Brain Research, 268 (1983) 339–343
Elsevier Biomedical Press

Short Communications

Evidence for late development of inhibition in area CA1 of the rat hippocampus

KRISTEN M. HARRIS* and TIMOTHY J. TEYLER

Northeastern Ohio Universities College of Medicine, Rootstown, Ohio (U.S.A.)

(Accepted February 1st, 1983)

Key words: recurrent inhibition — area CA1 — hippocampal slice

Development of recurrent inhibition in area CA1 of the rat hippocampus was monitored by a paired-pulse stimulation test. Inhibition of the second response was never observed before postnatal day six. In addition, a type of epileptiform activity, referred to as spontaneous unison firing, was observed frequently, only on postnatal days 4 and 5. Together these observations suggest that the inhibitory interneuronal network of area CA1 becomes effective on about postnatal day 6.

Recent experiments on the physiological development of synaptic inhibition and excitation in area CA1 of the rabbit hippocampus have demonstrated a relatively later development of inhibition[14,16]. Intracellular measures from the CA1 pyramidal cells revealed synaptic excitation in the fetal rabbit[16]. In contrast, long latency IPSPs were not seen in these cells before postnatal day 6[14]. Cells with some of the characteristics of inhibitory interneurons were first encountered on postnatal days 4–6, although these properties were not fully mature until postnatal day 14[14]. In addition, epileptiform activity was prominent in the rabbit hippocampus before postnatal day 14[14,16]. Ultrastructural studies revealed a relatively late development of synaptic contacts containing flattened vesicles, on CA1 cell somas[17]. We report here observations from area CA1 of the rat hippocampus that suggest a similarly late development of inhibition in this species. These observations were made during a study on the development of long-term potentiation in area CA1[8].

We used the method of paired-pulse stimulation[5,13] at inter-stimulus intervals of 15–30 ms to test for recurrent inhibition in area CA1 of the developing rat hippocampus. This inhibition is probably mediated through recurrent inhibition by basket cells on the CA1 pyramidal cells in adult animals[1–3,10]. In addition, we have observed a type of epileptiform activity in 4- and 5-day-old animals that could reflect the absence of recurrent inhibition[4,6,7].

Experimental parameters have been described in detail elsewhere[8]. Briefly, rats of the Long–Evans strain were randomly selected from their litters on postnatal days 1–10, 15, and 60. Hippocampal slices were prepared and maintained in vitro using standard procedures[18]. Following an incubation period of 1 h, a concentric bipolar stimulating electrode was positioned in stratum radiatum, and a recording micropipette was positioned in stratum pyramidale of area CA1. One slice from each of 37 animals was tested. Paired pulse stimulation was delivered at two or three stimulus intensities adjusted to produce sub-population spike threshold, population spike threshold, and supra-population spike threshold responses on the first stimulation. Two or three inter-stimulus intervals of 15, 20, and 25–30 ms were tested for each stimulus intensity. The amplitude of the population spike was measured from halfway between the two positive potentials to the negative peak for each response. If the response was sub-population spike

* To whom correspondence should be addressed at: Neurocytology Laboratory, Department of Neurology, 352 Warren Building, Massachusetts General Hospital, Boston, MA 02114, U.S.A.

