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Brain Research 658 (1994) 135–144

BRAIN RESEARCH

Research report

Brain temperature- and behavior-related changes in the dentate gyrus field potential during sleep, cold water immersion, radiant heating, and urethane anesthesia

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Accepted 21 June 1994

Abstract

The field potential evoked in the dentate gyrus (DG) by stimulation of the perforant path (PP) is known to vary with ongoing behavior and with brain temperature. To further study these phenomena chronic stimulating and recording electrodes were implanted into the PP and DG of rats, and a thermistor was implanted into the contralateral homotopic DG. Field potentials and brain temperature records were made during (1) slow wave sleep (SWS), (2) radiant heating, (3) immersion in cool water, (4) a control session during which no manipulations were made, and (5) under urethane anesthesia. In another group of rats field potentials were recorded during (1) baseline immobile wakefulness, (2) SWS, (3) before SWS or after gentle awakening from SWS (eyes open and presence of intermittent slow waves in the EEG), (4) immobile wakefulness, and (5) 24 h later. Findings were that field EPSP slope decreased and population spike (PS) amplitude increased by up to 60% of baseline values during conditions in which brain temperature was reduced (SWS, immersion in cool water, urethane anesthesia). Conversely, EPSP slope increased and PS amplitude decreased by up to 100% of baseline values during conditions in which brain temperature increased (awakening from SWS, radiant heating, and warming after immersion in cool water or urethane anesthesia). Product moment correlations between brain temperature and field potential measures confirmed the statistical reliability of these findings and accounted for up to 77% of the variance. These findings confirm the robust effect on hippocampal field potentials of brain temperature changes due to exogenous heating and cooling, and extend this effect to anesthetic- and sleep-induced brain temperature changes. They also identify a state that behaviorally resembles quiet wakefulness but resembles SWS in terms of neocortical EEG, brain temperature, and hippocampal field potential measures. The findings indicate the need to control for brain temperature-mediated changes in hippocampal research that uses the dentate gyrus field potential as a dependent measure.

Keywords: Hippocampus; Brain temperature; Behavior; Field potential; Sleep; Urethane anesthesia

1. Introduction

Brain electrical activity is known to vary from moment to moment as a function of ongoing behavior. Changes in behavior can influence the neocortical EEG [26] and neocortical- [20,25] and hippocampal-evoked field potentials. Winson and Abzug [30] stimulated the perforant path (PP) and recorded mono- and poly-synaptic responses in the hippocampus. They found that population spike (PS) responses recorded in the dentate gyrus (DG) and areas CA1 and CA3 were significantly larger during slow wave sleep (SWS) than during still alertness (evoked by startling the rat with a loud noise). On the other hand, the DG population EPSP was smaller during SWS than during still alertness.

Other researchers subsequently found that the responses recorded in various hippocampal sites are different during different waking behaviors [7,8,16,18,22]. Two groups, in addition to Winson and Abzug [30], recorded responses during both SWS and waking immobility. Leung [18], recording responses in hippocampal area CA1 to stimulation of the Schaffer collaterals, found that the population EPSP was larger during

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SSDI 0006-8993(94)00776-9

SWS and awake immobility than during walking, but that in most rats there was no difference in EPSP amplitude during SWS and awake immobility. Brankack and Buzsaki [7] found that the DG PS evoked by stimulation of the PP was larger during SWS and immobility than during walking and other Type 1 movements (see [26]), but they observed no difference in this measure between SWS and immobility. Thus, some workers find differences in both EPSP and PS measures between SWS and startle-induced immobility [30], while others find no consistent differences in the CA1 EPSP [18] or the DG PS [7] between SWS and spontaneous immobility. However, hippocampal electrical activity is different during startle-induced immobility and spontaneous immobility [18,29] (see section 4, Discussion), presumably reflecting different functional states of this structure. Thus, it is not clear whether the failure to find a difference between field potentials during SWS vs. awake immobility was due to the different anatomical locus studied [18], or the different methods of inducing immobility [7,18], or both.

