QUANTAL NATURE OF TRANSMISSION AT THE SYNAPSE BETWEEN HAIR CELLS AND EIGHTH NERVE FIBERS

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Summary 1. An intracellular recording technique was used to record excitatory postsynaptic potentials (EPSP's) from the eighth nerve fibers of goldfish. EPSP's were evoked by applying sound stimulus, and spontaneous miniature EPSP's were also observed.
2. Even when the stimulus sound was kept at a fixed intensity level, the EPSP amplitude showed some fluctuations including occasional failures of the response.
3. The applicability of Poisson's law was tested for several intensity levels of sound stimulus in the same fibers. The Poisson relation was found applicable to the amplitude distribution of EPSP's evoked by a weak sound, but some discrepancies were found for a strong sound.
4. It was concluded that the transmission between hair cells and primary eighth nerve fibers of goldfish occurred in quantal steps. Some theoretical implications of the present results were discussed.

It has been shown in previous reports (FURUKAWA and ISHII, 1967) that the excitatory transmission from hair cells to certain eighth nerve fibers in the goldfish sacculus takes place with mechanisms largely similar to those in other well known chemical synapses (KATZ, 1966). For example, the presence of a definite synaptic delay and the occurrence of spontaneous miniature EPSP's have been demonstrated. Further, EPSP's evoked by a repetitive application of the same sound stimulus often show some fluctuations in their size. Especially when the EPSP's are small in size, the fluctuation may become so remarkable that it is reminiscent of the quantal fluctuation of postsynaptic potentials as observed in other structures (MARTIN, 1965).

In the present paper, we examined the possibility of applying the Poisson analysis to the present case. The condition under which we observed the EPSP fluctuation, however, was a little different from that commonly used. EPSP's

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were reduced in size in the present case by applying a very weak sound, while the more usual procedure is to reduce the quantal content of the transmitter release by reducing the Ca^{2+} content of the medium or by elevating its Mg^{2+} content.

METHODS

Experiments were carried out on live and immobilized goldfish about 12 cm in size. Nerve fibers from the sacculus, *i.e.*, the inner ear of the goldfish, consist of two groups of fibers of different diameters. All the experiments in the present study were carried out on the large fiber group, *i.e.*, our SI fibers ($d: 15 \mu$). Potentials were recorded intracellularly with a glass micropipette electrode and a reference electrode of chlorinated silver wire placed in contact with brain tissue. Micropipettes were filled with 3 M KCl and their tip diameter was 1μ or less. As a sound stimulus, tone pips were delivered through a loudspeaker placed about 30 cm from the fish. Sound intensity was varied with two attenuators, one of which had 5 dB steps and the other 1 dB steps. The absolute level of sound intensity was checked with a condenser microphone and expressed in reference to the sound pressure level (SPL). Tone pips had a duration of 10–20 msec and were usually applied at a rate of two times per sec. Experiments were carried out at room temperature. Experimental methods including operation and other recording procedures have been reported elsewhere (FURUKAWA and ISHII, 1967).

RESULTS

1. *Fluctuations of the EPSP size*

Figure 1 shows an example in which EPSP's to the same sound stimulus showed a very marked fluctuation. The fluctuation was especially marked in the EPSP's that were evoked in response to the first compression phase of the stimulus sound. This fiber is obviously responding to both compression and rarefaction phases of the sound, but responses to the former are much greater than those to the latter. The response to the second compression phase of the sound developed into a spike and was much greater than other responses. As will be shown in Figs. 2 and 3, fluctuation in the EPSP size were generally more marked for a weak stimulus than for a strong one. In the case of Fig. 1 too, fluctuation in size of the first EPSP's is much more marked perhaps because the stimulus intensity for them is weaker than for latter ones. Figure 2 shows two cases in which fluctuation of EPSP size was examined for two different sound intensities. The fiber shown in A is responding only to the compression phases of the sound (see A3). As shown in A1, a random discharge of spontaneous miniature EPSP's was observed in this fiber. Responses to a weak stimulus sound, shown in A2, were very irregular, but the randomness was much less marked in A3 where the sound

Fig. 1. Sound-evoked EPSP's recorded intracellularly from an eighth nerve fiber. Note that the fluctuation in size is much more marked in the first EPSP's than the rest of them. Upward deflections in the sound monitor (lower trace) indicate compression phases of the sound. Stimulus: 300 Hz and 85 dB.

