RESPONSE OF CAT CEREBELLAR VERMIS INDUCED BY SOUND.
II. THE ROLE OF THE MOSSY AND CLIMBING FIBERS
IN ACOUSTIC TRANSMISSION TO THE CEREBELLAR
CORTEX AND INFLUENCE OF STIMULI PARAMETERS

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Abstract. Experiments were performed on cats under Chloralose or
Nembutal anesthesia. The parameters of the acoustic click stimuli were
found to have a strong influence on the responses registered from both
the surface of the cerebellar vermis lobuli V up VII as well as from
single units. It was shown that a stimulus frequency rate not greater
than 1/2 sec should be used, since higher frequencies caused strong at-
tenuation of the response. The type of anesthesia did not change the la-
tencies of reactions of both evoked potentials and single units. However.
decreasing the strength of the click resulted in increased response laten-
cies, in the case of single unit reactions. A very strong influence of weak
visual stimuli on units was also observed. It is suggested that mossy
fibers are the most important fibers in the transmission of acoustic in-
formation to the cerebellar cortex.

INTRODUCTION

It is known that only two systems of fibers, the climbing CF and
mossy fibers MF, reach the cerebellar cortex. The spikes transmitted by
mossy fibers excitate the granular cells, which next cause the Purkinje
cell discharges that are called simple spikes. This two stage process of
Purkinje cell excitation requires summation at both the level of the con-
nection between mossy fibers and granular cells and at the level of the
synapse of granular cells axons (parallel fibers) with Purkinje cells (1). The excitation that takes this route is strongly attenuated by Nembutal, which does not disturb the transmission by mossy fibers but blocks the transmission in granular cells (5–7).

Contrary to this system is that involving climbing fibers. One climbing fiber reaches one Purkinje cell and winds around its dendritic tree. The excitation of a climbing fiber always causes a short series of spikes of high frequency followed by inhibition in the Purkinje cell. This type of response, called the climbing fiber response (CFR), is characteristic enough to serve as one of the main criteria for the identification of the Purkinje cell (PC). In contrast to mossy fiber excitation, the excitation going through climbing fibers is not attenuated by Nembutal, and even in Nembutal anesthesia, facilitation of the CF pathway is observed (6, 7). The problem of which kinds of information reach the cerebellum by mossy or climbing fibers has been analyzed many times, mainly for the kinesthetic apparatus (2, 10). It has also been shown that visual information is transmitted by mossy as well as climbing fibers. Differing results have been obtained in the cerebellum with acoustic stimuli. For example, Shofer (13) asserted that acoustic information reaches the cerebellum by mossy fibers only, and Freeman (4) postulated that a system of climbing fibers is also engaged in this process.

In this paper the role of MF and CF fiber systems has been analyzed by suppressing MF transmission with barbiturates and by means of analyzing the influence of stimuli parameters on the response.

METHODS

The method of investigation, as well as the pattern of responses of single cells to clicks (103 db SPL) was described in a previous paper (9). The experiment was performed on 21 cats. Chloralose, Nembutal, or a mixture of the two was used for narcosis. The results were analyzed with an ANOPS 2.

RESULTS

Before discussing the reactions of cells to acoustic stimuli, it is necessary to note that some cells in the investigated area responded better to visual stimuli (flash of light) than to clicks or that they responded only to the visual stimuli. An “off” response for the visual stimuli was also observed, but this kind of reaction did not take place in response to the acoustic stimulus, for which only “on” responses to stimuli of long duration were registered. A distinct response even to very weak visual stimuli was noticed. As an example, Fig. 1 shows: 1, a histogram
made in the case of an acoustic stimulus and with the beams of the oscilloscope switched off, 2 a histogram of the spontaneous activity and 3, a histogram without any auditory stimulus accompanied by an onset of the oscilloscope beams, which made the darkened experimental room slightly brighter. It can be noticed that a distinct reaction occurs even to very weak visual stimuli. Because of this, the cat's eyes were covered, and switching off of the oscilloscopes was synchronized with onset of the acoustic stimulus during the analyzes.

