Longevity of synaptic depression in the hippocampal dentate gyrus

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This study used urethane-anesthetized rats to investigate the longevity of heterosynaptically evoked depression of the monosynaptic response generated by synapses between entorhinal cortical (EC) afferents and the cells of the dentate gyrus (DG). Brief, high-frequency activation of the converging ipsilateral EC-DG input depressed the synaptic response of the contralateral EC-DG synapses without prior experimentally induced potentiation. This depression lasted for hours. Such observations are consistent with a role for heterosynaptically induced long-term depression in the encoding functions of synapses.

Synaptic long-term potentiation (LTP) has received intensive study as a plausible cellular mechanism for learning and memory because of its longevity, the associative nature of its induction, and its synapse specificity (e.g., in the dentate gyrus). However, if synapses potentiate repeatedly, then there exists the possibility that synapses can become maximally strong, and therefore useless as memory stores. On the other hand, certain depression processes produce important properties such that synapses encode statistics. Furthermore, although experimentally induced LTP is long-lived in the dentate gyrus, it eventually decays. Thus, identifying conditions that produce such a decrease in synaptic efficacy is an essential part of understanding how synaptic modifications can store information.

One hypothetical process is passive decay. Because it is a common observation that some LTP experiments produce potentiation of greater longevity than others, it might be argued that passive decay does indeed exist, especially if we assume no spontaneous synaptic activity during the experiment. Even if passive decay actually exists as a mechanism to remove potentiation, however, it need not be the only mechanism. In fact, results in the dentate gyrus imply an activity-dependent process.

One of the best studied forms of activity-dependent depression of synaptic responses uses the bilateral monosynaptic projections from the entorhinal cortices (EC) to the dentate gyri (DG) of the hippocampal formation. Unfortunately, these previous studies do not deal effectively with the issue of the longevity of this activity-dependent depression. For reasons such as very short test periods (~15 min) and studying depression from a potentiated baseline, these previous studies are flawed in establishing the longevity of activity-induced depression. Even so, we always have referred to this heterosynaptic depression as long-term because of occasional, qualitative observations of depressed synaptic responses that lasted for a few hours.

Here we report a quantitative version of our previous incidental observations. Depression of the contralaterally evoked EC-DG response endures for 1 h with no indication of decay. An individual 15 h experiment further confirms the longevity of this depression. Some of these data appear in a preliminary report and a dissertation.

The methods were, in general, the same as those found in Lopez et al. However, no tetrodotoxin was injected into the entorhinal cortices. Eight Sprague-Dawley and one Long-Evans rats (180–340 g) received chloralose/urethane anesthesia (n = 8; 55 mg/kg and 0.2 g/kg, respectively) or urethane alone (n = 1; 1 g/kg). A hot water heating pad maintained body temperature at 36–37°C. Bipolar stimulating electrodes were placed stereotaxically in each angular bundle to activate EC axons (~5 mm incisor bar, 8.1 mm posterior to bregma, 4.4 mm lateral to the midline). Test stimulation consisted of constant current pulses (100 μs, 150 μA–1.5 mA). Saline-filled micropipettes (5–20 MΩ) were placed in the dentate gyrus (5° from sagittal plane, 2.5–2.8 mm posterior to bregma, 1.4–1.6 mm lateral to the midline) to record population excitatory postsynaptic potentials (pEPSP's). The final recording electrode position maximized the positive-going ventral leaf pEPSP's. The pEPSP's were
digitized and stored for off-line analysis. In the first experiment data storage followed the methods of Lopez et al.; in the second experiment, data storage followed Desmond et al. In both cases the pEPSP slope within the first 4 ms of the stimulus artifact was quantified to assess changes in synaptic efficacy.

Each experiment consisted of an initial baseline test period, a conditioning period, and a postconditioning test period. Test stimuli were given 1/60 s. The stability of the EC-DG system over several hours has been previously documented. In the first experiment \((n = 8)\), conditioning stimulation consisted of 5 sets \((1/5 \text{ min})\) of 4 trains \((1/10 \text{ s})\) of 8 pulses at 400 Hz. The postconditioning test period was 60 min \((n = 7)\) or 6 h \((n = 1)\). The magnitude of depression was analyzed statistically using a two-tailed, matched \(t\) test with \(P < 0.05\) considered significant. In the second experiment \((n = 2)\), conditioning stimulation consisted of one set of 8 trains \((1/10 \text{ s})\) of 8 pulses at 400 Hz with postconditioning test periods of 8 and 15 h.