Other studies have demonstrated a further influence on field potentials recorded from anesthetized preparations or the hippocampal slice: the effect of temperature change [3,4,21,24]. A recent in vivo study has extended this finding by showing that an increase in brain temperature, whether caused by radiant heating or by muscular activity, increases the magnitude of the EPSP and decreases the magnitude of the PS in the DG, and that a decrease in brain temperature had the opposite effects [19].

As SWS is known to be accompanied by a decrease in the temperature of the body and brain [1,5], it seems possible that the drop in brain temperature that accompanies SWS might contribute to the changed field potential properties that have been observed during SWS (see [30]). We were particularly interested in examining changes in both the EPSP and PS in the same animals during both behavioral immobility and SWS after various manipulations that changed brain temperature.

Our interest in the influence of behavior and temperature on hippocampal evoked responses stems from our use of chronic preparations in studies of long-term potentiation (LTP), in which we have confirmed that the DG evoked response differs during different waking behaviors [16]. Ongoing work with animals that were tested in long sessions during which they sometimes entered SWS gave us the impression that a state existed during which the animals appeared to be awake and quiescent with their eyes open, but during which a SWS-like DG field potential was obtained; that is, the PS was larger during this state than during waking immobility [10]. One purpose of this study was to further examine this state and the field potential changes that occurred at that time. Winson and Abzug's [30] conclusion that the DG response is the primary site at which changes in behavioral state affect neural transmission in the hippocampus prompted us to examine the PP-DG response in this study.

An understanding of these phenomena and their interactions would be useful in studies in which field potential measures are taken repeatedly over long periods, during which the behavior and the brain temperature of the subjects might change.

2. Materials and methods

2.1. Surgical and electrophysiological procedures

Naive male hooded rats used in the temperature experiment received implantation of a stimulating electrode into the perforant path (angular bundle: AP = −8.1 mm, ML = 4.4 mm) and a recording electrode into the hilus of the dentate gyrus (AP = −3.8 mm, ML = 2.4 mm) using conventional surgical techniques. Electrodes were made from Teflon-coated stainless steel wire 127 μm in diameter. Recordings from the monopolar hilus electrode were referred to a surgical screw in the skull. Measurements were from bregma, with bregma and lambda in the same horizontal plane. Final electrode placement was optimized using electrophysiological stimulation and recording before fixing the electrodes in place with dental cement. Test pulses throughout this study were biphasic square waves, with each phase 0.1 ms in duration. All rats displayed an evoked field potential, consisting of a positive EPSP with a superimposed negative PS, in response to stimulation currents of 100 μA or less. EEG was recorded from the DG electrode and from a lead attached to a skull screw overlying the neocortex. A thermistor (Fenwall Electronics Mini Probe) calibrated for accuracy and drift (less than 0.1 °C/h) was also implanted, aimed for the homotopic position in the contralateral hippocampus. The output of the thermistor was amplified using a Grass DC preamplifier, digitized, and acquired by a microcomputer using software developed in our laboratory.

Naive male hooded rats used in the sleep experiment received implantation of electrodes as described above.

2.2. Procedure

2.2.1. Temperature experiment

Before the start of the study the rats were placed in an empty enclosure, and field potentials were evoked at 0.1 Hz. Ten responses to pulses of a given intensity were averaged and analyzed by the microcomputer and software developed in our laboratory. The measures obtained were the maximum slope of the rising phase of the population EPSP, and the amplitude of the dentate granule cell PS measured as the length of the vertical line joining the negative peak and a line drawn tangent to spike onset and offset (see [16]). EPSP slope was also measured as the amplitude difference at two fixed latencies, the first of which was defined by the beginning of the EPSP response (see ref. 19). All electrophysiological recording took place between 10.00 a.m. and 4.00 p.m. during the light phase of the cycle (which began at 8.00 a.m.). EEG from neocortex and DG was recorded throughout both experiments to create a record of pulse delivery and to confirm the presence of SWS.

An input/output (I/O) curve was first obtained using 5 or 6 stimulation intensities. Stimulation was applied only when the rats were behaviorally immobile, at no more than 0.1 Hz. From this I/O curve, low and high stimulation intensities were chosen to represent the bottom and top of the I/O curve. During the collection of data during the temperature manipulations delivery of the two test pulse

intensities was alternated. Brain temperature measures were acquired at the same time that evoked response measures were acquired, and continuous records of EEG and behavioral state were made. Data were collected sequentially during the following five sessions spaced a few days apart.

