venous methylprednisolone (1 g daily for 5 days followed by a 1-week prednisone tapering schedule). Patients continued to take the myelin or placebo pills while receiving steroids.
17. The primary clinical outcome measures were the number of major exacerbations and change in the EDSS. Only well-defined attacks affecting pyramidal, cerebellar, brainstem, or visual functional systems were counted as major exacerbations. Sensory and bladder symptoms without objective findings on neurologic exam were not included. Patients were considered improved or worse if they changed two steps (one full point) on the EDSS. At the conclusion of the study and before unblinding, a global assessment rating was assigned for the course of each patient during the study.
18. Neither patient nor physician knew the treatment group. Compliance was measured by counting pills and by questionnaire. Patients rarely did not take a pill, missing at most 5 days during the year of the trial. Patients who did well predicted they were taking medication, irrespective of whether they were in the myelin or placebo group.
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1 October 1992; accepted 8 January 1993

# Association Between Brain Temperature and Dentate Field Potentials in Exploring and Swimming Rats

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**Attempts to correlate behavioral learning with cellular changes, such as increased synaptic efficacy, have often relied on increased extracellular potentials as an index of enhanced synaptic strength. A recent example is the enlarged excitatory field potentials in the dentate gyrus of rats that are learning spatial relations by exploration. The altered hippocampal field potentials do not reflect learning-specific cellular changes but result from a concomitant rise in brain temperature that is caused by the associated muscular effort. Enhanced dentate field excitatory potentials followed both passive and active heating and were linearly related to the brain temperature. These temperature-related effects may mask any learning-induced changes in field potential.**

The hippocampal formation is a phylogenetically old part of the cerebral cortex. Although there is strong evidence for its involvement in learning the spatial relation between objects (spatial learning) (*1*), neurophysiological correlates to learning such as synaptic weight changes have been difficult to find in freely moving animals (*2*). However, it was reported that exploration of an unfamiliar environment was associated with an increased dentate field excitatory postsynaptic potential (f-EPSP) after perforant path stimulation but with decreased population spike amplitude and latency. The effect was interpreted to reflect a changed synaptic weight due to the learning experience (*3*). We now examine an alternative possibility: These changes may be due to a temperature effect (*4*). This possibility would also explain why the enlarged f-EPSP was associated with a decreased population spike (*5*). Both exercise and feeding elevate the brain temperature in rats, whereas inactivity and sleep result in lower temperatures (*6*). The major factor that controls brain temperature is muscular heat production, which warms the cerebral arterial blood (*7*). In rats that were swimming in a Morris maze, we observed field potential changes that are exactly opposite to those reported above, namely, f-EPSP reduction with spike increase. Similar field potential changes are seen during brain cooling (*4*).

For these reasons we have recorded the hippocampal temperature in freely moving rats and correlated it with dentate field potentials during exploration and swimming (*8*). In both situations there was a strong, linear correlation between the behaviorally induced potential changes and the brain temperature.

When rats explored items on a platform the slope of the f-EPSP was increased (Fig. 1A), which confirms earlier reports (*3*). However, this enhancement was paralleled by an increase in brain temperature. In addition, the f-EPSP latency, the population spike amplitude, and latency all decreased. The brain temperature rose during 10 to 20 min of exploration from 37.0° ± 0.1°C (mean ± SEM) with a mean rate of 0.11° ± 0.01°C per minute ($n$ = 20); the largest increase was 3.2°C. After the exploration the brain temperature and the f-EPSPs declined along the same exponential time course, with both the f-EPSP and spike reaching base-line levels after 20 to 80 min. The temperature and f-EPSP curves were always parallel (43 sessions in nine of nine rats). The correlation factor ($r$) between the brain temperature and the f-EPSP slope during exploration was never <0.5 and in most runs was >0.75. For the exploring rat in Fig. 1, the $r$ values between the brain temperature and the following signal elements were: f-EPSP slope, 0.76 ($P < 0.001$); population spike latency, −0.78 ($P < 0.001$); and population spike amplitude, −0.34 ($P < 0.01$). In rats with two thermistors, implanted at the same depth in the same or opposite hemispheres, the bilateral activity-induced temperature changes were nearly identical (difference <0.15°C). Therefore, the temperature at the contralateral homotopic point could be used as a reference value. Warming the brain by radiant heating (Fig. 1B) gave a similar parallel increase in brain temperature and f-EPSP slope ($n$ = 19). Increasing the temperature of the animal by letting it run on a treadmill yielded comparable effects ($n$ = 15), with larger changes seen after the faster running speed (Fig. 1C). During treadmill runs of animals that were not fully habituated, there was an initial reduction of the f-EPSP slope, similar to the results of Green, McNaughton, and Barnes (*3*). Similar parallel changes of brain temperature and f-EPSP were seen in the responses of the olfactory bulb to lateral olfactory tract stimulation in exploring rats ($n$ = 5; Fig. 1D).

