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Long-term potentiation in the interpositus and vestibular nuclei in the rat

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Summary. Previous unpublished experiments from this laboratory had revealed only post-activation depression effects in the cerebellar cortex when its inputs were activated by high frequency trains. In the experiments reported in this paper, we found reliable long-term potentiation (LTP) effects in the deep nuclei (interpositus and vestibular) when stimulation trains were applied to the white matter at the point where inferior peduncle fibers enter the cerebellum. LTP effects were found in both acute and chronic preparations. In the chronic preparations, LTP lasted for at least 8 days in all but one animal.

Key words: Plasticity – Long-term potentiation – Memory – Cerebellum – N. interpositus – N. vestibular

Introduction

High frequency activation of forebrain pathways can result in a long-lasting increase in the efficacy of the activated synapses. This effect, known as long-term potentiation (LTP), has been demonstrated many times in pathways into, within, and out of the hippocampus (Bliss and Lomo 1973; Alger and Teyler 1976; Schwartzkroin and Wester 1975; Andersen et al. 1977; Lynch et al. 1977; Douglas and Goddard 1975; Racine et al. 1983). It has also been shown to occur in many other forebrain pathways (Racine et al. 1983; Wilson and Racine 1983). Because it is long-lasting and specific to the activated synapses, it has been used to model memory phenomena (Goddard and Douglas 1975; Teyler and Discenna 1984).

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The cerebellum is believed to be involved in learning and memory, particularly for motor skills (Marr 1969; Albus 1971), so we have been attempting to demonstrate LTP in cerebellar pathways. If the mechanisms of memory storage at the synaptic level are the same across systems, and if LTP really is based upon a similar synaptic mechanism, then LTP should occur somewhere within cerebellar or related circuitry. Our initial attempts to demonstrate LTP in the cerebellum failed. We concentrated our efforts on the cerebellar cortex, stimulating both surface pathways and deep white matter, but found no signs of LTP in the 40 animals tested. We did, however, reliably evoke a depression lasting for several sec to several min.

Recently, we succeeded in producing LTP by activation of inputs to the deep nuclei, including the vestibular nucleus, as reported below.

Methods

Animals and surgery

Twenty Long-Evans rats, 350–450 g, were used in this experiment. Ten of these animals were used in acute experiments and the remaining 10 animals were used in chronic experiments.

Acute experiments

Under urethane anesthesia (2 g/kg), bipolar stimulating electrodes were implanted into the inferior peduncle, and monopolar recording electrodes were implanted into the interpositus nucleus (5 animals) or the vestibular nucleus (5 animals). The electrodes were made from teflon coated stainless steel wire, 250 μM, either single strand for the monopolar electrodes or twisted together for the bipolar electrodes. The stimulating electrode was lowered first to within 0.25 mm of the target site. Stimulus pulses were then applied through the stimulating electrode as the recording electrode was lowered towards its target site. When an optimal response was recorded, the stimulating electrode was slowly

