

Age Differences in a Circadian Influence on Hippocampal LTP

KRISTEN M. HARRIS* and TIMOTHY J. TEYLER

Program in Neurobiology, Northeastern Ohio Universities, College of Medicine, Rootstown, OH 44272 (U.S.A.)

(Accepted July 20th, 1982)

Key words: long-term potentiation — hippocampus — development — circadian rhythms — hippocampal slice

Data from several experiments on long-term potentiation (LTP) in the rat hippocampus were examined for circadian influence. Incidence and magnitude of LTP produced in both area CA1 and area dentata were analyzed, and a reciprocal light/dark difference was found in the two areas, with pyramidal cells of area CA1 showing more LTP during the light period and granule cells of area dentata showing more LTP during the dark period. In addition, results from experiments on developing animals, suggested that the circadian influence on LTP in either area was not present before postnatal day 20. All of these experiments were from hippocampal slice preparations; therefore, it is important to note that circadian influences on hippocampal LTP are preserved in the *in vitro* environment where tonic extrahippocampal input has presumably been removed.

INTRODUCTION

Periodic variations in biological function are features of many behavioral, hormonal and neural systems^{1,6}. In 1977, Barnes et al.² described a circadian rhythm of synaptic excitability in the dentate gyrus of the rat. Monosynaptic responses of dentate granule cells to perforant path stimulation were measured extracellularly. They found that field EPSPs and population spikes were of greater magnitude during the animal's dark period than during its light period. The cyclic changes in response magnitude were synchronized by, but not due to environmental factors such as light, temperature and ambient noise. Cauller et al.³ have recently extended these experiments in the rat dentate gyrus and they have found similar results for the field EPSP, in that it was largest during the animal's dark period. However, in contrast, they found that the population spike was largest during the animal's light period. Their results are compatible with observations made by West and Deadwyler¹⁹, showing that dentate granule cells were more excitable during the light period.

Winson and Abzug^{20,21} have also investigated fluctuations in granule cell excitability. They found that natural variations in the behavioral state were correlated with changes in population response amplitude. During alert wakefulness, the dentate field EPSP amplitude was elevated relative to that observed during rapid eye movement sleep (REM) and slow-wave sleep (SWS). Conversely, the population spike was reduced during the alert state and elevated during SWS and REM. Examination of responses in area CA1 revealed a different pattern of relationships between amplitudes and behavioral states. In area CA1, the population spike was of maximal amplitude during SWS and reduced during the alert and REM states.

These experiments demonstrate that excitability of the hippocampal cells is modulated on a circadian rhythm and is influenced by the behavioral state of the animal. With these observations in mind, we have analyzed several data bases^{5,8-10} to determine whether any light/dark variations existed in either the incidence or magnitude of long-term potentiation (LTP) in area CA1 or area dentata of the rat hippocampus. LTP is an enduring form of synaptic

* This work was submitted as part of a doctoral dissertation by KMH to the Neurobiology Program at Northeastern Ohio Universities College of Medicine and Kent State University. Current address: Neurocytology Laboratory, Department of Neurology, 352 Warren Building, Massachusetts General Hospital, Boston, MA 02114, U.S.A.

plasticity found in several pathways of the hippocampus¹. Our data were collected over several years, in different laboratories, using essentially the same methodology¹⁸. Data from both developing and adult animals were analyzed for circadian modulation of LTP.

MATERIALS AND METHODS

Sprague–Dawley or Long–Evans rats, aged from 6 days to adult (55–200 days) and of both sexes, with the majority of the subjects being male, were used for these experiments. All animals were obtained from vivariums that maintained a 12:12 hour light/dark cycle. Animals were taken from the vivarium at various times during their circadian cycles, approximately 15–30 min before the slice procedure was done. All animals were in illuminated surroundings after removal from the vivarium, and before preparation of the hippocampal slices. Hippocampal slices were obtained according to standard procedures¹⁸ and responses monitored to ensure a stable baseline prior to delivering a single tetanus of 33 Hz for 3 s. For most of the experiments, the stimulus intensity was adjusted prior to tetanus to give about a 1 mV population spike. Exceptions are the data from developing animals presented in the first 4 columns of Fig. 2. In these experiments, the stimulus intensity was adjusted to give a population spike at half the magnitude which produced the maximal population spike obtainable from that slice.

For the experiments on area dentata, stimulation was delivered to the fibers in stratum moleculare, and extracellular population responses were recorded from the granule cells. For the experiments on area CA1, similar procedures were used except that the stimulating electrode was placed in stratum radiatum and the recording electrode was placed in stratum pyramidale. In addition, responses of CA1 pyramidal cells were tested in one group of developing animals by giving a tetanus of 100 Hz for 1 s at a stimulus intensity which produced a population spike averaging 1 mV in magnitude prior to tetanus, (columns 5 and 6 of Fig. 2). For all of these experiments, post-tetanus responses were obtained at 5, 20 and 30 min after delivery of the tetanic stimulation. As a measure of LTP, the amplitude of the population spike obtained at 20 or 30 min post-tetanus was

normalized with respect to the amplitude of the pre-tetanus population spike. Data from 174 experiments were analyzed for light/dark differences in the incidence or magnitude of LTP produced by these procedures.

