CHANGES IN UNIT POSTSYNAPTIC RESPONSES AT SENSORIMOTOR CORTEX WITH CONDITIONING IN RABBITS

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Abstract. A localized, conditioned startle reaction (LCSR) to a click (as conditional stimulus) could be established and recorded electromyographically in rabbits if complex unconditional stimulus was employed. The complex consisted of localized, electrical stimulation applied concurrently to sensorimotor cortex and hypothalamus. The latency of this LCSR was only 12-14 msec. Postsynaptic responses were recorded intracellularly or quasi-intracellularly within the sensorimotor cortex near the placement of the electrodes for the cortical stimulation. The latency distributions of the averaged postsynaptic responses were compared for the naive, conditioned and extinguished states. Latencies in naive animals were similar to those in the extinguished state, but were significantly \( p < 0.01 \) different from the latencies of click-responses in the conditioned state. An increase in the number of responses with 8-17 msec latencies was found after conditioning. Of 30 neurons recorded in conditioned animals six responded with an extremely short latency of 4 to 7 msec. Similar latencies were found in response to much more intense clicks capable of evoking an unconditional startle reaction in naive animals. Changes in postsynaptic potentials thus occur at the cortex independent of proprioceptive feedback from the conditioned movement. The short latency neuronal responses suggest that a pathway for the LCSR may pass through the sensorimotor cortex. The appearance of responses at less than 7 msec to a previously neutral click in conditioned animals supports the idea that an increase in synaptic effectiveness underlies the neuronal mechanism of conditioning.

INTRODUCTION

Recording from single units is presently employed by many investigators to study the cellular mechanisms and the neuronal organization of the conditioning process (for reviews, see 1, 5, 16, 20). However, the related recordings of intracellular postsynaptic potentials (PSPs) have been used only in simplified situations, the so-called cellular analogs of conditioning (5, 6, 22, 23, 25).
In an earlier publication from this laboratory (24) an experimental situation was described in which conditioned reflexes (CRs) of extremely short latency (12–16 msec) can be readily established. Wave-forms and latency of electromyographic (EMG) CRs were similar to those of the unconditioned startle reaction (14, 19). It had been shown by several investigators (3, 10, 11, 15) that the startle reaction can be conditioned to initially neutral stimuli. An important feature of the previously described (24) conditioned reaction was its very limited generalization to other muscles, contrary to the situation with generalization of the normal startle reaction (10). Thus the term “local conditioned startle reaction” (LCSR) will be used here to refer to the CRs previously described (24). In view of the extremely short and constant latency of the peripheral response, LCSRs promise to become a convenient paradigm for investigating the neurophysiological mechanisms of conditioning.

The level of the central nervous system at which the relevant changes occur is unknown. The short latency and similarity with unconditioned startle reactions suggests that the changes responsible for the elaboration of the LCSR are primarily subcortical. However, some changes in extracellularly recorded spike responses of cortical neurons were found in preliminary experiments (24) during conditioning and extinction of the LCSR.

In the present experiments postsynaptic responses of sensorimotor cortical neurons and EMG reactions were compared in the naive, conditioned and extinguished states. The data were analysed to determine: (i) whether significant changes in the cortical postsynaptic responses can be found independent of proprioceptive feedback; (ii) whether these changes can account for the elaboration of the LCSR.

METHOD

CRs were established and extinguished in rabbits using a procedure similar to that previously reported (24). The head was held rigidly fixed to a metal frame by means of screws previously attached to the skull under Nembutal anesthesia. Otherwise, the rabbit was loosely restrained in a hammock. To mask external sounds during conditioning and recording “white noise” of about 60 db (re. 0.0002 dynes/cm²) was applied. The conditional stimulus (CS) was a click produced by a 0.3 msec square wave pulse of 10 db intensity above the white noise level. LCSRs were elaborated by pairing the click with a complex stimulus consisting of direct stimulation (US) of the “motor” cortex and lateral hypothalamic “motivational reinforcement” (MR), stimulation of either locus alone being inadequate for production of the LCSR as had been shown in pre-
previous experiments (L. L. Voronin, G. Y. Gerstein, I. E. Kudriashov and S. V. Ioffe, in preparation). CS-US and US-MR intervals were 120–150 and 100 msec respectively. The intertrial interval was varied from 1.5–3 min during pairings and from 15–50 sec during extinction trials. The US (a train of two to five 0.1–0.5 msec, 0.3–1.2 ma pulses at 300–500 Hz) evoked a local movement of the contralateral foreleg. Parameters of the MR (0.5–2 sec of 0.3–1 msec, 100–300 µa pulses at 40–80 Hz) were those which produced a “feeding reaction” in the same animal as tested 1–3 days before the first recording session (7). Loud clicks (20 db above the white noise level) were used additionally in some experiments with naive animals to evoke a generalized unconditioned startle reaction.

