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Refining the temporal definition of an association at the neuronal level using long-term potentiation and long-term depression in the dentate gyrus

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This study sharpens the temporal definition of an association in the context of long-term synaptic modification of the monosynaptic projections from entorhinal cortex to dentate gyrus in the anesthetized rat. The ipsilateral projection produces a powerful postsynaptic excitation and shows synaptic potentiation following 7.5 ms trains. On the other hand, brief, high-frequency contralateral stimulation just after this powerful postsynaptic excitation is followed by long-term depression (LTD) at the synapses of this pathway. Therefore, based on the differential induction of long-term potentiation (LTP) or LTD, a cell can distinguish associated pre- and postsynaptic activation at a temporal resolution at least as small as 7.5 ms.

Associative synaptic modification has two faces in the dentate gyrus: long-term potentiation (LTP) [12] and long-term depression (LTD) [2, 8, 9]. Based on previous research [9], we can state, with a resolution of 25–40 ms, that whether or not a particular afferent response potentiates or depresses depends upon the temporal relationship between its afferent activity and a powerfully excitatory postsynaptic event. In particular, if weakly excitatory afferents are active just before or coincident with a synaptically induced postsynaptic excitation, then there is LTP of the synaptic response of the weakly excitatory afferents. On the other hand, if the weakly excitatory afferents are inactive or if they are active after the powerfully excitatory synaptic event, then there is LTD of the synaptic response of the weakly excitatory afferents.

The present experiments were undertaken to increase the temporal resolution of these observations below the time constant of the postsynaptic cell because such results are particularly challenging to biophysical models of LTP [6, 10, 16]. Intuition suggests that the integrating time constant of the postsynaptic cell ($\sim$10 ms) should limit the precision to which a cell can sense the timing of synaptic excitations.

Greater temporal resolution is achieved by using conditioning trains of three pulses rather than trains of eight pulses. At a resolution of 7.5 ms, the induction of LTP and LTD depends on the order of pre- and postsynaptic activation. A preliminary report of these results has appeared [5].

The experiments used male adult Sprague–Dawley rats anesthetized with urethane and maintained at a body temperature between 36.5 and 37.5°C. The basic electrophysiological methods have been described previously [8, 9, 11]. In each animal there were 4 measurable EC-DG responses — a right and left ipsilaterally evoked response and a right and left contralaterally evoked response. Stimulating electrodes were lowered into the angular bundle, and saline-filled micropipettes were positioned bilaterally at 2.7 mm posterior to bregma and 1.6 mm lateral to the midline. The pipettes were lowered approximately 3.6–3.8 mm into the hippocampal dentate gyrus. The final location of each recording electrode was determined by mapping the evoked field responses and by monitoring granule cell firing. The ipsilaterally and contralaterally evoked population excitatory postsynaptic potentials (pEPSPs) were required to map similarly across the dentate gyrus, positive in the somatic and hilar layers and negative in the middle molecular layer. The final depth of each recording electrode optimized the positive-going synaptic potential. At the end of each experiment, each response was again mapped to verify that there had been negligible electrode movement dur-