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Parallel changes in the brain temperature and f-EPSPs also occurred during spontaneous behavior in an opaque test cage similar to the rat's home cage (six rats followed for 8 hours). Changes of up to 2°C in the brain temperature, closely linked to the f-EPSP increase, were observed whenever the rat moved from rest to activity. Conversely, both types of signal declined during inactivity and sleep.

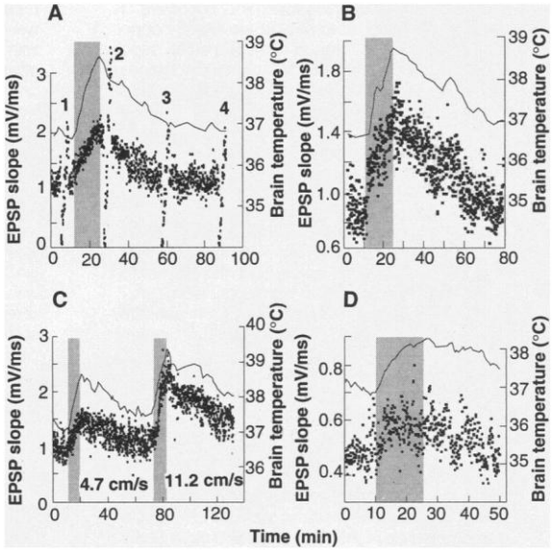



**Fig. 1.** Changes in f-EPSPs and brain temperature by exploration and treadmill running. (**A**) Brain temperature (continuous line, contralateral hippocampus) and dentate f-EPSP slope (dots) during and after 15 min of exploration (shaded column) of a platform (120 cm by 60 cm) with six to ten objects. Records that are marked 1 through 4 are input-output tests. Before and after exploration, the rat was left undisturbed for 10 and 60 min, respectively, in an opaque test cage (38 cm by 25 cm), to which the rat had habituated before the test. (**B**) Brain temperature and dentate f-EPSP slope in response to radiant heating (shaded column). The rat rested in the opaque test cage throughout the session. (**C**) Similar to (A) but dentate f-EPSP slope in response to 10 min of running on a treadmill (shaded columns) at speeds of 4.7 and 11.2 cm/s, respectively. (**D**) Brain temperature and f-EPSP in the olfactory bulb after stimulation of the olfactory tract during exploration (shaded column).

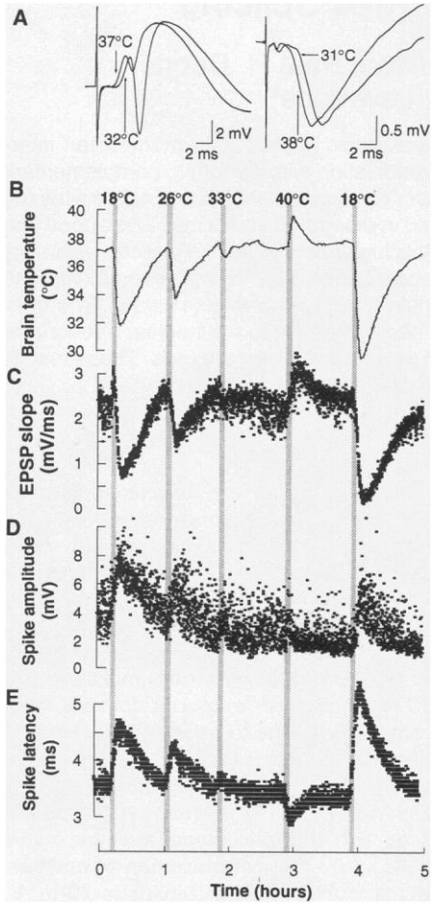
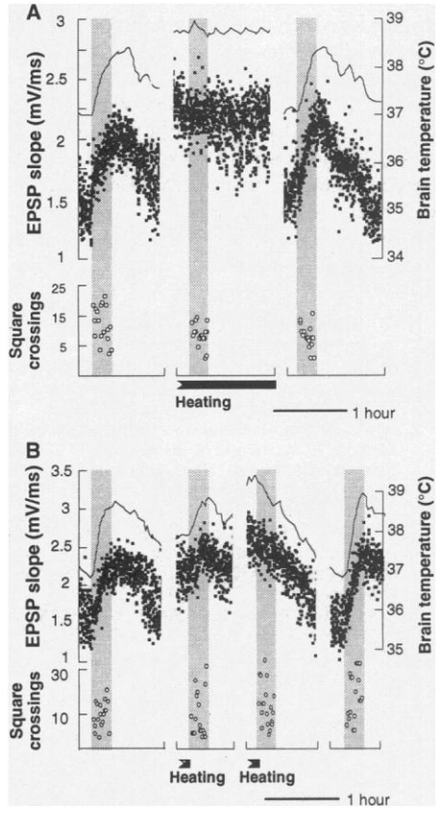