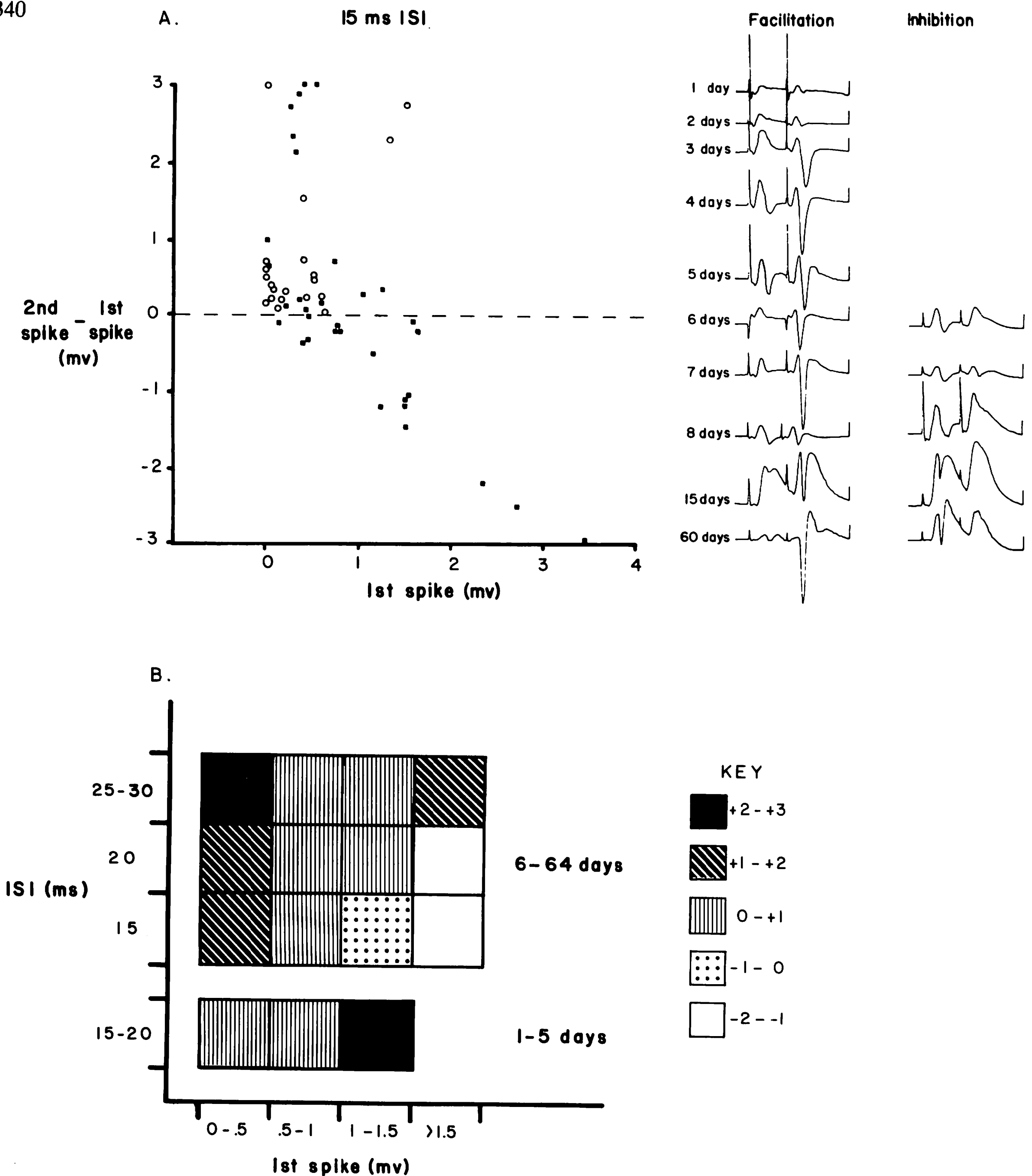

Fig. 1. Response characteristics from paired-pulse stimulation. A: the change in the population spike amplitude (2nd spike minus 1st spike) is plotted against the amplitude of the first population spike for 15 ms ISI. Each point is the value obtained from a single test of paired pulse stimulation. The dashed line at zero indicates no change in the amplitude of the response. Points above this line indicate facilitation and points below this line indicate inhibition. Data were combined into two age groups of 1–5 (14 animals, open circles) and 6–64 (23 animals, closed squares) because inhibition was never seen before postnatal day 6. Facilitation was always observed from 1–5-day-old animals (all open circles are above the zero dashed line). The 6–64-day-old animals showed facilitation or inhibition when the size of the first population spike was less than 1.5 mV, and only inhibition when the first population spike was greater than 1.5 mV. Columns to the right of these graphs show representative waveforms demonstrating facilitation and inhibition. A time calibration pulse of 5 ms precedes, and a voltage calibration pulse of 1 mV follows each pair of responses. B: influence of ISI and amplitude of the first population spike on the magnitude of facilitation or inhibition observed during paired-pulse stimulation. The average magnitude of change in the size of the population spike is plotted as a function of ISI and amplitude of the first population spike. The upper three rows are means from 6–64-day-old animals and the lower row is from 1–5-day-old animals (15 and 20 ms ISI combined). The key shows the density plotted if the mean was within the listed range. Facilitation is in the range of 0 mV to +3 mV and inhibition is in the range of —2 mV to 0 mV. (See text for discussion).

threshold, then the value assigned was zero.