Fig. 2. Spontaneous and evoked EPSP's in two fibers. Discharge of spontaneous miniature EPSP's was very frequent in the fiber A (see A1), but was almost nonexistent in the fiber B. Stimulus sound: 300 Hz, 78 and 83 dB in A2 and A3; 270 Hz, 78 and 83 dB in B2 and B3, respectively.

intensity was increased by 5 dB. Despite their much larger size, EPSP's in A3 have a short duration, their half width being less than 1 msec, indicating that the release of the transmitter packet quanta took place with better synchronization in A3 than in A2 (FURUKAWA *et al.*, in preparation). Figure 2B shows an instance in which the discharge of miniature EPSP's was so infrequent that they could not be recorded at all (see B1). This fiber was also responding to the compression as well as to the rarefaction phases of the sound. B2 and B3 show that there were very marked fluctuations in the EPSP size in this fiber.

2. Statistical analysis of the fluctuation in the EPSP size

When the release of the transmitter at a neuromuscular junction is suppressed by adding Mg^{2+} to the medium and/or by removing Ca^{2+} from it, the amplitude of the end-plate potential shows a marked fluctuation. The distribution of EPSP size at such a junction has been shown to obey Poisson's law, indicating that there are large numbers of contributing units at the junction, each with a small probability of response to nerve impulses (KATZ, 1966; MARTIN, 1965). Since, as is shown in Figs. 1 and 2, there were marked fluctuations in the size of EPSP's in the present material, some efforts were made to see whether a similar type of statistical analysis could be applied to describe the phenomena. However, our analysis has been very limited in extent due to the very infrequent occurrence of spontaneous miniature EPSP's and also due to technical difficulties in obtaining stable intracellular records. In the present investigation, we tried to calculate the distributions of EPSP size which would be expected from the statistical theory and tried to compare them with those observed (KATZ, 1966; MARTIN, 1965).

There are several different methods for calculating the mean quantum content m , *i.e.*, the average number of the quanta released per stimulus during a series of trials. In cases where miniature EPSP's could be recorded, m may be obtained by dividing the average amplitude of the EPSP's in the series by the average amplitude of spontaneous EPSP's, namely

$$m = \frac{\text{mean amplitude of EPSP responses } (v)}{\text{mean amplitude of spontaneous potentials } (v_1)} \quad (1)$$

Using m thus obtained, the expected number of failures, single unit responses and multiple responses could be calculated respectively based on the Poisson equation.

Figure 3 shows responses of a fiber on which the statistical analysis was carried out. Miniature EPSP's shown in A have a typical time course with a duration of 0.6–0.8 msec as measured at half their height (see also Fig. 2 A1). The histogram shown in Fig. 4D illustrates the size distribution of these spontaneous potentials. The mean amplitude of these spontaneous potentials, *i.e.*, the mean quantum size v_1 , was 0.43 mV and its standard deviation

Fig. 3. Sample responses of a S1 fiber. A: Spontaneous miniature EPSP's. B: Spike train evoked by a strong sound at 500 Hz and 90 dB. C: Responses to weak sounds at 200 Hz and 83, 85, and 90 dB for C1 to C3, respectively.

was 0.11 mV. Figure 3B shows the response of this fiber to a strong sound at 500 Hz. Although EPSP's and spike potentials were set up in response to each compression phase of the sound, small EPSP's were evoked also in response to rarefaction phases. Therefore, this fiber responds more strongly to compression phases but also responds weakly to rarefaction phases. This is also observed in records of C1-C3 in which weak sounds at 200 Hz were applied. The sound intensity was 83 dB SPL in C1 and was increased by 2 and 7 dB in C2 and C3, respectively. The statistical analysis was performed on the first responses that corresponded to the initial transient-like deflection in the sound monitor. As can be seen in C1, there was a considerable fluctuation not only in the response size but also in the time span of the response. The latter factor made it difficult