Brief high-frequency stimulation of the angular bundle ipsilateral to the recording electrode consistently reduced the pEPSP evoked by contralateral angular bundle test stimulation. The postconditioning response was 67.0 ± 6.0% \((\text{S.E.M.}; P < 0.001; n = 8)\) of the baseline response in the first 5 min of the 1 h postconditioning test period and 51.4 ± 6.5% \((P < 0.001)\) of the baseline response in the last 5 min of this same postconditioning test period. This trend toward additional depression across the test period was not significant \((\text{paired } t\text{ test})\). In the one animal that was followed for 6 h, the contralateral response was 69.7% of the baseline response after 1 h and 69.4% of the baseline response after 6 h. Thus, not only was there no trend for decay of depression in this experiment, but there was still more than 30% depression after 6 h.

In the second experiment, fewer conditioning pulses were used to elicit depression and the responses were followed for longer postconditioning periods. Both animals showed depression throughout the test period, so we reviewed the animal monitored the longest time. The EC-DG evoked responses recorded contralateral to the stimulation site (Fig. 1A) consist of a short latency monosynaptic component followed by a larger polysynaptic component, which is abolished by tetrodotoxin injection into the entorhinal cortices. Other examples of such responses can be seen in refs. 7, 9, 12 and 15. For the initial 5 min after conditioning, the mean postconditioning contralateral response was 72.6 ± 13.1% of the preConditioning baseline response (see Fig. 1A). For the final 5 min of the 15 h postconditioning test period, the mean contralateral pEPSP was 71.2 ± 8.4% of the pre-conditioning baseline response. Fig. 1B illustrates the relative stability of the postconditioning test response over 15 h.

The other pEPSP responses, i.e., the contralateral response on the other side of the brain and the two ipsilateral responses, also reflect the stability of this preparation and its evoked responses. None of these responses showed any decrement of mean response amplitude over the 15 h of postconditioning testing. All these responses responded to the unilateral conditioning train as predicted from our previous reports. That is, conditioning stimulation elicited no change in either response recorded from the other side of the brain, and there was potentiation of the conditioned ipsilateral response.

The present results demonstrate that the depression of contralaterally evoked EC-DG responses induced by ipsilateral conditioning stimulation is long-term and, indeed, longer term than any of the other reported forms of depression of which we are aware. Moreover using acute preparations, it is our experience that when potentiation and depression are symmetrically induced, they are of equivalent longevity.

As shown here, and in previous experiments that monitored responses only for shorter periods of time, activity-dependent depression can be induced from an experimentally unpotentiated baseline [see bottom graph of

![Fig. 1. A 15 h long postconditioning test period. A contralateral angular bundle test stimulation evoked population excitatory postsynaptic potentials (pEPSP's) in the dentate gyrus, and this response was scored for the early monosynaptic component. A: average of the 30 baseline test responses (Pre) superimposed on the average of the first 30 test responses (Post) of the postconditioning test period. Note the decreased early slope of the pEPSP following conditioning of the converging ipsilateral input. Scale 0.5 mV, 2.5 ms. B: time course. The mean of the baseline test period is set at 100%. Each datapoint is an average of 5 consecutive responses obtained at one per min. The downward arrow indicates the time of the conditioning stimulation. Note the persistence of the synaptic depression and its stability across the 15 h postconditioning test period.](image-url)
Fig. 1 of ref. 12; see also ref. 15. By studying the longevity of activity-dependent depression from an unpotentiated baseline, we can discount the possibility that this depression is limited solely to experimentally potentiated synapses or that the longevity of this depression is due to a passive decay of experimentally induced LTP. Of course, if one believes that synapses develop normally by starting out small and weak and then grow larger and stronger due to a natural version of experimentally induced LTP, then the depression studied here is also a removal of potentiation.

The apparently equivalent longevity in the dentate gyrus of activity-induced potentiation and activity-induced depression at all poststimulus durations tested is consistent with an associative synaptic modification that does not saturate to an equal value at all synapses13. Thus the experimentally studied associative modifications of the dentate gyrus together constitute a viable memory storage process.

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