrently (Fig. 2A). Other explanations could also account for these data, such as covariation in respect to other unmeasured variables, or the masking effects of other transitory phenomena. These and any other interpretations of these correlations must be tested directly in other experiments.

The mechanisms responsible for these physiological changes and possible morphological changes need to be investigated further. At least two mechanisms might produce dynamic morphological and physiological effects. (i) The morphology of the synapse could be directly modulated by contractile proteins of the cytoskeleton to change the shape of the synaptic complex, and thus (by a number of factors) the overall sensitivity of the synapse. (ii) The morphology of the synapse might be influenced by the rate at which presynaptic membrane is added through exocytosis of synaptic vesicles and sequestered by endocytosis of coated vesicles (*14*). Thus synaptic morphology and synaptic function may be changed as a direct result of the processes that maintain the homeostasis of presynaptic membrane recycling. Particular types of stimulation might outstrip processes that maintain this homeostasis, resulting in a feedback mechanism to regulate the sensitivity of the synapse.

Sustained periods of oscillation in spontaneous rate and sensitivity have been reported in other sensory systems after sudden intense stimulation (*15*). The ampullae of Lorenzini respond to strong mechanical stimulation (*16*) and might have received different amounts of intense stimulation in these experiments as a result of the dissection and in vitro procedures. Such oscillations could result when electroreceptors are subjected to strong electrical or mechanical stimulation in nature. If these differences in synaptic morphology reflect a dynamic process of functional and morphological modulation, this could be one regulatory mechanism to affect adjustments in sensitivity for optimal sensory reception under differing conditions.

These observations provide evidence that synapses might be structurally as well as functionally plastic and that these two properties may be interrelated to modulate the efficacy of transmitter release. The ribbon synapse in the ampulla of Lorenzini is an elaborate structure, which makes structural deviations more apparent. Similar processes might also occur in other synapses in which systematic changes in morphology are more difficult to detect. Since the precise operation of a synapse depends on an intricate structural mechanism for the packaging, transport, and metered release of neurotransmitter chemicals into the synaptic cleft, it is not unreasonable to expect alterations in synaptic structure to affect the physiological properties of synaptic transmission or that modifications in synaptic morphology may occur to modulate the physiology of transmitter release. A redistribution of organelles and membrane proteins active in transmitter release might be important in modulating synaptic function on time

scales of several minutes or longer and thus might participate in such processes as habituation, sensitization, and long-term memory.

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5. Bipolar silver hook or suction electrodes connected to a high-impedance A-C–coupled preamplifier were used to monitor responses from axons of the afferent neurons. The temperature was held near 10°C with a solid-state cooling device, and oxygen was bubbled into the well containing the sensory epithelium.
6. These changes were not significantly associated with postmortem time, recording time, the animal, the date of the experiment, or minor differences in the size organs (analysis of variance).
7. Tissue was fixed in the recording chamber, placed in cold fixative for several hours, and fixed again with 1 percent osmium tetroxide. The recording chamber was thoroughly washed, soaked in 5 percent lysine to quench reactivity of any remaining aldehydes, and rinsed in distilled water and buffer before reuse.
8. After secondary fixation for 1 hour in buffered 1 percent osmium tetroxide, tissue was washed in cacodylate buffer (*p*H 7.3), dehydrated in a graded series of ethanol and acetone, and embedded in epoxy resin. Sections were stained with uranyl acetate and lead citrate. Fixative contained 2 percent paraformaldehyde and 2.5 percent glutaraldehyde in 0.15*M* cacodylate buffer (*p*H 7.3), with 4 percent sucrose; osmolality = 1150 mOsm. Ringer solution contained the following: NaCl, 300 m*M*; $CaCl_2$, 2 m*M*; KCl, 4 m*M*; $MgCl_2$, 2 m*M*; $NaCHO_3$, 2.5 m*M*; urea, 315 m*M*; trimethylamine oxide (Sigma), 76 m*M*; Hepes buffer, 7.5 m*M* (*p*H 7.3); and glucose, 5 m*M*.
9. Ribbon synapses also occur in the retina [F. S. Sjostrand, *J. Ultrastruct. Res.* **2**, 22 (1958); J. E. Dowling, *Proc. R. Soc. (London) Ser. B.* **170**, 205 (1968)], the pineal body [J. A. McNulty, *Cell Tissue Res.* **215**, 491 (1981)], and electroreceptors of teleosts [A. W. Wachtel and R. B. Szamier, *J. Morphol.* **119**, 51 (1966)].
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11. Multiple sections of the same organ in different planes, and serial sectioning and reconstruction show that the synaptic morphology is bilaterally symmetrical, and the differences in morphology are not an artifact of the orientation or location of sectioning.
12. Mean squares of the depth of synaptic troughs among groups (organs of different thresholds) = 2527 nm (d.f. = 3), $P < 0.01$; among subgroups (among sense organs) = 400.8 (d.f. = 26), $P << 0.001$; within subgroups (within sense organs) = 59.5 (d.f. = 232). The mean depths of synaptic troughs were 429, 272, 265, and 238 nm for groups of organs classed according to thresholds of 0.001 to 0.01, 0.01 to 0.1, 0.1 to 1, and >1 μA.
13. The depth of the synaptic trough did not differ significantly among organs that were declining in sensitivity, increasing in sensitivity, or showed no change in sensitivity in the period prior to fixation (Bonferroni *t*-test for multiple comparisons).
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Dynamic Modification of the Vestibulo-Ocular Reflex by the Nodulus and Uvula