**Fig. 2 (left).** Changes in f-EPSP and brain temperature in a rat as it swam in a water maze 198 cm in diameter without a submerged platform at four different water temperatures. (**A**) Superimposed f-EPSPs of the dentate gyrus (left) and the olfactory bulb (right) in response to stimulation of the perforant path and the lateral olfactory tract, respectively, taken at the indicated brain temperatures before and after swimming in water of 18°C. (**B**) Brain temperature of the right hippocampal formation in a rat as it swam for five 5-min periods (shaded columns) with water at the indicated temperatures. (**C** to **E**) The EPSP slope and the population-spike amplitude and latency, respectively, of simultaneously recorded perforant path–dentate field potentials.

**Fig. 3 (right).** Dissociation of dentate f-EPSP changes from the exploratory behavior. (**A**) Records of brain temperature (continuous line, contralateral hippocampus), dentate f-EPSP slope (dots), and motor activity (open circles) during two 15-min control explorations that started at normal brain temperature (shaded columns, left and right panels) and during a similar period in which the rat had been preheated (horizontal thick bar) with an infrared lamp (middle panel). The exploratory activity was recorded by an observer and plotted as the number of border crossings of squares (8 cm by 8 cm) in the exploration area per 50 s. (**B**) Similar to (A) but the explorations (shaded columns) were started at various brain temperatures produced by preheating. The heating lamp was turned off at the start of exploration.

Because exploration was accompanied by relatively large changes in brain temperature, we tested whether swimming in a water maze produces brain cooling and, if so, whether any changes in f-EPSPs could be observed. Therefore, we measured the brain temperature and dentate f-EPSPs in rats that were swimming at different temperatures in a Morris water maze without a platform (9). In water of 18°C, the rat brain temperature rapidly decreased about 5°C (Fig. 2B). In parallel, the f-EPSP slope diminished and its onset was delayed (Fig. 2, A and C), whereas the population spike paradoxically increased both in size and latency (Fig. 2, D and E); these effects are the exact opposite of those observed during exploration. Similar but smaller changes were seen in water of 26°C. Hardly any change occurred at 33°C, and all changes reversed direction in water of 40°C. The observations were robust, appearing in all 55 trials in 15 animals. There was a strong correlation between brain temperature on the one hand and the f-EPSP slope (for the rat in Fig. 2, $r = 0.97$ and $P < 0.001$), population spike latency ($r = -0.99$ and $P < 0.001$), and population spike amplitude ($r = -0.58$ and $P < 0.001$) on the other. Equivalent results were observed for responses in the molecular layer of the dentate gyrus, the intrahippocampal synapses between the Schaffer collaterals and CA1 pyramidal cells (three rats), and the olfac-



tory bulb (five rats) (Fig. 2A).

Finally, attempting to dissociate the f-EPSP changes from the exploratory behavior, we used radiant heat to bring the brain temperature to the maximum value that was obtained during exploration on the previous day. By intermittent infrared heating, the brain temperature was kept just above this temperature (Fig. 3A, middle). In five of five rats, exploratory activity had normal intensity under these conditions, but no further change in the f-EPSP was observed (Fig. 3A). Input-output tests (as in Fig. 1B) showed that the lack of additional changes in the f-EPSP was not due to a ceiling effect. In another series of experiments, the brain was warmed before the exploration. The magnitude of the exploration-induced potential changes depended on the increment in brain temperature and disappeared altogether at a sufficiently high starting temperature (Fig. 3B). Again, the exploratory intensity was unchanged from that of the control sessions. The dissociation of the exploratory behavior from the f-EPSP changes argues against a causal relation between the two processes.

Our results show a consistent relation between the field potential parameters and brain temperature, whether the latter is changed by heat produced by muscle activity or by artificial warming. In essence, the observed f-EPSP changes during exploration appear to be caused primarily by an increased brain temperature due to muscular heat production rather than by a learning-induced change in synaptic strength. The results represent a caveat for the interpretation that in freely moving rats changed f-EPSPs are signs of altered synaptic efficiency. They do not rule out the possibility that f-EPSP changes are produced by learning but they do indicate that such changes must be evoked independently of changes in brain temperature that are induced by activity, environment, or drugs.