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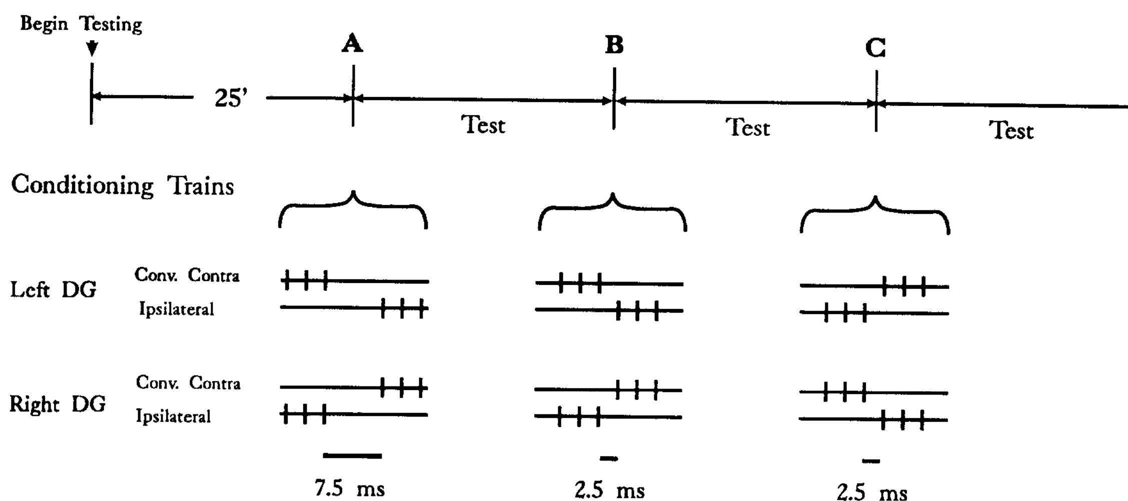

Fig. 1. The experimental paradigm. The upper part of the figure depicts the overall sequence of the 3 conditioning paradigms (A–C) and the interposed 25 min test periods. The lower part of the figure illustrates essential aspects of these 3 conditioning paradigms from the viewpoint of both the left and of the right dentate gyrus. Note that the weak converging (conv.) contralateral (contra) response must follow the strong ipsilateral response on one side of the brain if the converging contralateral response leads on the other side. In conditioning period A there is a 7.5 ms interval between ipsi- and contralateral trains while in periods B and C there is a 2.5 ms interval. Between periods B and C the ordering of the ipsi- and contralateral trains are reversed. In each conditioning paradigm the interpulse interval of the 3 pulse trains is 2.5 ms. In each conditioning paradigm trains are given 16 times at 1/200 ms.

ing the experiment. Electrode tracks were confirmed histologically in some animals.

To monitor synaptic efficacy, the two angular bundles alternately received test stimulation once every 15 s (i.e. each angular bundle received test stimulation every 30 s). Test stimulation was a single monophasic, constant current square pulse of 0.1 ms duration. The stimulation intensity was above the threshold to elicit an ipsilateral population spike and was sufficient to evoke a minimum of a 0.5 mV (but never more than 1 mV) contralateral pEPSP at the outset of the experiment. Each EC-DG response was fed through high-impedance preamplifiers (Getting model 5 and WPI model M-707) to AC-coupled amplifiers with low pass filter settings of 3 kHz. The analog response was then digitized at a 20 kHz sampling rate using an A-D converter (Analog Devices RTI-800-F) and stored for subsequent off-line analysis.

The stimulation protocol (Fig. 1) consisted of 7 sequential intervals: (1) a preconditioning baseline test period; (2) conditioning period A; (3) an intermediate test period; (4) conditioning period B; (5) an intermediate test period; (6) conditioning period C; and (7) a final test period. All test periods had a duration of at least 25 min.

The conditioning paradigm employed trains applied bilaterally but asynchronously to the two angular bundles (see Fig. 1). On each side, the ipsilateral response served as a powerfully excitatory input, and the converging contralateral response served as the weak test input. In all experiments, conditioning stimulation consisted of 16 trains of 3 pulses at 400 Hz with 200 ms between trains. Because the EC-DG projection is bilateral, the asynchrony of converging contralateral and ipsilateral activity is reversed in each dentate gyrus (see Fig. 1). In conditioning period A, the leading train delivered to one angular bundle led the train applied to the other angular bundle by 12.5 ms (7.5 ms last pulse to first pulse). In conditioning period B, the leading train led the trailing train by 7.5 ms (2.5 ms last pulse to first pulse). In conditioning period C, the previously trailing train led by 7.5 ms. The intensities of test and conditioning stimulation were constant throughout.