Sleep. the rats were allowed to enter and remain in SWS (i.e. eyes closed, curled up sleep posture, immobility, and continuous slow waves in the EEG) for 40 to 60 min. They were then gently awakened, kept awake for 10 to 20 min, and then allowed to go back to sleep.

Radiant heat. A 20-min baseline recording period was followed by 15 to 20 min of radiant heating by a heat lamp placed 60 cm above the rat. The lamp was removed and the rats were then allowed to regain normal temperature.

Cool water immersion. A 20-min baseline recording period was followed by 10 to 15 min immersion in a large tub of water at 22–24°C. The rats were allowed to float with their head, but not their torso, above water by clinging to a piece of wood at the surface of the water; the wood would not support their whole body weight out of water. During the immersion they generally clung quietly to the piece of wood and did not attempt to escape. After being removed from the water the rats were warmed until their temperature was again normal.

Control. A 60-min period during which the rat was kept in an empty recording chamber at room temperature; the rat was allowed to rest quietly but not to sleep.

Urethane. A 20-min baseline recording period was followed by injection of an anesthetic dose of urethane (ethyl carbamate, 1.5 g/kg); during this time no supplemental heating was applied. After the brain had reached a stable low value, the rat's temperature was then gradually raised using a heat lamp.

Three animals were studied in the five sessions. This experimental design allowed for three replications per animal using brain temperature-reducing treatments (SWS, cool water immersion, urethane anesthesia) and three replications per animal using brain temperature-increasing treatments (awakening from SWS, radiant heat, radiant heat after urethane anesthesia).

2.2.2. Sleep experiment

Ten days before the start of the study the rats were placed in an empty enclosure, and field potentials were evoked at 0.1 Hz. The same basic procedures for stimulation, recording, and I/O curve construction were again used, with the exception that 3 stimulation intensities were chosen for an abbreviated I/O curve, to represent low, medium, and high intensities that were at the bottom, middle,

Table 1
Correlations between brain temperature and evoked potential measures

Session	Rat	Brain temperature change degrees C	Test pulse intensity	Brain temperature and EPSP maximum slope		Brain temperature and EPSP rise (Fixed Latencies)		Brain temperature and PS amplitude	
				r	P	r	P	r	P
Slow wave sleep	1	1.9	Low	+0.41	< 0.001	+0.61	< 0.001	−0.08	n.s.
			High	+0.32	< 0.001	+0.71	< 0.001	−0.09	n.s.
	2	2.4	Low	+0.65	< 0.001	+0.75	< 0.001	0.00	n.s.
			High	+0.50	< 0.001	+0.71	< 0.001	−0.38	< 0.001
	3	3.7	Low	+0.49	< 0.001	+0.62	< 0.001	−0.19	< 0.001
			High	+0.50	< 0.001	+0.73	< 0.001	−0.48	< 0.001
Radiant heat	1	2.3	Low	+0.27	< 0.005	+0.27	< 0.005	−0.07	n.s.
			High	+0.37	< 0.001	+0.48	< 0.001	−0.19	< 0.05
	2	2.1	Low	+0.36	< 0.001	+0.51	< 0.001	−0.18	n.s.
			High	+0.35	< 0.001	+0.45	< 0.001	−0.42	< 0.001
	3	2.6	Low	+0.66	< 0.001	+0.70	< 0.001	+0.01	n.s.
			High	+0.53	< 0.001	+0.69	< 0.001	−0.37	< 0.001
Cool swim	1	5.2	Low	+0.34	< 0.001	+0.75	< 0.001	−0.29	< 0.001
			High	+0.32	< 0.001	+0.68	< 0.001	−0.53	< 0.001
	2	6.9	Low	+0.25	< 0.02	+0.34	< 0.001	−0.59	< 0.001
			High	+0.39	< 0.001	+0.67	< 0.001	−0.67	< 0.001
	3	5.1	Low	+0.41	< 0.001	+0.66	< 0.001	−0.58	< 0.001
			High	+0.47	< 0.001	+0.86	< 0.001	−0.70	< 0.001
Control	1	0.4	Low	+0.25	< 0.02	+0.28	< 0.015	+0.08	n.s.
			High	+0.31	< 0.005	+0.34	< 0.003	−0.24	< 0.05
	2	< 0.1	Low	+0.01	n.s.	−0.08	n.s.	0.00	n.s.
			High	+0.05	n.s.	+0.11	n.s.	+0.12	n.s.
	3	0.4	Low	+0.32	< 0.005	+0.35	< 0.001	−0.25	< 0.02
			High	+0.46	< 0.001	+0.51	< 0.001	−0.05	n.s.
Urethane	1	4.9	Low	+0.55	< 0.001	+0.62	< 0.001	−0.41	< 0.001
			High	+0.73	< 0.001	+0.88	< 0.001	−0.86	< 0.001
	2	4.8	Low	+0.78	< 0.001	+0.82	< 0.001	−0.49	< 0.001
			High	+0.81	< 0.001	+0.86	< 0.001	−0.87	< 0.001