Changes in the amplitude of the population spike on the second stimulation were measured for each stimulus intensity and inter-stimulus interval (ISI). Paired-pulse facilitation occurred if the second population spike was larger than the first population spike, and paired-pulse inhibition occurred if the second population spike was smaller than the first population spike. Paired-pulse inhibition was never seen before postnatal day 6. This absence of paired pulse inhibition before postnatal day 6 raised two questions. First, were the CA1 pyramidal cells stimulated strongly enough to activate the inhibitory network? Second, how did the amplitude of the first response interact with the length of the ISI to produce facilitation or inhibition of the second response?

In order to analyze how the size of the first response influenced the probability of seeing facilitation or inhibition on the second response, the data for each ISI were graphed as the difference between the amplitudes of the second and the first population spikes relative to the amplitude of the first population spike. The graph in Fig. 1A illustrates this relationship for 15 ms ISI. Paired-pulse inhibition occurred across the entire range of first population spike amplitudes for the animals older than 5 days (closed squares below the dashed line). In contrast, the 1–5-day-old animals showed only paired-pulse facilitation (all open circles are above the dashed line).

The probability of observing facilitation or inhibition was related to the size of the first response and the length of the ISI for the 6–64-day-old animals, but not for the 1–5-day-old animals. In Fig. 1B, the average magnitude of facilitation or inhibition is plotted as a function of ISI and size of the first population spike. First, compare the two age groups at 15 and 20 ms ISI. For the 6–64-day-old animals, the amplitude of the second population spike decreased as the amplitude of the first population spike increased, thus showing more inhibition. Contrast the data for the 1–5-day-old animals, where the amplitude of the second population spike increased as the amplitude of the first population spike increased, thus showing more facilitation. Slices from 6–64-day-old animals that showed inhibition at 15 and 20 ms ISI, usually showed facilitation at the longer ISIs of 25–30 ms. For animals 1–5 days old, only 4 tests were given at these longer ISIs. They were comparable to the results obtained at 15 and 20 ms ISI and are not included here.

Together these results suggest that the absence of inhibition in the 1–5-day-old animals is probably due to an immature circuitry for recurrent inhibition and not merely an absence of sufficient stimulation of the CA1 pyramidal cells. Some caution must be exercised when interpreting these results, because not all stimulus intensities or ISIs were tested. It is possible that with other ISIs and stimulus intensities, the 1–5-day-old animals would also show inhibition. Other ISIs were tested in one 5-day-old animal; and intervals from 4 ms to 200 ms were all seen to be facilitatory. Before postnatal day 3, much larger stimulus intensities were required to evoke a response, and frequently, no population spike was seen on the first stimulation. In these animals, it was possible that the inhibitory interneurons were not activated. However, for the 4- and 5-day-old animals, population spikes elicited from the first stimulation were as large as some of those seen to produce inhibition in the older animals, suggesting that if the inhibitory interneu-