Fig. 1. Cellular (ACEG) and EMG (BDFH) responses to clicks of two different intensities in two naive animals (A–D and E–H). s, five superimposed single traces; av, average of 10 responses; A, B, E and F, click of low intensity (onset marked by a thin bar); C, D, G and H, loud click (onset marked by a thick bar) which evoked the unconditioned startle response (DH). Peak of action potentials (here and in Fig. 2) off screen. Note latency of 4–5 msec in unit response (G, av) preceding EMG (H) for loud clicks.

Recording glass microelectrodes were filled with K+ citrate and had a resistance in the range of 30 to 100 magaohms. They were inserted in the sensorimotor cortex near the place of US application. A modified method developed by Shvirkov (18) was used for the microelectrode insertion.

Two needles placed subcutaneously upon a muscle with a clear-cut
reaction to the US were used for recording the EMGs of the responses to the clicks. In most of the experiments the EMG of an other muscle (mainly from a hindleg) was recorded to monitor the possible effector generalization of the responses to the clicks.

Neuronal and EMG activity were recorded from a “Disa” oscilloscope in parallel with an ART-1000 computer for on-line averaging. In cellular recordings PSPs and spikes were averaged together. It was possible to distinguish between averaged PSPs and spikes by comparison of the averages (see right part of the Figures) with the corresponding single sweeps (as examplified by left part of the Figures). For example, early responses in Fig. 1C and 2A represent pure PSPs, those in Fig. 1G and 2D consists mainly of spikes. Frequency bands were 10–1,000 Hz for EMG recordings and 0–1,000 Hz or 1.6–1,000 Hz for neuronal recordings in most experiments.

RESULTS

PSPs and spike discharges were recorded intracellularly and quasi-intracellularly (12) at the sensorimotor cortex. Recordings of spike discharges of 10–20 mv with steady potential shifts of 15–40 mv were common. Field potential responses to the same stimuli (clicks) were recorded routinely (Fig. 2C) to check the influence of gross activity on the single neuron recordings. This influence was found to be negligible especially for early components with latency \(<\) 20 msec (compare Fig. 2C with 2AD).

Four types of responses were analyzed (Table I). For control, responses were recorded from naive animals. The future click CS did not evoke any EMG response (Fig. 1BF). Neither in about half of the neurons were significant responses found in averaged recordings in the period of 0 to 100 msec after the click presentation (Table I). An example is shown in Fig. 1A. The two humps in Fig. 1A, \(av\) represent two random spikes (not PSPs). In the rest of the cells in this type (Fig. 2E) postsynaptic responses were evoked by clicks after a latency of 8–43 msec. Inhibitory postsynaptic potentials (IPSPs) were the most prominent component of the responses. The IPSPs were or were not preceded by excitatory postsynaptic potentials (EPSPs) or by spike discharges.

The second type of responses was recorded in the conditioned state, the LCSR latencies being 12–14 msec (Fig. 2BE). The relative number of the responsive neurons is greater in this type in comparison with the control (Table I). The averaged neuronal responses were more prominent. Of special significance seems to be the appearance of EPSPs (Fig. 2A) and spike discharges (Fig. 2D) with latency \(\leqslant 7\) msec. Such short latency responses were never found before conditioning.
The responses of the third type (Table I) were recorded after extinction of the LCSR by presenting the click alone. Comparatively low amplitude EMG responses were evoked only by 10–20% of clicks in the extinguished state or there were no EMG responses at all. As shown in Fig. 2G only the first click was followed by a clear EMG response (Fig. 2).