n.s. = not significant
PS = population spike

and top of the I/O curve, respectively. An abbreviated I/O curve with 3 well-spaced intensities was used because it appears to be the best compromise for providing a description of the different changes in response properties that occur at different stimulation intensities [11] (see section 3, Results, below), yet avoiding the potentiating effects of repeated presentation of many low-frequency test pulses [12,14,23]. All data were obtained while the rats were in a chamber to which they were well habituated.

Sleep and sleep replication sessions. Each rat was then studied during two sleep sessions spaced a few days apart, the second of which was a replication of the first.

During each session an abbreviated I/O curve was obtained using test pulses of ascending intensity during each of the following conditions in the following order.

(i) Baseline: awake immobility characterized by eyes open, head held up against gravity, no movements apart from breathing, and normal waking EEG.

(ii) Sleep/Closed: SWS for approximately 1 h, characterized by closed eyes, curled up sleep posture, immobility, and continuous slow waves in the EEG.

(iii) Sleep/Open: a period approximately 5 min after gentle awakening from SWS, characterized by eyes open, immobile resting posture (ventral body surface flat on the chamber floor, with head lowered onto forepaws), and a preponderance of slow wave bursting in the EEG. The rats typically displayed these properties before entering SWS, but it was not always possible to distinguish its onset with certainty. This condition was sometimes more reliably obtained by gently awakening the rats from SWS, after which they would pass back into the Sleep/Open condition.

(iv) Awake: awake immobility, as described for Baseline, after awakening from the Sleep/Open condition.

(v) 24 h: awake immobility 24 h later.

Pulses applied during the Sleep/Closed and Sleep/Open conditions were delivered at 0.1 Hz. Notes were kept on the state of the animal throughout.

Four-measure control session. A few days later the same procedure was again repeated except that the rats were kept awake throughout by gentle stroking of the body when necessary or by slow intermittent rotation of the chamber whenever it appeared that they might go to sleep. The measures were taken at the same times as in the Sleep session. All pulses were delivered during awake immobility as described above. This session was to control for the passage of time.

Two-measure control session. A few days later this same procedure was repeated except that only the Baseline, Awake, and 24 h measures were taken. Care was taken to obtain the Awake measure after the passage of the same amount of time as in the Sleep session. This session was to control for the passage of time. Seven rats completed all of the Sleep Experiment sessions.

At the end of the experiments the rats were deeply anesthetized if not already under urethane-induced general anesthesia and perfused with formal saline. The brain was removed and processed using standard histological techniques to determine the location of the electrodes, and thermistor if present.

