ORIGIN OF THE SEIZURE DISCHARGE EVOKED
IN VITRO IN THIN SECTIONS FROM THE
GUINEA PIG DENTATE GYRUS

Chosaburo YAMAMOTO AND Nobufumi KAWAI

Behavior Research Institute, University of Gunma Medical School,
Maebashi, Japan

It was reported in the preceding communications7,9) that thin sections prepared from the guinea pig hippocampus generated a train of afterdischarges in the artificial medium in which Cl ion was replaced by propionate or other large anions. The afterdischarge was considered to be akin to the seizure discharge.

In the experiments reported here, the origin of the seizure potential was studied. For this purpose, potentials were recorded from the slices in which component layers of the dentate gyrus were successively removed. Also, the laminar distribution of the potential within the slice was studied.

METHODS

Thin sections about 0.3 mm thick were obtained from the dentate gyrus of the guinea pig hippocampus and incubated in chemically-defined media. The general procedures of preparation of tissue and techniques of incubation were the same as given previously7,8,9). Slices were placed on a nylon mesh either with their cut surface upwards or with their pial surface upwards. The orientation of the slice is indicated below by diagrams in the most of the illustrations or described in the text. In order to stimulate the slice, a stimulating electrode consisting of a pair of ball-tipped silver wires was placed on the upper surface of the slice. The surface recording electrode was also a ball-tipped silver wire applied on the slice about 1 mm away from the stimulating electrode. The reference electrode was placed in the medium. Unless otherwise mentioned, stimulus was delivered once every 30-60 sec. In order to avoid "post-afterdischarge extinction9)", the experiments were started at least 60 min after the preparation of the slice. The composition of the standard incubation medium was given before9). To prepare the Cl-free medium, Cl ion in the standard medium was replaced by propionate ion.

Conventional glass pipettes filled with 3 M KCl and having resistance of 1-5 megohms were used for recording the electric field potentials from within the brain slice. Just before insertion of the microelectrode, the surface of the medium in which the slice was immersed was lowered to the level of the nylon mesh supporting the slice.
The microelectrode was inserted into the slice through the upper surface of the slice thus exposed. For marking the site of potential recording, stainless steel microelectrodes were used: After depositing a minute amount of iron electrolytically, the slice was stored for 24 hours in 10 per cent formalin with 1 per cent potassium ferrocyanide added and examined histologically in sections cut at 5 μ and stained by the Klüver-Barrer method.

RESULTS

Elimination of the component layers of the slice. As described in the preceding paper, in the medium in which all or most part of Cl ion was replaced by propionate ion, a single shock applied to the slice elicited a short-latency response (the primary response) which was followed by a train of seizure discharge. When recorded from the cut surface, the primary response consisted of one to three sharp negative deflections (arrow in Fig. 1-A2) and the seizure discharge was composed of a train of negative spike potentials superimposed on a positive wave lasting 300-500 msec.

As to the origin of the seizure discharge, it was questioned whether or not all three layers composing the dentate gyrus (the polymorphic, granular and molecular layers) were essential to generate the seizure discharge. This was studied in the experiments shown in Fig. 1. In the first experiment (Fig. 1-A) thickness of the slice was reduced to 200 μ and a slice lacking the polymorphic layer was prepared. In response to electric stimulation, a typical seizure discharge was picked up from the cut surface of this slice (record A-2).

Fig. 1. Potentials induced in the slices lacking the deep layer(s) of the dentate gyrus. In A, the polymorphic layer was not included in the slice, but the seizure discharge was elicited (A-2). B: in another experiment, a great part of the granule cell layer was further removed. The slow positive wave alone was elicited (B-2). Recorded in Cl-free medium. In this and following figures, upward deflections indicate negative potential.
In another experiment, the thickness of the slice was further slightly reduced and the bulk of the granular layer was eliminated (Fig. 1-B). In this slice the seizure discharge was no longer elicitable. Though a slow positive wave followed the stimulus, its amplitude was not more than 150 $\mu$V. These findings suggest that the seizure potential was generated by the granule cells but it could not be evoked by their apical dendrites in the molecular layer alone.

**Laminar Distribution of the Spike Potential.** In order to obtain further information about the origin of the spike potential, the seizure discharges were recorded at different depths as a microelectrode was successively advanced into the slice. In these experiments, it was preferable to add a small amount of Cl ion to the medium, because the presence of Cl ion in a low concentration reduced the discharge frequency of seizure potential (see ref. 9) and made it easier to study changes of the configuration and amplitude of the individual spikes accurately. Therefore, the experiments from which Figs. 2-5 were cited were performed in the medium which contained Cl ion in a concentration of 9 mM.