Abstract. *The time constant of the decay of slow-phase eye velocity of postrotatory nystagmus or optokinetic after-nystagmus is reduced during exposure to a stationary visual surround (visual suppression). It is also reduced after tilting the head (tilt suppression). A "dump" mechanism in the vestibulo-ocular reflex has been proposed to rapidly discharge activity from the central vestibular system during both types of suppression. Monkeys lost this mechanism after lesions of the nodulus and uvula. They also lost the ability to habituate the time constant of nystagmus on repeated exposure to optokinetic and vestibular stimuli. Periodic alternating nystagmus, which is believed to represent an instability in the vestibulo-ocular reflex, was observed in two of three monkeys. These data indicate that the nodulus and uvula play an important role in suppressing, habituating, and stabilizing the vestibulo-ocular reflex.*

Rotation of the head causes activation of the vestibulo-ocular reflex (VOR), producing compensatory eye movements that maintain gaze. If the visual surround moves with the head, signals from the VOR responsible for compensatory eye movement must be suppressed if images are to remain stable on the retina. The visual system accomplishes this by several processes. One, involving the flocculus, directly opposes activity from the VOR (*1*). This mechanism is responsible for the rapid rise in slow-phase velocity during optokinetic nystagmus (OKN) (*2*) and is utilized in mediating ocular pursuit (*1, 3*). Another process shortens the dominant time constant of the VOR by quickly discharging or "dumping" activity stored in the vestibular system, thereby reducing residual eye velocity (*4*). Specific brain structures that are associated with the dumping process have not been identified.

Eye velocity can be suppressed during nystagmus in other ways that do not involve the visual system. If the head is tilted during postrotatory nystagmus, eye velocity decays rapidly to zero (*5, 6*). Since tilting probably does not modify the time constant of activity coming from the semicircular canals, the shortened time constant is not likely to be due to a change in the peripheral signal that drives the system. Instead there must have been a discharge or "dump" of activity stored in the VOR (*4*). Consistent with this is a rapid decay in slow phase eye velocity of optokinetic after-nystagmus (OKAN) after tilting (*6, 7*). We have proposed that the same dump mechanism that rapidly alters the time constant of the VOR during visual suppression (*2, 4*) is used by the vestibular system to discharge stored activity in response to head tilt (*6*). We now provide evidence in support of this idea and show that the dynamic modification of the VOR time constant is mediated through the nodulus and uvula of the vestibulocerebellum.

Eye movements of three rhesus monkeys (*Macaca mulatta*) and one nemestrina monkey (*M. nemestrina*) were recorded with electrooculography (EOG). Animals were tested in a three-axis vestibular and optokinetic stimulator to establish baseline values for horizontal and vertical per- and postrotatory nystagmus, OKN, and OKAN (*2, 4, 6, 8*). The nodulus and uvula were removed by suction-ablation under anesthesia. Animals were tested for periods of 2 to 3 months, and the extent of lesions was determined in three animals after their deaths. In each monkey the nodulus and ventral uvula were destroyed. The fastigial nucleus was invaded on the left side in one animal, but the other roof nuclei were intact.

OKN, OKAN, and per- and postrota-