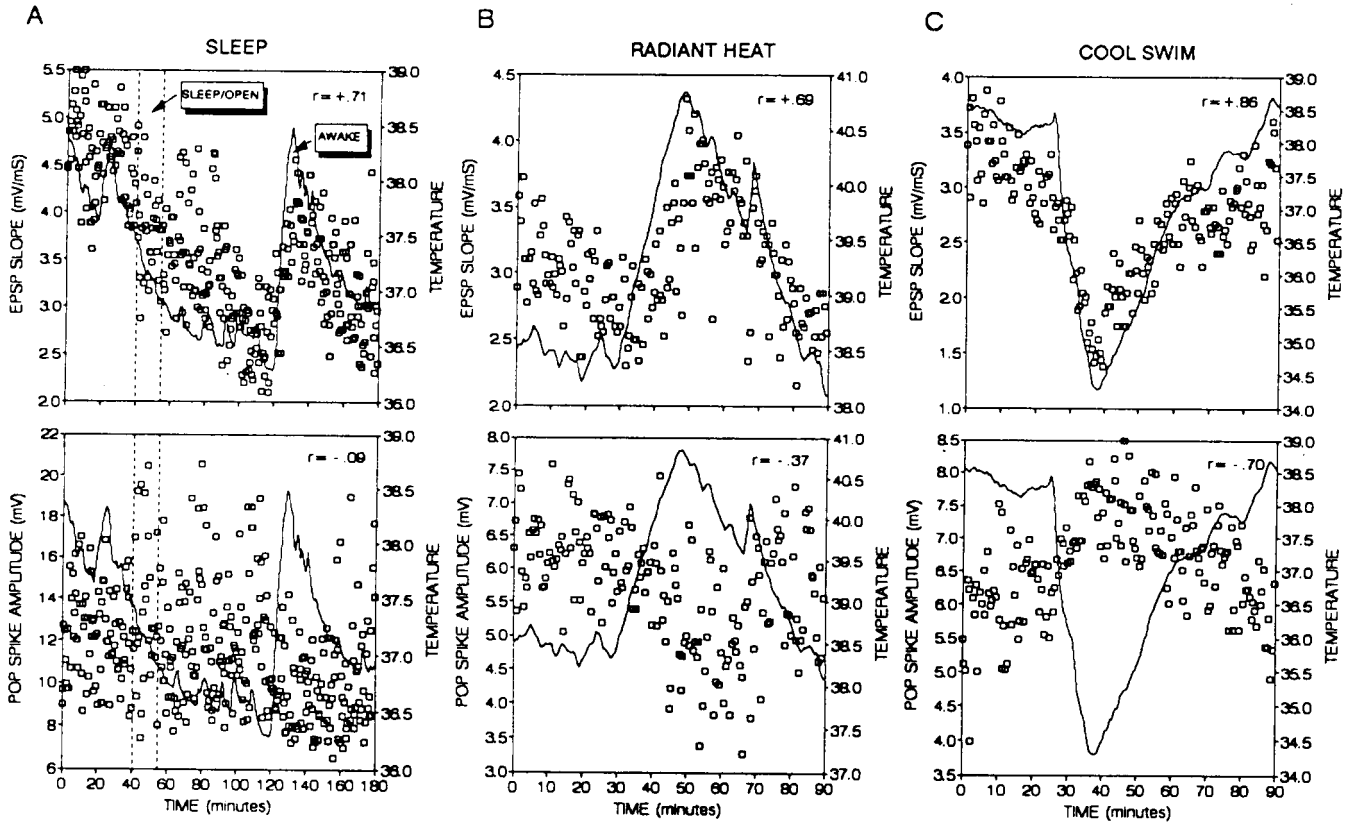

Fig. 1. Changes in brain temperature (solid line), field EPSPs (small squares, top) and PS amplitudes (small squares, bottom) during Sleep, Radiant Heat, and Cool Water Immersion sessions. A: Sleep session for rat 1, which included a Sleep/Open period (indicated by 'Sleep/Open'), and subsequent awakening (indicated by "Awake"). B: Radiant Heat session for rat 3. Heating was by a heat lamp, and coincided with the steep rising phase of the temperature curve, beginning at minute 28 and ending at minute 48. C: Cool Water Immersion for rat 3. Water immersion coincided with the steep falling phase of the temperature curve, beginning at minute 23 and ending at minute 38. Correlation coefficients calculated for brain temperature and EPSP slope or PS amplitude are given in the top right corner of each panel.