In Fig. 2, a recording microelectrode was inserted into a slice from its pial surface while putting it on a nylon mesh with its cut surface downwards (see diagram). The polarity of the seizure potential recorded from the pial surface was completely opposite to that recorded from the cut surface: The seizure discharge was composed of monophasic positive spikes superimposed

![Fig. 2. Laminar potential distribution of the primary response and seizure discharge. Electrode was inserted from the pial surface. Each numeral to the left indicates the depth at which the corresponding response was recorded. In this and the following figures, M and G denote the molecular and granule cell layers, respectively. Medium contained Cl ion in 9 mM in Figs. 2-5.](image-url)
on a slow negative wave (upper record in left column). As the electrode was gradually inserted, the spike potential was transformed into the positive-negative biphasic one (at 150 μ) and then became predominantly negative at a depth of 200 μ. Progressing further into the deeper layer, the negative spike was followed by a small positive deflection (at 250 μ) and it decreased in amplitude (at 350 μ). The discharge trains shown in Fig. 2 were preceded by the primary responses composed of two sharp waves. With the advance of the electrode, the shape and amplitude of the primary response were altered in similar manner as those of the spike potential.

As shown in Fig. 6-B, similar reversal of the spike potential took place when the slices were placed on the nylon net with its pial surface downwards and the microelectrode was inserted into the slice from its cut surface.

The above findings indicate that the spike potential was generated in the granule cell soma and it was conducted along the principal dendrite toward the pia mater (see Discussion). Further, the finding that the spikes were monophasic positive on the pial surface suggests that the conduction of the spike was blocked at some distance from the cell soma and the spike could not invade the most distal part of the dendrite.

It is also worth while to note in Fig. 2 that the spikes generated early in a spike train changed their configuration at layers more superficial than did the spikes generated later in a train. At a depth of 100 μ, the first spike in a train already took biphasic contour while spikes following it were still monophasic positive. At a depth of 150 μ, the initial three spikes took the biphasic configuration with large negative deflections, while last eight spikes were still mainly positive. These findings mean that the conduction of the spikes in a train was not blocked at the same point, but the spikes generated later in a train were conducted successively shorter distance before being blocked. Thus, early spikes were able to invade more distal portions of the dendrite than could the late spikes.

Scrutinizing Fig. 2, some variations are noted in the latency and duration of the seizure trains in different traces. This was merely the spontaneous fluctuation of the latency and duration of the seizure discharges always observed in the present experiments and not related to the depths at which these potentials were recorded.

The level of Conduction Block. As mentioned above, the spikes generated in the cell soma were conducted toward the pial surface along the dendrite shafts. But, at some levels in the molecular layer they ceased to conduct and did not invade the most distal part of the dendrites. In Fig. 2, the most superficial level to which the spike could ascend was about 100 μ below the pial surface. As the thickness of the molecular layer was about 200 μ, this means that the conduction block occurred at approximately half way between
the cell soma and the distal end of the dendrites.

The above consideration was based upon the depth measured by the distance of the microelectrode insertion. The insertion of the electrode, however, might make a dimple in the slice, which could cause a considerable error in estimating the depth of the electrode tip. Consequently, in Fig. 3, a more direct technique was applied to estimate the level of the conduction block. A stainless steel microelectrode was inserted from the pial surface. At a depth of 100 μ, spikes were predominantly positive but just below this point, the spikes generated early in a train started to change their configuration and at a depth of 150 μ all the spikes in a train became biphasic. A small amount of iron was deposited at this point. The histological section shown in Fig. 3-B demonstrates that the deposit was found at a point slightly deeper than the middle level of the molecular layer (arrow). This point indicates the level at which the late spikes in a train were blocked. Taking into account that the early spikes in a train started to change their contour about 50 μ above this point, we may think that the early spikes in a train could ascend up to the middle point between the cell soma and the dendritic terminal.

**FIG. 3.** Approximate level at which the spike was transformed from the monophasic positive potential to biphasic one. Records in A were taken with a steel microelectrode inserted from the pial surface. Histological section in B shows the iron deposit (arrow) indicating the point at which the bottom record in A was taken.

