Neuronal plasticity recorded from cat hippocampus during classical conditioning

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It has been demonstrated recently that hippocampal neurons show a very early
and marked increase in activity during classical conditioning which continues to grow
over the course of training but does not develop in animals given unpaired control
training. The preparation used is the rabbit nictitating membrane (NM) res-
pone, which has several advantages for analysis of brain substrates of learning. Because the conditioned increase in hippocampal unit activity is so large (an average
increase of 15–20 standard scores) and consistent, appears to involve a large
proportion of the pyramidal neurons, and is 'projected' from the hippocampus to a
major efferent target (lateral septum), it is at least possible that these findings have
tapped an important functional role of the hippocampus in learning and memory. If
this is indeed the case, it is necessary to demonstrate generality of the findings across
species.

A method for classical conditioning of the cat nictitating membrane response
under conditions essentially identical to those used for the rabbit has been reported
recently. The purpose of the present study is the initial characterization of possible
increases in hippocampal unit activity during classical conditioning of the NM response
in the cat, using conditions identical to those previously employed with the rabbit.

The experiments were performed on 6 cats obtained from local animal pounds.
Each animal was anesthetized with pentobarbital (Nembutal) and a multiple unit
microelectrode (insulated stainless steel insect pin, size 0) with a 40–50 μm tip exposure
implanted into the hippocampus. Electrode placement was determined by stereotaxic
coordinates and by monitoring spontaneous unit activity during descent. Histological
results showed that all electrode recording sites were in or near pyramidal or dentate
granule cell layers.

Following a 10–14 day recovery, animals were placed in an experimental
enclosure consisting of a restraint box and a double-walled, ventilated isolation
chamber. The first day of training consisted of adaptation to restraint and the

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enclosure, with no stimuli presented to the animal. Paired or unpaired control training began on the second day. The CS was an 85 dB, 1000 Hz, 350 msec tone measured at the animal's head. The UCS was a 100 msec, 210 g/sq.cm air puff directed at the cat's cornea. The external eyelids were held open mechanically and NM movements recorded via a thread attached to a suture in the membrane. Movement of the NM rotated a wire on the shaft of a minitorque potentiometer, and was recorded both on an oscillograph and through an FM adapter on a Sony 4-channel tape recorder. Signal markers were placed on a second channel and brain activity on a third channel. Programming equipment presented stimuli automatically and timed three 250 msec recording periods, one prior to CS onset, one after CS onset, and a third after UCS onset. The paired experimental session consisted of 117 trials, the first of each 9 being a CS-alone test trial. For the remaining trials, UCS onset occurred 250 msec after CS onset, with the stimuli being coterminous. The inter-trial interval was 50, 60 or 70 sec with a 60 sec average. In the control condition, 208 CS and UCS presentations were separated by 20, 30 or 40 sec and were given in a pseudorandom sequence. Separate groups were given paired conditioning training (n = 4) and unpaired control stimulation (n = 2). The data were analyzed on a PDP-12 computer which provided counts of neural unit activity, standard scores of unit activity in the CS and post-UCS periods relative to the pre-CS period, and area under the curve of the NM responses. All neural results described here are from analysis of the largest amplitude units (multiple unit counts) in each recording.

Previous studies of rabbit hippocampal activity have shown that, for animals receiving conditioning training, neural responses in the UCS period increased above spontaneous rates within the first few pairings of tone and air puff. Heightened UCS period discharges continued to grow following subsequent paired training, with CS period increases appearing approximately at the onset of behavioral conditioning. In addition, the neuronal firing pattern within trial periods showed a close correspondence with the topography of the behavioral NM response. Unpaired group data showed only small evoked unit responses during UCS-alone presentations, and no increases in unit activity across trials.

Fig. 1 reveals that hippocampal activity in the cat demonstrates a very similar form of neuronal plasticity during NM conditioning. After the first block of paired training trials (Fig. 1A), UCS period activity has increased substantially above background pre-CS levels, and is significantly greater than responses of unpaired animals to air-puff-alone trials (Fig. 1C). The hippocampal response of conditioning animals grows to this level within the first block of 8 paired trials, as shown by the results of a single trial analysis. In Fig. 2A, the relative amount of hippocampal activity in the UCS period for the first 8 conditioning trials of paired animals is compared to activity recorded during the first 8 UCS-alone trials of unpaired animals. Clearly, the results show that while unit activity of unpaired animals decreases slightly within the first block, unit responses of paired animals increase over the first few CS-UCS pairings.