3. Results

At no time during either experiment did epileptiform afterdischarge occur in response to any test pulse. No animal struggled, vocalized or showed other signs of stress (e.g. exopthalmus) during any test condition; thus, stress did not appear to be a factor in the results.

3.1. Temperature experiment

The range of brain temperature change is documented for individual rats and the various test sessions in Table 1 (see also Figs. 1 and 2). Associations between temperature and field potential measures were evaluated by product–moment correlations calculated for each of the low and high test pulse intensities for each rat, giving 56 correlations in all, which accounted for up to 77% of the variance. (The field potential in one rat deteriorated before the Urethane session, which prevented data collection for this session for this rat.) As indicated by significant positive correlations, EPSP slope changed in the same direction as brain temperature for every rat for both stimulation intensities during every session, with the exception of rat 2 during the Control session (see next paragraph). The two measures of EPSP slope yielded similar correlations with temperature, although the correlations using the measures obtained by two fixed latencies were almost always higher (see Table 1). PS amplitude changed in the opposite direction in most cases, as indicated by predominantly significant negative correlations. No statistically significant positive correlations between brain temperature and PS amplitude were found.

Brain temperature changed considerably less during the Control sessions than during the other sessions (see Table 1). In rats 1 and 3 there was a small drop in brain temperature of 0.4 °C, which reflected their behavioral inactivity. For both of these rats there was an associated small reduction in the EPSP measure, as reflected by small but significant positive correlations between brain temperature and EPSP slope. PS amplitude appeared to change less than the EPSP slope during this time. In rat 2 the net temperature change was less than 0.1 °C, and both field potential measures