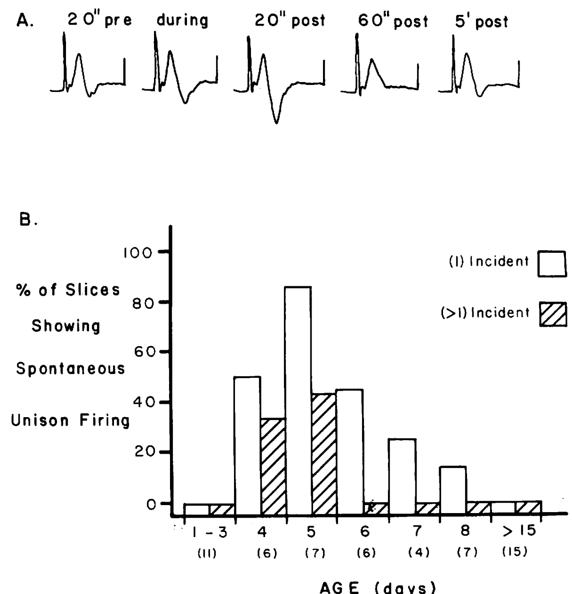

Fig. 2. Spontaneous unison firing. A: a representative example of spontaneous unison firing is plotted. The times are indicated in seconds ('') and minutes (') around and during a single incident. Calibration pulses are 5 ms pulses before the stimulus artifact and 1 mv pulses at the end of each waveform. B: the percentage of animals (one slice per animal was tested) showing one or more incidents of spontaneous unison firing are graphed for the indicated ages. The total number of animals tested in each age group are listed in parentheses under the graph.

rons were effective, they should have inhibited the second response.

Spontaneous background activity of CA1 cells was monitored throughout these tests and during further experimentation. Changes in the rate of spontaneous activity were noted, and an unusual pattern of increase in activity was seen particularly on postnatal days 4 and 5. We refer to this activity as 'spontaneous unison firing'. 'Spontaneous unison firing' was distinguished from spontaneous firing of individual cells in 3 ways. First, an increase in spontaneous background activity was heard on the audiomonitor. Second, when a stimulus was given during this increased spontaneous activity, the evoked population response was enhanced (Fig. 2A, during and 20'' post) relative to that elicited when little or no background activity was heard (Fig. 2A, 20'' pre). Third, at the cessation of the spontaneous unison firing, the evoked population response was somewhat depressed (Fig. 2A, 60'' post), but recovered to pre-spontaneous unison firing levels within 5 min.

It is possible that spontaneous unison firing is a developmental form of seizure activity. In hippocampal slices from adult animals, seizure activity is followed by a complete suppression of the evoked and spontaneous responses, and recovery to pre-seizure response amplitude usually requires at least 20 min[9] (and Harris and Teyler, unpublished observations). We distinguished spontaneous unison firing in the developing animals from adult-like seizure activity because the response was not completely suppressed following spontaneous unison firing since, a field EPSP could be evoked, and complete recovery occurred in less than 5 min.

The percentages of slices showing spontaneous unison firing during an experiment are plotted in Fig. 2B. Multiple incidents of spontaneous unison firing were only seen in slices from 4- and 5-day-old animals. For these slices spontaneous unison firing was seen periodically throughout all phases of the experiment, and was not related to the type or magnitude of stimulation given. The incidence of spontaneous unison firing decreased markedly after postnatal day 6.

The observation that spontaneous unison firing occurs just prior (postnatal days 4–5) to the time when paired-pulse inhibition is first observed (postnatal day 6), lends further support to the hypothesis that inhibitory innervation on CA1 pyramidal cells is not present in the rat hippocampus before postnatal day 6. Spontaneous epileptic seizures are thought to be prevented in the normal adult hippocampus by recurrent inhibition[7]. If the inhibitory interneuronal network were functionally effective in the 4–5-day-old animals, we would expect the spontaneous unison firing to be similarly prevented by recurrent inhibition.

These observations are in close agreement with results of intracellular recordings from the rabbit hippocampus, where long-latency IPSPs were rarely seen in CA1 pyramidal cells, and cells with response characteristics of inhibitory interneurons were not encountered before postnatal days 4–6[14,16]. They are in contrast with results from CA1 pyramidal cells of the kitten hippocampus where IPSPs were seen at birth[12,15] (except see argument in Schwartzkroin[14]). Minkwitz[11] has shown, via light microscopy of Golgi impregnations, that CA1 interneurons are present prenatally in the albino rat. Our results suggest that some postnatal maturation is required before these interneurons begin to exert their inhibitory effect on the pyramidal cells.

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