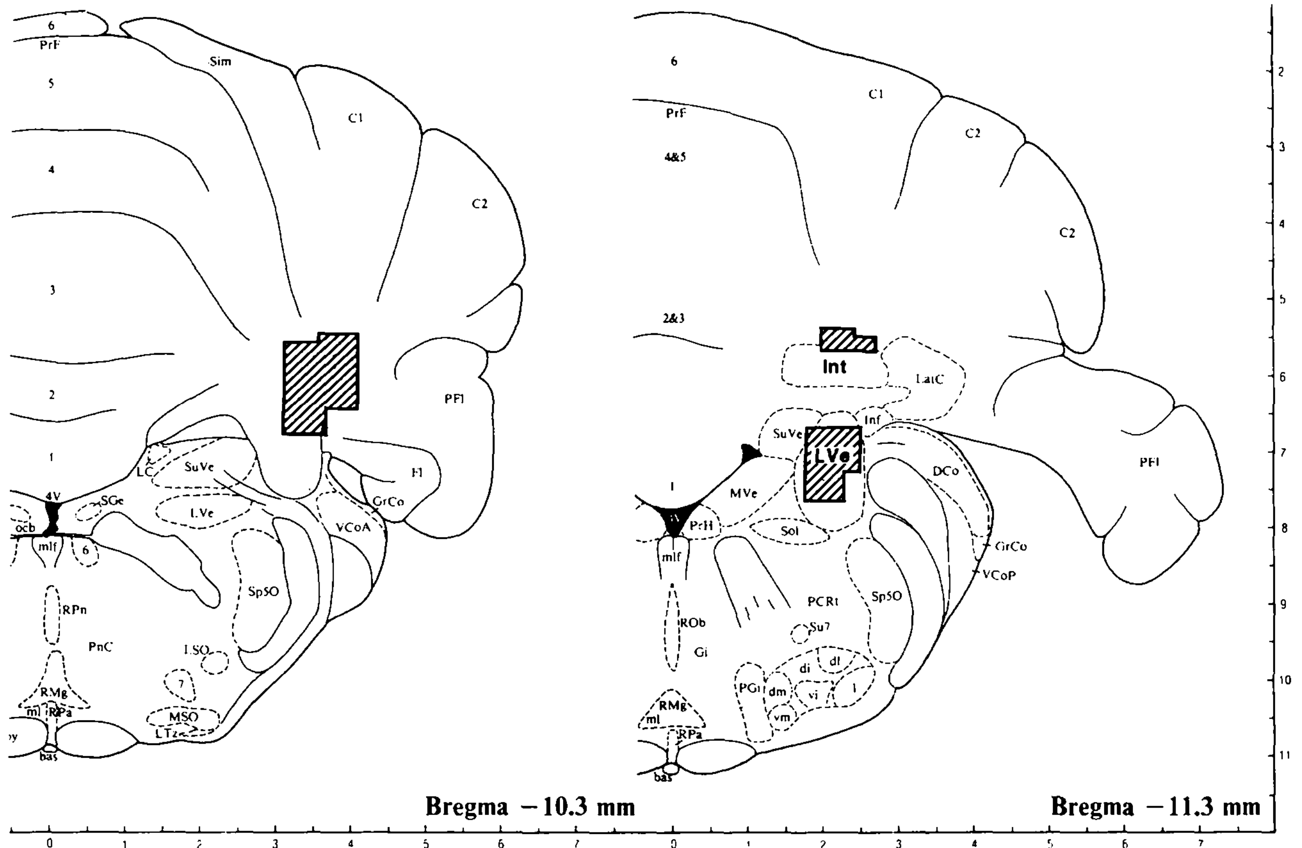

Fig. 1. The distribution of electrode tips for the animals used in these experiments are shown above. The stimulating electrode locations are indicated in the left atlas diagram and the recording electrode placements are indicated in the right-hand diagram. LVe: Vestibular nucleus; Int: Interpositus nucleus. Coordinates shown are with respect to Bregma. Diagrams are from Paxinos and Watson (1982)

advanced until the response no longer increased in size. The electrodes were then cemented in place. The stimulating pulses used during surgery were 100 μs in duration and 100 μs between pulses in the biphasic pulse pair. The intensity was set at 1000 μA peak to peak, and the frequency was 0.1 Hz.

Chronic experiments

The surgical procedures for the chronic experiments were the same as described for the acute experiments, except that stimulation pulses were not applied (the coordinates established during the acute experiments were used), and the anesthesia was sodium pentobarbitol (60 mg/kg). In addition, the amphenol pins to which recording and stimulating leads were attached were gathered together in a plastic connector which was cemented to the animals' skull. Three anchor screws were used to keep the connector in place and one of these screws was attached to a ground lead.

Stimulation and recording

With a few exceptions, the stimulation and recording procedures were the same for acute and chronic animals. Initially, stimulation pulses were applied via the peduncle electrode and the responses evoked in the nuclei were observed on a storage oscilloscope screen. The parameters were as described above except that the intensity was begun at 100 μA, peak to peak, and increased until the approximate threshold and maximal responses were found. An intensity/response (I/O) curve was then determined. Starting 25% below the estimated threshold intensity, the intensity was increased in 6 equal steps to end on an intensity that was 25% above that estimated to evoke a maximal response. This provided 7 points on which to construct an I/O curve. Five responses were evoked, sampled by the computer and averaged at each intensity.

Once the I/O testing was complete, the intensity which evoked a response that was 50% of maximal amplitude was used as the test intensity for the remainder of the experiment. LTP tests were then run on the acute animals using an ascending intensity train series. One hundred and twenty test pulses were delivered to the peduncle at 0.1 Hz to provide a baseline measure of response amplitude. A set of five trains was then delivered, one train every 2 s. The train duration was 50 ms and the intra-train pulse frequency 300 Hz. The train intensity was initially set at 200 μA peak to peak. Following delivery of this set of five trains, the test pulses were resumed for another 60 sweeps (10 min). The train intensity was then raised by 200 μA and another 5 trains delivered, followed by 60 test pulses. This was continued until the train intensity had reached its final value of 2000 μA peak to peak. Following the final set of 60 test pulses, the animals had received a total of 10 sets of trains and 780 test pulses. The 780 sweeps were stored on hard disk and later analyzed by an LSI 11 computer. Upon completion of this phase of the experiment, the I/O measures were repeated. The acute animals were then sacrificed,