RESULTS

The results of this analysis are presented in terms of both the incidence and magnitude of LTP as a function of brain region (area dentata vs area CA1) and time of day (light vs dark). In Fig. 1, data from animals aged 20 days to adult are presented; Fig. 2 presents the data from animals aged 6–20 days. In both figures, data from males and females have been combined because no sex-related differences in the incidence or magnitude of LTP were observed (χ^2 - and *t*-tests¹⁵, $P > 0.1$). The ages were separated into the two groups because it was only after 20 days that the adult pattern in light/dark differences appeared. When data from animals aged 20–30 days was analyzed separately from the adults, the pattern of incidence and magnitude of LTP was similar to the adult pattern, so their data were combined with the adult results. Results from animals less than 6 days old are not presented, because LTP is not produced reliably in area CA1 before 6 days of age⁹, and further experiments will be necessary to determine the exact onset of LTP production in area dentata of developing animals^{6,10}.

Fig. 1A shows that the incidence of LTP was different for the light and dark periods. Plotted are the percentage of slices tested during the light or dark period that showed more than 120% of pre-tetanus response levels during the post-tetanus tests. The dentate region was more likely to show LTP when the animal was taken from the vivarium during its dark period than during its light period, and a smaller reverse pattern was seen for area CA1 (dentate, $\chi^2 = 8.58$, $P < 0.01$, CA1, $\chi^2 = 0.91$, $P > 0.1$). When the incidence of LTP was compared across the two regions, area CA1 showed LTP more frequently than area dentata during the light period ($\chi^2 = 16.66$, $P < 0.001$). During the dark period, area dentata showed LTP more frequently than area CA1, although this difference was not statistically significant ($\chi^2 = 0.25$, $P > 0.1$).

The magnitude of LTP produced by the two

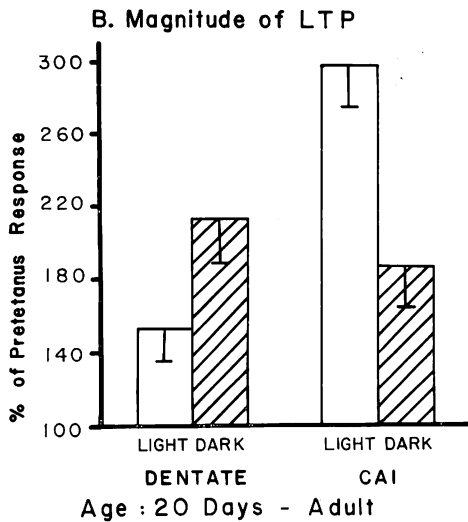
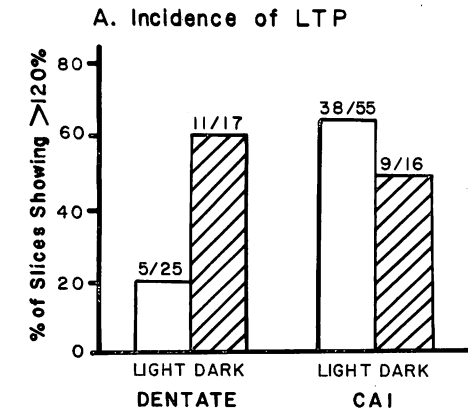


Fig. 1. A: incidence of LTP production is presented as a percentage of the total number of animals tested. The ratios reflect the actual numbers of slices (one hippocampus slice per animal) showing LTP out of those tested. B: average magnitudes of LTP attained by those slices showing more than 120% of pretetanus control are presented as means with S.E.M. See Results for further discussion.

regions, was also analyzed for light/dark variations. Fig. 1B shows LTP magnitude (as a percent of pretetanus response magnitude) for area dentata and area CA1 in the light and dark periods. Only those slices showing LTP, as defined by a post-tetanus value of at least 120% of the pretetanus control values, were averaged. The pattern of light/dark differences in magnitude of LTP paralleled that seen for incidence of LTP, with area dentata having a larger magnitude of LTP (in those slices showing LTP) during the dark period, and area CA1 showing more LTP during the light period. Within

each area, the light/dark differences were tested by a *t*-test for independent means. For area dentata, the magnitude of LTP averaged $153.6 \pm 17.1\%$ during the light and $213.6 \pm 23.3\%$ during the dark ($t = 1.63$, $P > 0.1$), and for area CA1, LTP magnitude averaged $297.1 \pm 20.1\%$ during the light and $187.8 \pm 21.5\%$ during the dark ($t = 2.55$, $P < 0.02$). Comparisons between the two regions revealed a greater magnitude of LTP in area CA1 than in area dentata during the light period ($t = 2.54$, $P < 0.02$). During the dark period, the magnitude of LTP was not significantly greater in area dentata than in area CA1 ($t = 0.08$, $P > 0.1$).