**Conduction Velocity of the Spike Potential.** If the spike were conducted along the dendritic shafts from the proximal towards the distal portions, we may expect that the lengthening of the latency of the negative peak occurs as the recording electrode proceeds from the granule cell layer towards the pial surface. This, however, can not be detected in Fig. 2, because in this figure, the spontaneous fluctuations in the latency of the discharge trains mask the lengthening of the spike latency with respect to depth. In order to eliminate the spontaneous fluctuation of the latency, in the experiment shown in
Fig. 4, the temporal relationship between the spikes recorded at different depths and the spikes recorded simultaneously from the surface of the slice was studied. In this illustration, lower traces (broken lines) were recorded with a microelectrode inserted into a slice from its cut surface and upper traces (solid lines) were recorded with a silver wire placed on the cut surface within a fraction of a millimeter of the point of entry of the penetrating electrode. The spike recorded 100 μ below the surface was maximum in amplitude and purely monophasic. At this level, there was no marked latency difference between peaks of surface and deep spikes. As the microelectrode was advanced, the spikes became biphasic and their negative peaks were elicited with longer latencies as compared with the peak of the surface spike. The relative time relations between the surface and deep spikes measured in this way did not show any detectable fluctuation even if the absolute latencies of the spikes as measured from the stimulus artifacts varied spontaneously from train to train. In Fig. 4, advance of the electrode tip by 100 μ caused a delay of 0.9 msec. Therefore, the conduction velocity was 11 cm/sec in this slice. From experiment to experiment, it ranged between 8-15 cm/sec.

Fig. 4. Simultaneous records of spike potentials on the cut surface (records a) and at indicated depths below it (records b), traced from films. Note successive delay of the negative peaks at deeper levels.
Facilitation of Spike Invasion. As shown in Fig. 2 and in records in column 1 of Fig. 5, spikes occurring early in a spike train invaded the more distal portion of the dendrites more than did the spikes occurring late in the same discharge train. The late spikes in the discharge train, however, were made to invade the more distal portion by repetition of stimulus at an adequately low frequency. In the experiment depicted in Fig. 5, a microelectrode was inserted to the layer at which early spikes in the train were biphasic with large negative deflections and late spikes were predominantly positive (record 1, upper trace). While keeping the electrode tip at this level, a train of four shocks were applied at 0.5 cps. In the discharge train elicited in response to the fourth shock, the late spikes were biphasic in contour with prominent negative phase (record 2, upper trace). The appearance of the negative phase in the late spikes in the train suggests that during the sequence of shocks, the invasion of the late spikes was facilitated and they could invade the more distal portion of the dendrite which they could not invade before. Subsequently, the electrode was successively advanced by 30 and 50 μ, and the effect of repeated stimulation was observed at each level. At a level of 200 μ, except for initial two or three spikes in a train, the spikes evoked by the first shock were mostly positive in polarity (record 1 middle trace), but after repetition of the shocks, considerably large negative deflections appeared in all of the spikes (record 2). At a depth of 250 μ, at which all the
spikes were monophasic positive in control response (record 1 lower trace), stimulus repetition was not able to bring about negative deflections in the spikes (record 2). These observations indicate that though the spikes occurring late in the spike train were facilitated to invade the distal dendrite by repetition of stimulus shock, they could not exceed the level at which conduction block occurred in the early spikes. Therefore, the most distal portion of the dendrites was still free from the invasion of the spike.

*Laminar Potential Distribution of the Slow Wave.* The potential distribution of the slow wave superposing the spike potential was studied in the separate experiments in which Cl ion was completely removed from the incubation medium, because the slow wave was not large enough in the medium partially containing Cl ion in which Figs. 2-5 were taken. Fig. 6-A was recorded while inserting an electrode from the pial surface into the slice. Until the electrode tip reached the depth of 150 μ, the slow wave was negative and of fairly large size. At level of 200 μ, it abruptly decreased in amplitude and became undetectable in the deeper layers. Similar potential distribution observed in another experiment is represented graphically in Fig. 6-A'. In contrast to the spike potential, the surface negative slow wave had no counterpart in depth. The abrupt reduction of the slow wave always occurred at the depth at which the spike potential was transformed from positive to biphasic...
contour with a large negative deflection.

As the electrode was advanced from the cut surface toward the pia mater (FIG. 6-B), the positive slow wave recorded on the cut surface also declined suddenly at the level at which the spikes took the biphasic configuration (200 μ in Fig. 6-B). Fig. 6-B' is a graphic representation of the potential distribution of the slow wave recorded in another experiment. Likewise, no counterpart of the surface positive wave was found in the depth of the slice.