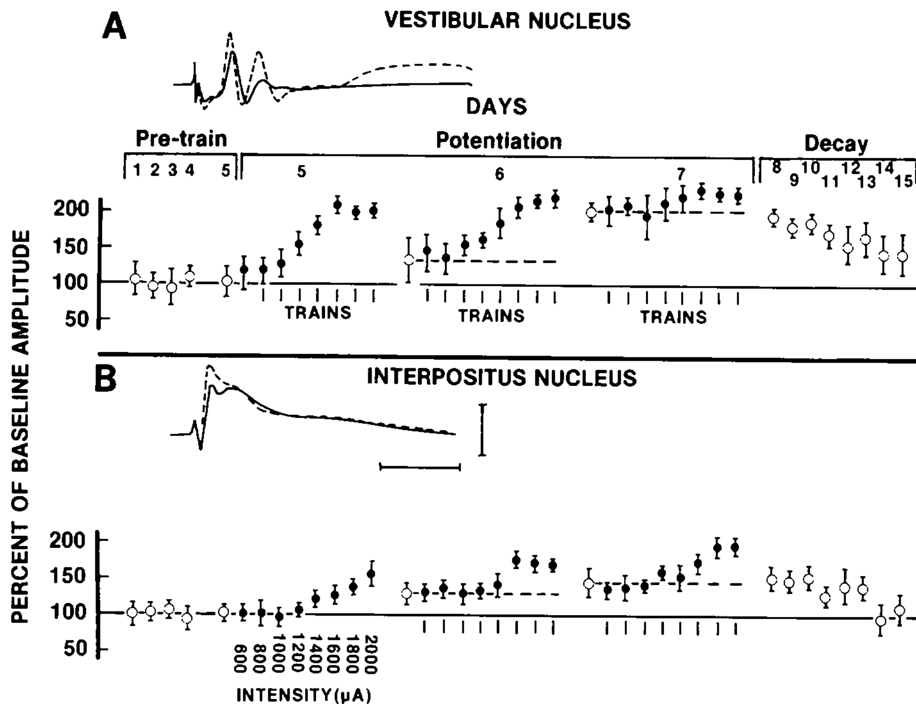

Fig. 2A and B. The results of the potentiation treatment are shown above for 2 chronic animals. **A** Vestibular nucleus; **B** Interpositus. The response amplitudes (± SEM) are all plotted as a percent of the day 5 pretrain average. The amplitudes are also determined for averages taken between 8 and 10 min after the application of each train in an ascending intensity train series on days 5, 6, and 7 (the first 2 sets of trains, at 200 and 400 μA, are not shown, because they produced no detectable effect in any of the animals). The decay of potentiation is shown for the 8 days from day 8 to 15. Also shown are averaged responses from day 5 (pretrain – solid line) and day 8 (24 h post-train – dashed line). Calibration: 500 μV (negative up) and 5 ms

the brains removed, fixed, sectioned and stained with thionine.

The procedures for the chronic animals were similar, except that an average based on 20 responses was determined every day for 5 days to determine the stability of the response over time. The LTP tests for the chronic animals were begun on day 5. Also, the chronic animals were administered the ascending intensity train series each day for 3 days (days 5, 6 and 7). Finally, the chronic animals were given 20 test pulses each day for 8 days to determine the duration of the LTP effects, after which they were also sacrificed and their brains sectioned to determine the position of the electrode tips.

The responses evoked in the vestibular nuclei had two or more components that appeared to be population spikes. The amplitude of both spikes was used as measures of the amplitude of the response. The interpositus responses were more variable. The amplitude of the first, negative, component of the potential was measured for purposes of comparison.

Results

Electrode placements and baseline responses

The location of the electrode tips is shown in Fig. 1. The vestibular placements were all within the nucleus. The interpositus electrodes were less accurately placed, and centered on the dorsal border of the nucleus. The interpositus electrodes in one acute and one chronic animal were just outside the nucleus, but the responses and LTP effects, though slightly weaker, were similar to those of the other animals. Two of the acute and one of the chronic interpositus animals were discarded because of inaccurate electrode placements and poor responses. One chronic interpositus animal lost his electrode assembly before the LTP tests. The inferior peduncle placements were somewhat shallower than originally intended and the final location was where the peduncle fibers enter the cerebellum.

The baseline responses were consistent across animals, particularly in the vestibular nucleus. Sample responses from both nuclei are shown in Fig. 2. As can be seen, the vestibular responses contained one or more fast negative components that appeared to be population spikes. Confirmation of this was obtained by single cell recording experiments in 4 additional animals. Cells in the vestibular nucleus tended to fire in 'doublets', with inter-spike intervals equivalent to the interval between our presumed population spikes. The double and triple population spikes seen in that nucleus, then, were at least partly due to individual cells firing two or more action potentials. The amplitude measures shown in Table 1 and Fig. 2 were taken from the 2nd population spike. The 1st population spike showed similar effects, but they were not as large. A rapidly decaying short-term component of potentiation was evident in some, but not all, animals. It was not analyzed.