The incidence and magnitude of LTP obtained in

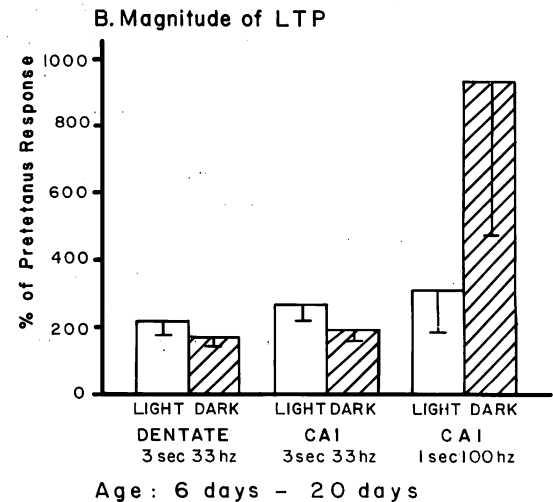
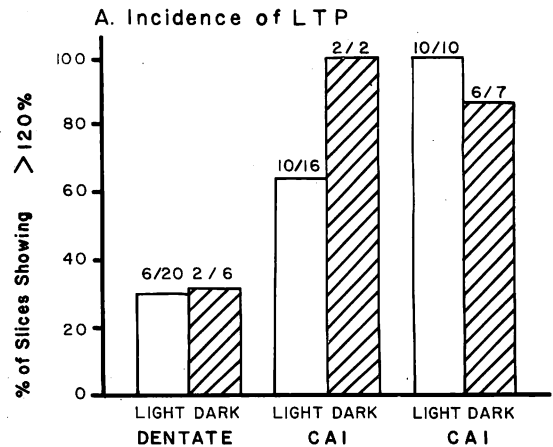


Fig. 2. Data are presented for animals (one hippocampal slice per animal) aged 6-20 days in the same format as for Fig. 1. See Results for further discussion.

slices from animals younger than 20 days, was also analyzed for light/dark differences. Fig. 2A shows the incidence of LTP in both areas during the light and dark periods. Note that two sets of data are presented for area CA1, with the first from experiments where tetanic stimulation of 33 Hz for 3 s was given, and the second from experiments where tetanic stimulation of 100 Hz for 1 s was given. When a χ^2 analysis was done on each of the 3 data sets, no differences were seen to be related to the light/dark cycle. In Fig. 2B, the magnitude of LTP attained under each of the 3 experimental conditions is presented. A *t*-test analysis revealed no differences related to the light/dark cycle for either area or stimulation paradigm. The pattern for LTP magnitude in area CA1 under the 100 Hz for 1 s paradigm appears to be reversed from the adult pattern. However, this reversal reflects that more 15-day-old animals were tested in the dark period than in the light period. LTP production reaches a peak during development sometime around 15 days; therefore, combining these data artificially raises the dark mean⁹.

DISCUSSION

This analysis indicates that the incidence and magnitude of hippocampal LTP are influenced by the light/dark cycle in rats older than 20 days. LTP is observed more frequently and in greater magnitude, during the light period for area CA1 and during the dark period for area dentata. With the results of Barnes et al.², we expect that these data reflect circadian rhythms, rather than modulation by transient environmental stimuli.

The combined results from developing animals less than 20 days old, show that circadian influence on LTP is not present from birth, but suggest that the modulation begins sometime during the third postnatal week. This developmental trend is in accordance with reports that circadian modulation

of other biological functions is also immature or not present in the rat before the third postnatal week^{4,7,11-13,16,17}.

While the underlying mechanism for this circadian influence on LTP is not understood, it is of interest that the circadian influence is preserved in the *in vitro* slice. In our studies, the slices were normally maintained in the *in vitro* environment for 1-3 h prior to experimentation, and were then studied further for several hours. Presumably, tonic extrahippocampal influences have been removed under these circumstances; therefore attention should be directed toward modulators that either are lost very slowly from the hippocampus, or have an enduring influence once they are activated.

The significance of this circadian influence on LTP production is unknown. To the extent that LTP might be a substrate for long-term information storage in the brain, it is conceivable that the attenuation of LTP in area dentata during the behaviorally inactive light period is correlated with an absence of active memory encoding during this period. Conversely, the enhanced LTP measures seen during the light period in area CA1, which is two synapses removed from area dentata, could reflect some aspect of post-encoding consolidation of recently acquired information.