Fig. 4. A, B, and C: A comparison of expected distribution of EPSP size with those observed. Histograms show observed amplitude distributions of the first EPSP's for different intensities of sound. Sample records for A, B, and C are shown in Fig. 3 C1, C2 and C3, respectively. Smooth curves connecting open circles are expected amplitude distributions calculated for m of 1.04, 2.25 and 3.07 for A, B, and C, respectively. v_1 and σ were 0.43 and 0.11 mV, respectively. D: Amplitude distribution of spontaneous miniature EPSP's shown in Fig. 3A.

to decide whether a given deflection might represent a true response or not. As a compromise, the response size was measured between two arrows shown in Fig. 3 C1. The histogram shown in Fig. 4A illustrates results of 78 measurements. The mean quantum content m , being calculated from the equation (1), was found to be 1.04. Then the expected number of failures, single unit responses, double unit responses, and so forth were calculated from the Poisson theorem:

$$n_x = \frac{Nm^x e^{-m}}{x!} \quad (2)$$

where $N = 78$, $m = 1.04$, and $x = 0, 1, 2, \dots$. Next, the expected distributions of n_1, n_2 , and so forth were plotted. Namely, the distribution of n_1 was drawn around the mean of v_1 (i.e., 0.43 mV) with a variance of σ^2 , that of n_2 around the mean of $2 v_1$ with the variance of $2 \sigma^2$, and so forth. The sum of these individual curves would constitute the expected distribution. This is shown in Fig. 4A with the line connecting open circles. The expected number of failures, n_0 , was 28.5, a little greater than the actual value of 22. Also the expected distribution curve is in fairly good agreement with the histogram experimentally obtained. Results of similar analysis applied to the experiments of Fig. 3 C2 and C3 are shown in Fig. 4B and C, respectively. In these two cases, the calculated distribution did not fit the observation. The mean quantum content was estimated too low in both of them. This seems to indicate that the Poisson theorem has only a limited applicability to the present case (*cf.* Fig. 6), although the discrepancies may be partly attributable to a temporal dispersion and non-linear summation of unit EPSP's, *etc.* (KUNO and MIYAHARA, 1969).

A different approach was employed for the analysis of the experiment shown in Fig. 2B. In this case the occurrence of spontaneous EPSP's was so infrequent that it was practically impossible to find the mean quantum content from the equation (1). Therefore, the mean quantum content was calculated with the following equation:

$$m = \log_e \frac{\text{number of stimulus (N)}}{\text{number of failures of EPSP responses } (n_0)} \quad (3)$$

Fig. 5. Amplitude distribution of evoked EPSP's calculated using m derived from the number of failures. m : 1.34 mV; v_1 : 0.89 mV; σ : 0.22 mV. See text for further details.

Fig. 6. Another instance in which amplitude distributions were calculated using m 's derived from the number of failures. v_1 : 0.60 mV; σ : 0.20 mV.

Using the values of m thus obtained, the mean quantum size v_1 was obtained from the relation:

$$v_1 = \frac{v}{m}$$

As in the previous example, the expected figures for n_1 , n_2 , n_3 , and so forth were calculated using equation (2). However, the standard deviation of the miniature EPSP's σ is not known. Therefore, in drawing the Gaussian curve for n_1 , the variance σ^2 was obtained by a trial and error method. The Gaussian curves for the second and the third groups were then drawn about the mean of v_2 and v_3 , using $2\sigma^2$, and $3\sigma^2$ as their variances. The theoretical distribution curve which was obtained by adding four Gaussian curves together fits again fairly well with the histogram obtained experimentally shown in Fig. 5. In this case, the mean quantum content m , the quantum size v_1 , and the standard deviation σ were 1.34, 0.89, and 0.22 mV, respectively. The stimulus sound was 300 Hz at 75 dB SPL.

Figure 6 shows the same type of analysis employed in Fig. 5. The stimulus sound at 300 Hz was varied in its intensity from 80 to 82 dB by 1 dB steps. As expected, EPSP's increased in size with an increase in the sound intensity. Mean quantum content calculated separately using equation (3) turned out to be 1.50, 2.15, and 2.20, respectively. The theoretical distribution curves were drawn by employing common values of $v_1 = 0.60$ mV and $\sigma = 0.20$ mV. As can be seen in the figure, all the curves showed fairly good agreement with the histograms experimentally obtained.