LTP effects in interpositus and vestibular nuclei

LTP was reliably produced in the vestibular nuclei in both acute and chronic preparations, and in the interpositus in the 3 chronic preparations and in 2 of

Table 1

A. Chronic preparations			
		Pre-train day 5 amplitudes as percent of day 2 amplitudes	Post-train day 8 amplitudes as percent of pre-train day 5 amplitudes
Vestibular nucleus (2nd "population spike")	1	107	193
	2	102	158
	3	92	170
	4	96	126
	5	105	195
Interpositus nucleus	11	100	150
	12	106	137
	13	104	128

B. Acute preparations			
		Pre-train amplitudes at 18–20 min as percent of amplitudes at 0–2 min	Post-train amplitudes 8–10 min after last train as percent of pre-train amplitudes
Vestibular nucleus	6	100	113
	7	102	110
	8	103	145
	9	103	122
	10	98	125
Interpositus nucleus	16	108	118
	17	95	120
	18	102	96

the 3 acute preparations. The effects were larger in the vestibular nucleus than in the interpositus. The responses actually appeared to be slightly depressed at the low end of the I/O curves (first 2 or 3 intensities) but were clearly potentiated at all remaining intensities. The potentiated responses also reached higher asymptotic amplitudes.

The LTP effects in the chronic animals had half-decay times ranging from 4 to beyond the 8 days tested. Fitting these data with single exponentials yielded estimated half-decay times ranging from 4 to 12 days (with a mean of 9 days). There were no clear differences between the nuclei in the duration of LTP. The data for these experiments are summarized in Table 1.

Discussion

LTP was produced in both the interpositus and vestibular nuclei, and the effect was found to last for several days. The effects were stronger and more reliably produced in the vestibular nucleus, but it is not yet clear if this is due to a real difference in plasticity or to non-optimal electrode placements in the interpositus nuclei.

There are indications that both of these nuclei are involved in behavioral plasticity. The vestibulo-occular motor reflex (VOR), for example, has been shown to be capable of modification in experiments using inverting lenses or telescopic lenses (Davies and Melville-Jones 1976; Miles and Eighmy 1980; Miles and Baitman 1980; Ito 1982). Humans or animals wearing these lenses gradually correct the resulting error in the VOR. If telescopic lenses increase the angle of apparent movement of the visual field, the subjects will initially show a corresponding error in the VOR. Over a period of several days the VOR shows a change in gain which lasts for as long as the distorting lenses are worn. When removed, it takes several days to adjust back to a normal visual displacement. There is still some question about where in the VOR system this change is taking place, but the cerebellum appears to be involved (Ito 1982). Several lines of evidence indicate that the plasticity may be centered within the vestibular nucleus itself (Miles and Lisberger 1981; Galiana and Outerbridge 1984; Galiana et al. 1984).

Plasticity is also demonstrated in the nictitating membrane reflex which can be classically conditioned. Thompson and co-workers (McCormick et al. 1982; Lincoln et al. 1982; McCormick et al. 1982; McCormick and Thompson 1984; Clark et al. 1984) have carried out a number of experiments in an attempt to determine the neural substrates of this learned response. Although several structures appear to show altered responses as a result of conditioning of the nictitating membrane reflex (e.g., the hippocampus), few of them are critical to the learned response. Exceptions are the deep nuclei of the cerebellum, including the interpositus nucleus (Clark et al. 1984; Glickstein et al. 1983). Damage to this system abolishes the learned response.

As can be seen in Fig. 2, short latency components appeared to be potentiated as well. The largest component of the interpositus response, in fact, appeared with a very short latency. These may have been due to fiber responses, but there is very little compelling evidence for potentiation of such responses in the potentiation literature. We have not yet attempted an electrophysiological analysis of the components of these nuclear responses. Until that is done, we are limited in the conclusions that we can draw from these data. The characteristics of the LTP effect in these nuclei, however, appears superficially similar to that reported in several other brain pathways (e.g., Racine et al. 1983).

All of our previous observations suggested that the cerebellar cortex does not support LTP in response to the usual experimental inputs. The deep nuclei, including the vestibular nucleus, do appear to support LTP. As described above, some evidence in the literature suggests a similar distinction with respect to more normal types of plasticity (e.g., classical conditioning). The cerebellum thus provides a system in which one set of cells (the Purkinje cells) appears to lack the type of plasticity monitored here, while other sets of cells (those within the deep nuclei) support LTP (and possibly information storage as well). Further comparisons of these sites may shed some light on the necessary conditions for LTP.

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