The rate of stimulus presentation is the parameter that has a large influence on the response. Figure 2 shows the influence of stimulus presentation rate on evoked potentials. It appeared that a rate not greater than 1/2 sec should be used, since higher frequencies cause strong attenuation of the response, a fact not usually considered by some authors.

During the experiment it appeared that evoked potentials can be registered from cerebellar vermis lobuli V to VII and that the area of optimal responses overlaps with the area reported by Snider and Stowell. The systematic changes of latency with varying placements of the recording electrode, which was suggested by Levy (11), were not observed.

As was found in the previous paper (9) the type of the narcosis had no influence on either the latency of the evoked potentials or the single cell responses. However, it appeared that the modification of stimulus strength changed the latency of single cell reactions, but did not change the latency of the evoked potentials.
Figure 3 presents average evoked potentials for the following stimuli: 1, standard; 2, -54 db; 3, -48 db; 4, -45 db; 5, -38 db; 6, -30 db; 7, -3.5 db; and 8, for a stimulus that was 6 db stronger than the standard. The increase of stimulus strength produced later waves on the surface of the cerebellum. The second wave was seen for a stimulus that was 45 db weaker than the standard one. A fully developed potential was registered for a stimulus that was 38 db weaker than the standard. The standard click exceeded the threshold of the fully developed response by 10 to 30 db. Further increase of stimulus strength above the standard did not yield any changes, other than sometimes producing a slight increase of the amplitude of late component, and had no effect on the first waves. There were also responses to stimuli that were 60 db weaker than the standard. Figure 4 presents the reactions of three cells under Chloralose anesthesia parallel with the decrease of stimulus level. Note that a distinct reaction occurs to stimuli 60 db weaker than the standard.

Besides the general decrease of wave amplitude on PSH and the disappearance of particular components (Fig. 4 4–6), a decrease of reaction latencies from 14 to 26 msec was observed. It may be considered that this extension is not due to a general shift of all waves but results from a lack of the initial part of the first sharp excitation (Fig. 4 7–8).

Fig. 2. Influence of stimulus presentation rate on the evoked potentials. Cat under Chloralose anesthesia. Upper beam, cerebellum; lower beam, round window. 1, 0.3 Hz; 2, 1 Hz; 3, 2 Hz; 4, 3 Hz.
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Fig. 3. Influence of stimulus strength on the evoked potentials. Chloralose anesthesia. 1, standard stimulus 0 db; 2, -54 db; 3, -48 db; 4, -45 db; 5, -38 db; 6, -30 db; 7, -3.5 db; 8, +6 db. Numbers 1, 5, 6, 7 have the same amplifications. For the nonexpanded potentials in 3, 4, 8, amplification was twice as great as the standard, for 2, four times standard. For potentials in 3, 4, 8, amplification was 2.5 times the standard; and for 2, five times greater than the standard. Stimulus onset is shown by the black triangle.

DISCUSSION

It is evident from the data presented that the kind of narcosis does not affect the latency of evoked potentials and single units reactions. However, in Nembutal anesthesia the shape of the evoked potential is simplified. Administration of chloralose anesthesia with multiple small doses of Nembutal to the cat does not change the latency of the diminishing reaction. This finding may be explained as due to the first component's representing the volley of afferent pulses that reaches the cere-
Fig. 4. The influence of stimulus strength on cerebellar single unit reactions. 1–3, 4–6, and 7–8 are from three different cats. 1–6, Chloralose and Flaxedil, 7–8, Chloralose only. 1, standard stimulus, 2, –40 db; 3, –60 db; Note that the excitatory period shortens with the weakening of the stimulus and that the latency grows from 14 msec to 26 msec. One bin equals 1.6 msec. 4, standard stimulus; 5, –12 db; 6, –30 db; one bin equals 0.8 msec. The latencies are 16 msec, 20 msec, 26 msec, respectively, 7, –35 db; 8, –65 db. The latencies are 21 msec and 34 msec respectively.

bileum through mossy fibers and possible adding to this potential the slow potential generated by the synapses of these fibers. The later components of this potential are due to the discharging granular and Purkinje cells and climbing fibers (3). The attenuating influence of Nembutal on the transmission of pulses from mossy fibers by granular cells probably accounts for the lack of the excitation of these cells. As a consequence, Nembutal results in a simplified potential.