DISCUSSION

Due to technical difficulties involved, the number of cases in which successful analysis could be performed was very limited in the present experiment. But the results described above seem to be consistent with the idea that the EPSP's in the eighth nerve fiber of goldfish are built up of all-or-none units which are identical in size and shape with the spontaneously occurring unit potentials. However, there are some problems to be considered.

The first one is concerned with the organization of the synaptic connection. Each eighth nerve fiber branches at its terminal region to innervate number of hair cells. But hair cells innervated by a single afferent fiber do not necessarily perform identical actions. We already know that hair cells of opposite spatial orientations are innervated by a single S1 fiber (FURUKAWA and ISHII, 1967). As for quantitative aspects, hair cells innervated by a single afferent fiber may be different in their sensitivity to sound and in their ability to evoke EPSP's. Also it is possible that EPSP's produced at individual nerve terminals suffer different amount of electrotonic decrement before reaching the recording site. From a practical point of view, however, these possible differences did not present any serious

obstacle for the statistical treatments, as indicated, for example, by the fact that the amplitude of the spontaneously occurring miniature EPSP's distributed normally around the peak of the mean amplitude without disclosing multiple peaks.

The second problem concerns the mechanism of quantal release of the transmitter substance. It is generally admitted that a single presynaptic impulse releases only a fraction of the so-called immediately available store of transmitter substance from nerve terminals, as can be expressed in the following equation:

$$m = np$$

where m is the quantal content of the response, n is the number of quanta in the readily available store, and p is the fraction of the release (CHRISTENSEN and MARTIN, 1970; MARTIN, 1965). Usually, the applicability of the Poisson theorem is tested on preparations in which m is made very small. The condition can be attained by reducing the Ca^{2+} concentration of the medium or by increasing its Mg^{2+} concentration or by a combination of both. It has been established that these procedures reduce m by making p small, while n remains large (DEL CASTILLO and KATZ, 1954; KATZ and MILEDI, 1967). In the present experiment, however, we took advantage of the fact that the presynaptic elements are sensory hair cells, and applied very weak sound stimulus for obtaining small EPSP's. A difficulty here is that it has not been settled yet whether the magnitude of the presynaptic depolarization should affect n or p . If it is assumed that p is affected, there would arise no special problem at all, for the results could be interpreted basically in the same term as in other instances in which the modification of m was attained by adjusting Ca^{2+} - Mg^{2+} concentrations. But there are some indications which seem to suggest that the stimulus intensity, *i.e.*, the magnitude of the presynaptic depolarization, and Ca^{2+} - Mg^{2+} concentrations in the medium are quite different in their mode of action, although the amount of the transmitter release may be modified by either of these factors. More concretely, our impression is that changes in the stimulus intensity interfere with the release by changing n , for the results on the "early tetanic run down" of EPSP's (ELMQVIST and QUASTEL, 1965) showed that p remained at a constant value of about 0.6 (mean of 12 measurements; range being 0.73-0.52) for different stimulus intensities (FURUKAWA and MATSUURA, in preparation). A similar notion that the magnitude of the presynaptic depolarization may affect m by changing n has been expressed by HUBBARD and WILLIS (1968).

Why is it then that the Poisson theorem could be applied, though to a limited extent, to our present case? We tentatively postulate the presence of multiple release sites in presynaptic cells each with different sensitivity to a depolarization. We assume that the transmitter is released at each site when the membrane depolarization exceeds the threshold for that site. As suggested by the fact that the curves relating the amount of release to the amplitude of the presynaptic depolarization are sigmoid in shape (ISHII *et al.*, 1971), the threshold at the majority of

release sites lies within a rather narrow range. Therefore, if it could be assumed that the thresholds at these large number of release sites show some random temporal variations around their equilibrium positions, our experiments meet the Poisson condition with only a very small number responding each time out of a large population of release sites.

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