**DISCUSSION**

The neurons existing in the slice were mainly the granule cells, the somata of which were closely packed in the granule cell layer and their principal dendrites extend toward the pial surface across the molecular layer. The observation that the spike was negative on the cut surface and positive on the pia mater suggests that the spike potential represents the electric dipole generated on the granule cell dendrites, the sink of the dipole being located in the cell soma or proximal dendrites8). To this sink, the current flowed from the source situated in the distal portion of the dendrites.

It was found that the spike potentials composing the seizure discharge resembled the sharp waves in the primary response in contour and duration. Also, these two types of potentials took the maximum amplitude at the same depth and their shape and amplitude changed in a similar manner at different depths. Therefore, these two types of potentials seem to reflect the same electrical activity of the granule cells. Already it has been found that the sharp wave of the primary response represents the action potential elicited in the granule cell soma8). Consequently, it may be concluded that the negative spikes of the seizure discharge recorded in the granular layer reflect the synchronized discharge of the granule cells in the slice.

The spike potential as well as the sharp wave in the primary response changed from negative to positive-negative biphasic potential as the distance between the cell soma and the site of recording progressively increased. Concomitantly, gradual lengthening of the latency of the negative peak was observed in these potentials. These findings indicate that, in accordance with the conclusion reached by Andersen et al.13 in in vivo experiments, the action potential generated in the cell soma conducted along the principal dendrite of the granule cell toward its distal end. As pointed out by Nelson and Frank6), the lengthening of the latency of the potential is merely circumstantial evidence of the conduction of the potential, because similar latency delay could be expected when the potential spread electrotonically along the dendrite. But the change of the spike shape from a negative to positive-negative biphasic one can not be explained without assuming the active conduction of the spike.

The spike potential was monophasic positive and lacked any detectable
negative deflection on the pial surface and immediately below it. As the lack of negativity means that the spike could not arrive at the site of recording, the conduction of the spike seems to be blocked at some distance from the cell soma. Similar block of spike conduction along the dendrite was observed in the cerebellum by Eccles and his associates. Recently, Katz and Miledi presented a question as to whether the positive action potential is sufficient evidence of the conduction block. They showed that the mainly positive potential could be recorded at the closed end of the motor fiber even though the nerve impulse actively invaded the terminal right up to its tip. The recorded positive action potential was considered as due to the characteristic local current flow at the closed end. One may argue, therefore, that in the present experiment the spike might invade the most distal terminal of the dendrite and the observed positive spike may be explained as due to the characteristic local current flow at the dendritic terminal. However, this argument can not explain the observation that the positive spikes were transformed into the positive-negative biphasic spikes during a sequence of low frequency stimulation, because repeated stimulation can not modify the physical condition of the closed end. This observation is easily explained by considering that the positive spike represents the action potential blocked at the more proximal part of the dendrite and the transformation of the positive spike to the negative one was due to the facilitated invasion of the spike into the more distal part of the dendrite.

Andersen et al. showed in vivo in the dentate gyrus of rabbits that the action potential in the granule cell could invade the most distal terminals of the dendrites. They reported further that the conduction velocity was about 50 cm/sec. It was found to be 8-15 cm/sec in these experiments. The difference between their results and ours may be accounted for by the diversity of animal species and/or of the experimental conditions.

The slow wave recorded from the cut surface reversed in polarity when the slice was inverted and the potential was recorded from the pial surface. This suggests that, like the spike potential, the slow wave represents an electric dipole existing in the slice. However, in contrast to the spike potential, the surface slow wave had no counterpart in the deep layer of the slice. This indicates that the origin of the slow wave is not a simple dipole within the slice. However, the exact origin of the slow wave could not be elucidated in these experiments. The causal relation between the slow wave and the spike potential is also a problem to be studied in future.

SUMMARY

1. The origin of the seizure discharge evoked in vitro in thin sections obtained from the guinea pig dentate gyrus was studied.
2. The polymorphic layer of the gyrus was not essential for the generation of the discharge but the slice without the granule cell layer could not produce it.
3. The discharge recorded from the pial surface of the slice was composed of a train of positive spikes superposed on a negative slow wave.
4. By inserting the microelectrode into the slice, it was found that the spike took biphasic configuration at intermediate levels and reversed in polarity at the deep layer of the slice.
5. The spikes generated early and late in a discharge train changed their contour at different depths.
6. In contrast with the spike, the surface slow wave had no counterpart in the deep layer.
7. It was concluded that the spike represented the action potential generated in the granule cell body and it was conducted along the principal dendrite. This conduction was blocked at some distance from the cell body.

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REFERENCES