On the other hand, it is known that the excitation going through the climbing fibers is attenuated under Chloralose anesthesia and that the action of this system is enhanced by Nembutal (6, 7). This fact indicates a small role for the climbing system in the transmission of acoustic information to the cerebellar cortex. In addition, a correlation of CFR with the stimulus was not observed.
The second set of data which support the above hypothesis are the observation of the relationship between the strength of stimulus and the latency of single unit reactions; and, at the same time, the lack of a correlation between stimulus strength and latency of evoked potentials. Potentials evoked by clicks were observed after about 8 msec. The latency of single unit reactions to the standard stimulus was approximately 15 msec and grew to 30 msec in parallel with a lowering stimulus intensity. The lack of correlation between the strength of the stimulus and latency of the first wave of evoked potentials, which is probably due to the synchronized volley of spikes going by MF, allows one to the deduce that temporal summation in the pathways transmitting acoustic information to the cerebellum, is not a strong effect. Probably, the cerebellum receives information from rather low levels of the acoustic system (cochlear nuclei, superior olive or the system controlling the stapedius reflex) as well as from higher levels (inferior colliculi, auditory cortex), which causes the decrease in the effect of temporal summation occurring in the acoustic system.

Changes of the latency of single unit reactions in the cerebellar cortex that parallel the changing level of stimulus intensity indicate the existence of strong temporal summation in the cerebellar cortex loop. This fact strongly supports the hypothesis about the dominating role of MF in the transmission of acoustic information ot the cerebellar cortex. It is known that temporal or spatial summation cannot exist in the CF system, which is due to fact that one PC is excited by one CF, and, what is more important, that excitation going this way always evokes reactions of PC. The opposite situation is seen in the mossy fiber system. Excitation going through the MF evoked a volley of spikes in the granular cells, which drive PC. This two-step process is under the inhibitory influence of Golgi cells, and needs the summation of the excitation from several (one to four) mossy fibers toward granular cell excitation, and, further, requires the summation of many granular cells with the goal of exciting Purkinje cell (1, 12). When the stimulus intensity is lowered the excitation going by MF decreases, and a longer time is needed for summating excitations and setting off reactions of granular cells as well as Purkinje cells.

It was said that under Nembutal the majority of the cells did not show a reaction to acoustic stimuli and that it is necessary to have very low levels of Nembutal anesthesia in order to observe single cell reactions. Treatment of cats which were under Chloralose anesthesia with a small dose of Nembutal (5 mg/kg) resulted in diminishment and disappearance of the response (8, 9). Since Nembutal blocks the mossy fiber path and does not disturb the climbing fiber (6, 7) the data strongly sup-
port the hypothesis that the MF-system plays a basic role in this case. It is possible to explain the constant latency of responses under different anesthesia and levels of anesthesia in the following manner. The standard stimulus was about 60–70 db stronger than the threshold stimulus so that it evoked a strong and similar excitation in the mossy fibers system under both types of anesthesia. This was demonstrated by the fact that the first wave of the evoked potential exhibited only small variations of amplitude with different anesthesia. In this case granular cells always received more than enough information. In the Chloralose, excited granular cells caused excitation of PC. However, in strong Nembutal anesthesia the passage by granular cells was blocked (6, 7) so it was not possible for stimulation to excite the granular and Purkinje cells.

In the case when the level of Nembutal anesthesia was already low and transmission was only partially attenuated, our standard stimulus was strong enough to allow excitation of the higher cerebellar cortical level. Since excitation at the level of MF was strong, the latency of single unit reactions (when they occurred) is the same as in the Chloralose. If the excitation had traveled by the CF system, which is not attenuated by Nembutal, it would not have been possible to observe these effects. This discussion supports the hypothesis that the acoustic information is transmitted mainly by means of the mossy fiber system.

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