After initial increases within the first phases of conditioning, hippocampal UCS period activity in cats continues to grow with further training. The UCS response is markedly greater at the end of conditioning (Fig. 1B) compared to the first block (Fig.
On the other hand, unpaired animal responses maintain low and/or decreasing levels throughout additional trial exposures (Fig. 1E). The differences between paired and unpaired animals are substantial and significant ($t = 6.26, df = 76$; see Fig. 2B).

The group data indicate that growth in UCS period activity of paired animals asymptotes by the sixth conditioning block, and is followed by a small decrease. This differs from results found for rabbit hippocampus, which showed increases throughout the entire first 13 blocks of training. This difference may be partially species-dependent. While the present $n$ is admittedly small, hippocampal recordings from cat show a definite tendency to increase above control levels more quickly than rabbit. The difference in growth rates is especially apparent for unit increases within the first block of conditioning trials (Fig. 2A).

Near the end of the first session of paired training, cats begin to develop

![Fig. 1. Hippocampal unit activity recorded during conditioning. Upper trace: average NM response for one block of 8 trials. Lower trace: hippocampal unit poststimulus histogram for same block of trials. Early cursor indicates tone onset; late cursor indicates air puff onset. Total trace length equals 750 msec. A and B show results from one conditioning animal. A: first block of paired trials. B: later block, after behavioral conditioning is evident. C-F show results from one unpaired control animal. C and E: blocks of UCS-alone trials from early and late phases of training, respectively. D and F: blocks of CS-alone trials from early and late phases of training, respectively.](image)
conditioned behavioral responses. Again paralleling the rabbit data, increases in cat hippocampal activity were seen to occur in the CS period, as shown in Fig. 1B.

Finally, spontaneous firing rates were recorded during the pre-CS period throughout training. No consistent variation was seen to occur across trials for either paired or unpaired animals in this background activity.

A major difference between cat and rabbit was seen in hippocampal responsiveness to tone and air puff stimulation. All cat hippocampal recordings showed a significant initial response to tone, whereas this was rarely seen in rabbit hippocampus\(^1\)\(^-\)\(^5\). The sensory response to CS is especially clear under the unpaired tone-alone condition (Fig. 1D, F). In addition, cat recordings showed a much more substantial hippocampal response to air puff UCS than did rabbit\(^1\)\(^-\)\(^3\).

Earlier analyses of rabbit hippocampal plasticity stressed the close correspondence between the pattern of hippocampal cell discharge and NM topography within trial periods\(^1\)\(^-\)\(^5\). The present cat results also reveal this fundamental similarity between hippocampal and behavioral measures (see Fig. 1A, B). In both species, hippocampal unit response patterns precede NM extension in time and closely correlate with the behavioral topography.

In summary, cat and rabbit hippocampal activity exhibit strong similarities during classical conditioning of the nictitating membrane response. Species differences are apparent with respect to hippocampal responsiveness to tone and air puff stimuli, and with respect to the rate of increase in hippocampal unit activity across early phases of conditioning. In both cat and rabbit, however, hippocampal activity increases rapidly in the UCS period with initial CS–UCS pairings, moving forward in time to the CS period with behavioral conditioning. The neural plasticity which occurs under paired

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**Fig. 2.** Standard scores of hippocampal unit activity recorded during the UCS period for the conditioning animals (solid lines) during paired trials and unpaired control animals (broken lines) during UCS-alone trials. Results for single trial analysis of first block of trials are shown in A. Those for block-by-block analysis are shown in B. Single trial scores (A) were computed relative to a standard deviation of pre-CS period unit activity occurring during first 8 trials. Block standard scores (B) were computed relative to a standard deviation of pre-CS activity occurring during entire session.
conditions does not develop with control procedures. Cat and rabbit hippocampal unit histograms show high correlations with NM topographies and precede the behavioral response in time. Thus, the primary characteristics of hippocampal neuronal plasticity during classical conditioning of the nictitating membrane are not specific to one species of animal.

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