Pilot experiments revealed that trains of 3 pulses result in smaller amounts of synaptic modification than trains of 8 pulses. In order to enhance the size of the modifications, we used the 'theta' or prime burst paradigm [3, 7, 13]. In addition the sensitivity for measuring depression was enhanced by conditioning periods A and B (Fig. 1). Even though depression can be induced before experimentally induced potentiation [2], it is our experience [8] that it is much easier to observe depression after experimentally induced potentiation. Thus, we used the initial pair of potentiating trains, A and B (see Fig. 1), to enhance the sensitivity for detecting the smaller amounts of LTD produced by these very short trains.

Quantification of the digitized responses consisted of off-line measurements of the initial slope (within the first 4 ms of the stimulus artifact) or amplitude of the positive-going pEPSPs. In some animals, quantification of the contralateral pEPSP was difficult either because of its small size or because of contamination by volume-con-

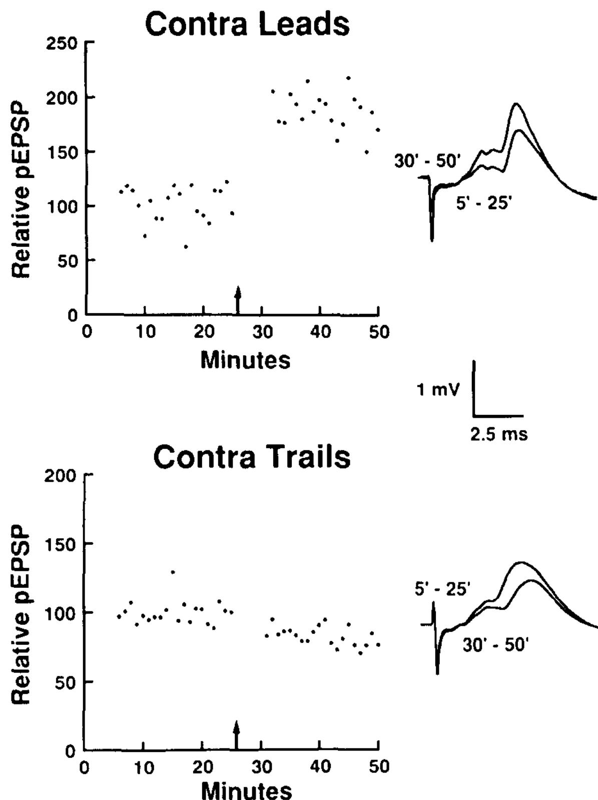

Fig. 2. Contralateral modification after condition C. This figure shows the mean contralateral responses ($n=7$) for the test periods immediately preceding and following the last conditioning period (C of Fig. 1 and indicated by an arrow in this graph). The responses of each animal were normalized to 100% by comparison to the 20 min average of the appropriate test response of each animal just before conditioning C. The average of each test response is plotted for the last 20 min of each of the two test periods. The pairs of waveforms to the right of each graph show a contralateral response of an individual animal before conditioning (average of test responses running from minutes 5 to 25) and after conditioning (average of test responses running from minutes 30 to 50). The response pairs were chosen as representative because they each come closest to matching the overall average changes.

ducted potentials generated simultaneously on the opposite side of the brain by ipsilateral activation. Of the 7 animals and 14 contralateral responses in this study, the two such contaminated contralateral responses were quantified by measuring response amplitude (at a fixed latency) rather than response slope. In all cases that could be evaluated, the correlation between slope and amplitude as synaptic modification measures was high. The first 5 min of responses after conditioning were ignored to avoid studying short-term changes. Response values were normalized by considering the mean of the preceding test period as 100%. The means of such test periods were analyzed statistically using a *t*-test. For a result to be considered statistically significant, a $P<0.05$ was required. Linear regressions over time were used to evaluate trends in response size.

The ipsilateral responses potentiated significantly after conditioning period A ($57.7 \pm 13.2\%$, $P<0.005$ on the leading side and $44.1 \pm 12.8\%$, $P<0.014$ on the trailing side). There was little change in either ipsilateral response following subsequent conditioning periods (less than 3.5% increase for all cases).