![Fig. 2. Responses of three different neurons (ADF), successively recorded in a single penetration in the sensorimotor cortex of a conditioned animal, and EMG (BEG). C, extracellular field potential recorded just after the death of the first neuron (A) and before obtaining response from the second cell (D). Some of the EMG responses (BE) are limited by the amplifier (note changes in gain). Clicks were delivered alone without succeeding cortical and hypothalamic stimulation. Extinction can be seen by comparison of EMG averaged responses, av, average of 6 (A–C) and 15 (D–G) responses. For additional explanation see Fig. 1 and text.](image)

It was found that in most of the naive animals tested with a loud "startling" click (see Method) a generalized EMG response was evoked (Fig. 1DH). The latencies of the unconditioned startle response did not differ significantly from those of the LCSR. The postsynaptic responses
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Distribution of postsynaptic latencies for cortical neuronal responses to click

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Number of neurons recorded</th>
<th>Number of neurons with latency</th>
<th>Significance of the difference from the control distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-7 msec</td>
<td>8-17 msec</td>
<td>18-43 msec</td>
</tr>
<tr>
<td>I. Control (naive state)</td>
<td>25</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>II. Conditioned state</td>
<td>30</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>III. Extinguished state</td>
<td>22</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>IV. Startle reaction to loud click</td>
<td>20</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

Evoked by the loud click were significantly larger than those to the neutral click in the same neuron (see IPSP in Fig. 1E, av with that in Fig. 1G, av). Most of the unresponsive neurons (Fig. 1A) were found to show significant responses to the loud click (Fig. 1C) and some were comparable to those in the conditioned type in responding with a latency $\leq 7$ msec (Fig. 1G, av). The latency distributions were similar in the two types of responses recorded during the performance of the two types of motor reaction: the local, conditioned movement to previously neutral clicks (Table I, Type II) or the generalized, unconditioned startle reaction to loud clicks (Table I, Type IV).

DISCUSSION

Statistically significant changes in the latency of the click response suggest that changes in the postsynaptic responses relevant to the conditioning procedure occur at the sensorimotor cortex. An increase in the number of responses with latency $\leq 17$ msec excludes explanations based on proprioceptive feedback. This is clear from the comparison of the 17 msec latency of the postsynaptic responses to click with the 12-14 msec latency of the LCSR and the minimal 7 msec latency of postsynaptic responses to forelimb stimulation (21).

In addition, the appearance of extremely short latency (less than 7 msec) responses even in a small number of neurons, suggests that at least one of the crucial changes during this type of conditioning takes
place at the cortical level. It does not mean, of course, that information related to the performance of a LCSR is transmitted exclusively through the cortex. Transmission exclusively through the cortex also does not seem probable if account is taken of the essentially subcortical nature of the unconditioned startle reaction (see 9, 11, 14, 28) and the striking similarity between the latter and the LCSR.

Nevertheless, the latency of the postsynaptic responses is consistent with transmission through the cortex. The difference between the latency of the postsynaptic response (4–7 msec for the shortest latencies) and that of the LCSR (12–14 msec) is equal to 5–10 msec. This difference corresponds to a 4–7 msec delay between direct cortical stimulation and the EMG responses found in experiments with suprathreshold surface and intracortical stimulation. It appears, therefore, that at least one path of the LCSR passes directly through the sensorimotor cortex.

The extremely short minimal latencies of the click-evoked postsynaptic responses (4–7 msec) suggest a very fast afferent conduction through paucysynaptic pathways. Similar pathways must be postulated to account for the 4 msec latency of neuronal discharges in the cat auditory cortex (17) and latencies ≤ 6 msec for the rat sensorimotor cortex after conditioning (13). The latter data are especially closely related to the present study.

No significant short latency responses (< 7 msec) to a neutral sound in naive animals were found either in our study or in that of Olds et al. (13). The short latency responses appear only to sounds which evoke a motor response (a conditioned reaction or unconditioned startle response).

One might speculate that it is the “short latency” neurons participating in the unconditioned startle reaction that form the “learning site” for the elaboration of the LCSR. If this hypothesis is close to the truth there would be two criteria in choosing neurons for intracellular study of learning mechanisms in the future experiments. The first, based on data of Woody et al. (26, 27), is the efferent projection of the cell to the target muscles of the conditioned response (of the LCSR in our case). The second criterion is the short latency postsynaptic response to loud (starting) sound as suggested above.

The increase in amplitude of PSPs in the conditioned state may be considered to support the well known postulate (2, 4, 5, 8) that increased synaptic effectiveness underlies neuronal mechanisms of conditioning. This has not been previously demonstrated in experiments with elaboration of a behavioral conditioned response. The LCSR seems to be a promising situation to find direct evidence for this hypothesis and to delineate it neurophysiologically.
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