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5. During exploration there was an unexpected relation between the f-EPSP and the population spike. Normally, when the f-EPSP increases the population spike grows and its latency diminishes. The paradoxical spike amplitude reduction with reduced latency during exploration is probably an effect of increased brain temperature. The larger f-EPSP and its shorter latency are both probably caused by a temperature-induced increased speed of transmitter release [B. Katz and R. Miledi, *J. Physiol. (London)* **181**, 656 (1965)], an effect of physiological significance. The reduced spike latency results from the f-EPSP increase. The effects of warming and of cooling on the population-spike amplitude are exactly opposite. Cooling gives three effects: (i) a small depolarization lowers the threshold for cell discharges, which causes more cells to fire; (ii) each discharging cell contributes a larger signal [A. L. Hodgkin and B. Katz, *J. Physiol. (London)* **109**, 240 (1949)]. and (iii) because each action potential in the cooled state is broader than it is in the warm condition [G. M. Schoepfle and J. Erlanger, *Am. J. Physiol.* **134**, 694 (1941)], the algebraic summation of the individual units to a compound potential results in a larger sum, in spite of the fact that the onset times are more spread out (less synchronous) than they are in the warm condition.
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8. Male Long Evans rats (250 to 500 g) were anesthetized with a mixture of chloral hydrate and pentobarbital (Equithesin, 1.0 ml per 250 g of body weight). Bipolar stimulation electrodes (SNEX 100; Rhodes Medical, Woodland Hills, CA) were implanted in the right angular bundle (7.5 to 8.0 mm posterior and 4.3 mm lateral to bregma), and a tungsten recording electrode was placed in the dentate hilus or granule cell layer (4.0 mm posterior and 2.6 mm lateral to bregma). Responses in the CA1 pyramidal layer to stimulation of Schaffer collaterals or olfactory bulb responses to lateral olfactory tract stimulation were recorded in some animals. A thermistor (0.5-mm diameter; 111-802 EAJ-B01, Fenwal Electronics, Milford, MA) was implanted contralaterally at the homotopic point of the recording electrode. Electrode and thermistor leads were connected to a socket fastened to the skull with dental acrylic. Before implantation, each thermistor was calibrated in a water bath against a precision thermometer. The thermistors allowed temperatures to be measured at a precision of 0.05° to 0.1°C. The rats were allowed 1 week of recovery before testing started. Behavioral testing was performed at 23°C (air temperature) and began 1 to 2 hours after the rat was connected to the recording equipment, when both the electrical and temperature records had reached stable values. Test f-EPSPs were elicited by a constant stimulus (100 to 500 μA, 50 μs) at 0.2 or 0.07 Hz. The slope of the f-EPSP was measured near its maximum as the amplitude difference at two fixed latencies. The population spike was taken as the vertical distance between the peak and a joint tangent to the preceding and succeeding positivities. The spike latency was defined as the time from stimulus onset to the spike peak.
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10. We thank T. Eriksen, E. Aaboen Hansen, B. Piercey, and T. Reppen for technical assistance. Supported by the Norwegian Medical Research Council.

11 September 1992; accepted 25 November 1992


# Higher Level Organization of Individual Gene Transcription and RNA Splicing


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**Visualization of fibronectin and neurotensin messenger RNAs within mammalian interphase nuclei was achieved by fluorescence hybridization with genomic, complementary DNA, and intron-specific probes. Unspliced transcripts accumulated in one or two sites per nucleus. Fibronectin RNA frequently accumulated in elongated tracks that overlapped and extended well beyond the site of transcription. Splicing appears to occur directly within this RNA track, as evidenced by an unambiguous spatial separation of intron-containing and spliced transcripts. Excised introns for neurotensin RNA appear free to diffuse. The transcription and processing site of the fibronectin gene localized to the nuclear interior and was associated with larger transcript domains in over 88 percent of the cells. These results support a view of nuclear function closely integrated with structure.**


The long-standing interest in the spatial organization of transcription and splicing within the interphase nucleus has been heightened by several observations (*1*). Visualization by fluorescence microscopy of highly localized nuclear "tracks" of specific viral RNAs (*2*), preserved in chromatin-depleted nuclear matrix extracts (*3*), indicated that these RNAs are not free to diffuse but rather are associated with an underlying nuclear substructure (*4*). The results of an autoradiographic study have indicated that intron sequences in acetylcholine receptor mRNA preferentially localize around the nuclear periphery (*5*). Total nuclear polyadenylate [poly(A)] RNA has been shown to accumulate within 20 to 40 discrete "transcript domains" that coincide with the location of small nuclear ribonucleoproteins (snRNPs) (*6*, *7*). These snRNPs were previously reported to exhibit a clustered nuclear distribution (*8*) coincident with the spliceosome assembly factor SC-35 (*9*). The concentration of microinjected globin RNA within these 20 to 40


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