For the contralateral responses, conditioning trains A and B produced the changes expected from our earlier studies, but as anticipated, the depression was not statistically significant.

Fig. 2 shows the preceding 20 min baselines and the results of the final conditioning period (conditioning C; see Fig. 1). Regression analysis indicates that these baselines were stable and that any trends were in the opposite direction of the changes to be induced. The baseline period for the leading contralateral response (upper panel of Fig. 2) changed at the rate of -0.06%/min. The baseline period for the trailing contralateral response changed at the rate of 0.01%/min. Following these baselines, conditioning stimulation with a 2.5 ms intertrain interval produced the anticipated results. On the side where weak contralateral conditioning stimulation followed the powerfully depolarizing ipsilateral stimulation, significant depression resulted (mean decrease $= 19.7 \pm 5.8\%$; $P<0.015$). Simultaneously, on the other side of the brain, the reverse ordering (weak precedes strong; see Fig. 1) produced potentiation (mean increase $= 84.6 \pm 28.7\%$; $P<0.026$).

The results of this study extend earlier observations on the temporal requirements for synaptic modification in the dentate gyrus [9], and the results concerning LTP are consistent with observations in CA1 [1, 4].

A comparison of the temporal characteristics of the different conditioning paradigms used here leads to the conclusion that a cellular process can distinguish between the associated synaptic activity leading to LTP or LTD with a resolution of 7.5 ms or less. Because the ipsilateral system provides its own powerful postsynaptic activation, we conclude that a 3-pulse 7.5 ms train of an afferent test system, simultaneous with strong postsynaptic activation, potentiates the afferent test synapses (the ipsilateral system in this case). On the other hand, a slight delay in the activity of the afferent test system during conditioning prevents LTP. More precisely, contralateral trains beginning only 2.5 ms after the last strong pulse — or, equivalently, 7.5 ms after the first strong pulse — are followed by depression of the test synapses. Thus by virtue of the differential induction of LTP or LTD with the different paradigms, we hypothesize a cellular process that can qualitatively distinguish between the small timing difference.

It is a challenge, however, to explain such timing data in quantitative models of associative synaptic modification [6, 10, 16]. For example, one model of a dentate

gyrus granule cell [6] has been able to simulate the events that do — and the events that do not — cause LTP. These successful simulations include the earlier, less precise timing results (but do not include the results presented here). The model correctly predicts the older timing data because of the relatively slow turn-on ($\sim$5 ms) and turn-off ($>$30 ms) kinetics of the *N*-methyl-D-aspartate (NMDA) receptor. (The delayed turn-on produces the weak-then-strong ordering requirement for LTP of the weak system; the slow turn-off determines how great the latency between weak and strong excitation can be and still produce LTP in the weak system.) However, it is not at all clear that this biophysical model [6] will be able to fit the present data. The difficulty is that current estimates of the cell time constant (at least 12 ms but probably more [14, 15]) and of the quantitative on-off kinetics of the synaptically activated NMDA receptors and voltage activated channels do not seem sufficiently short to produce the subtle temporal dependence reported here for potentiation and depression.

A modification to the biophysical model may result in a suitable fitting of the present data. Recall that depression of the weak pathway results even when there is activation of the strong pathway without any activation of the weak pathway at all [8]. It is therefore sensible to include at each synapse a depression process that opposes potentiation there *whenever* sufficient postsynaptic excitation exists to induce LTP. That is, the change in efficacy of each synapse would be a superposition of both synaptic modifications. Net potentiation would then result when the subcellular events leading to a stronger synaptic strength outweigh the subcellular events leading to a weaker synaptic strength.

In this hypothesis the synaptic weakening process could compensate for a small tendency toward potentiation that would occur at short intervals of strong ipsilateral conditioning followed by weak contralateral conditioning. Thus, the model, now with two opposing modification processes, might reproduce the present results even though neither modification process alone has sufficient temporal resolution to explain the data.

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