These data should be considered as only preliminary indicators of a circadian modulation of hippocampal LTP and further systematic studies in both slices and intact animals will be required to determine the underlying mechanisms. However, the results suggest the potential existence of a circadian variable in experiments using the hippocampal slice.

ACKNOWLEDGEMENT

Supported by NIMH Predoctoral Fellowship 1 F31 MH08313-01 to K.M.H. and NSF Research Grant 78-23947 to T.J.T.

REFERENCES

- 1 Alger, B. E. and Teyler, T. J., Long-term and short-term plasticity in CA1, CA3, and dentate regions of rat hippocampal slice, *Brain Res.*, 110 (1976) 463-480.
- 2 Barnes, C. A., McNaughton, B. L., Goddard, G. V., Douglas, R. M., and Adamec, K., Circadian rhythm of synaptic excitability in rat and monkey CNS, *Science*, 197 (1977) 91-92.
- 3 Cauler, L. J., Boulos, Z. and Goddard, G. V., Contrary circadian rhythms of EPSP and population spike in hippocampal dentate gyrus and their relation to state,

- Soc. Neurosci. Abstr.*, 7 (1981) 18.
- 4 Davis, F. C., Ontogeny of circadian rhythms. In J. Aschoff, (Ed.), *Biological Rhythms. Handbook of Behavioral Neurobiology*, Vol. 4, Plenum Press, NY, 1981, pp. 257-274.
 - 5 Duffy, C., Teyler, T. J. and Shashoua, V. E., Long-term potentiation in the hippocampal slice: evidence for stimulated secretion of newly synthesized proteins, *Science*, 215 (1981) 1148-1151.
 - 6 Duffy, C. J. and Teyler, T. J., Development of potentiation in the dentate gyrus of rat: physiology and anatomy, *Brain Res. Bull.*, 3 (1978) 425-430.
 - 7 Ellison, N., Weller, J. L. and Klein, D. C., Development of a circadian rhythm in the activity of pineal serotonin N-acetyltransferase, *J. Neurochem.*, 19 (1972) 1335-1341.
 - 8 Harris, K. M. and Teyler, T. J., Evidence for late development of inhibition in area CA1 of the rat hippocampus, (1982) in preparation.
 - 9 Harris, K. M. and Teyler, T. L., Developmental onset of long-term potentiation in area CA1 of the rat hippocampus, (1982) in preparation.
 - 10 Harris, K. M., Teyler, T. J. and Cruce, W. L. R., A developmental comparison of LTP in areas CA1 and fascia dentata of the rat hippocampal formation, *Soc. Neurosci. Abstr.*, 6 (1980) 31.
 - 11 Itoh, S., Hirota, R. and Katsuura, G., The rate of phase shift of plasma corticosterone circadian rhythm during early developmental stages in neonatally blinded rats, *Jap. J. Physiol.*, 30 (1980) 41-48.
 - 12 King, T. S. and Dougherty, W. J., Neonatal development of circadian rhythm in 'synaptic' ribbon numbers in the rat pinealocyte, *Amer. J. Anat.*, 157 (1980) 335-343.
 - 13 Lenn, N., Bruce, B. and Moore, R. Y., Postnatal development of the suprachiasmatic hypothalamic nucleus of the rat, *Cell Tiss. Res.*, 178 (1977) 463-475.
 - 14 McNaughton, B. L., Douglas, R. M. and Goddard, G. V., Synaptic enhancement in fascia dentata: cooperativity among coactive afferents, *Brain Res.*, 157 (1978) 277-293.
 - 15 McNemar, Q., *Psychological Statistics*, Wiley, NY, 1970.
 - 16 Rusak, B. and Zucker, I., Neural regulation of circadian rhythms, *Physiol. Rev.*, 59 (1979) 449-526.
 - 17 Takahashi, K., Hanada, K., Kobayashi, K., Hayafuji, C., Otani, S. and Takahashi, Y., Development of the circadian adrenocortical rhythm in rats: studied by determination of 24- or 48-hour patterns of blood corticosterone levels in individual pups, *Endocrinology*, 104 (1979) 954-961.
 - 18 Teyler, T. J., The brain slice preparation: hippocampus, *Brain Res. Bull.*, 5 (1980) 391-403.
 - 19 West, M. O. and Deadwyler, S., Circadian modulation of granule cell response to perforant path synaptic input in the rat, *Neurosci.*, 5 (1980) 1597-1602.
 - 20 Winson, J. and Abzug, C., Neuronal transmission through hippocampal pathways dependent on behavior, *J. Neurophysiol.*, 41 (1978) 716-732.
 - 21 Winson, J. and Abzug, C., Gating of neuronal transmission in the hippocampus: efficacy of transmission varies with behavioral state, *Science*, 196 (1977) 1223-1225.