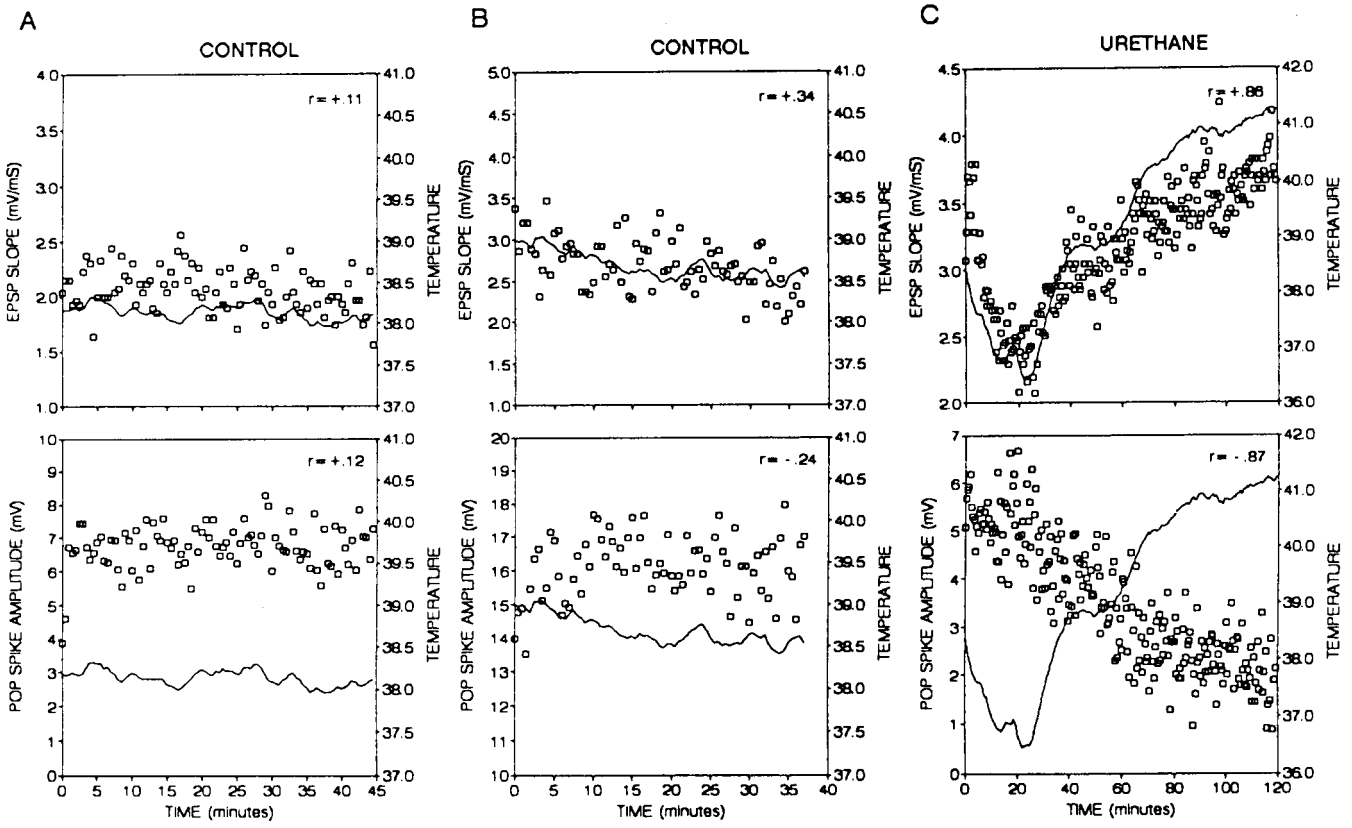

Fig. 2. Changes in brain temperature (solid line), field EPSPs (small squares, top) and PS amplitudes (small squares, bottom) during Control and Urethane sessions. A: Control session for rat 2, which exhibited a net change in brain temperature of less than 0.1 °C and stable field potential measures. B: Control session for rat 1, which exhibited a net change in brain temperature of 0.4 °C. Correlations between brain temperature and each field potential measure, which appear in the top right corner of each panel, are small but statistically significant. C: Urethane session for rat 2. The initial drop in brain temperature from minute 0 to 21 occurred under urethane anesthesia without supplemental heating. The subsequent rising phase of the brain temperature curve coincided with application of supplemental heat using a heat lamp, from minute 21 onward.

remained stable throughout, as reflected by the lack of significant correlations for this rat (see Table 1). The lack of significant correlations for this rat during the Control condition may be due to the unchanging nature of the measures (see Fig. 2A), a situation that would be expected to yield a correlation close to 0.

3.2. Sleep experiment

3.2.1. Baseline electrophysiological results

Mean stimulation intensities and electrophysiological results for the Baseline measures taken during the Sleep and Sleep Replication sessions are presented in Table 2. Repeated measures ANOVA performed on all of the data from these two sessions (Baseline, Sleep/Closed, Sleep/Open, Awake, and 24 h measures) for both the EPSP slope and PS amplitude indicated that the results did not differ significantly between the two sessions ($P > .50$). Therefore the data for these two sessions were collapsed for subsequent analyses.

Averaged field potentials obtained during the different conditions appear in Fig. 3. To compare results from the various test sessions, all measures were transformed to difference scores relative to the Baseline measure taken at the beginning of the original Sleep session, as described previously [9,11,16]. The data for the Sleep and Sleep Replication sessions were collapsed together and are presented in Fig. 4.

3.2.2. Population EPSP

The data for the population EPSP collapsed across both Sleep sessions (Fig. 4, top) were subjected to a repeated measures ANOVA, which yielded significant main effects of behavioral condition ($F_{4,24} = 9.82$; $P < 0.001$) and stimulation intensity [$F_{2,12} = 7.01$; $P < 0.01$]. Thus the EPSP slope differed across behavioral conditions, and the effect varied with test pulse intensity. Post-hoc contrasts showed that the following measures were significantly ($P < 0.05$) below baseline: low intensity: Sleep/Closed; medium intensity: Sleep/Closed, Sleep/Open; high intensity: all points.

Because the greatest depression of the EPSP slope occurred at the highest stimulation intensity these data were examined further. Results obtained with the high intensity test pulses were plotted as difference scores relative to the Sleep session Baseline (Fig. 5, top). To examine whether the baselines remained stable across recording sessions, mean baseline measures were also plotted as difference scores relative to the Sleep session Baseline (Fig. 5, top). Repeated measures ANOVA indicated that the four Baseline measures did not differ ($P > 0.50$).

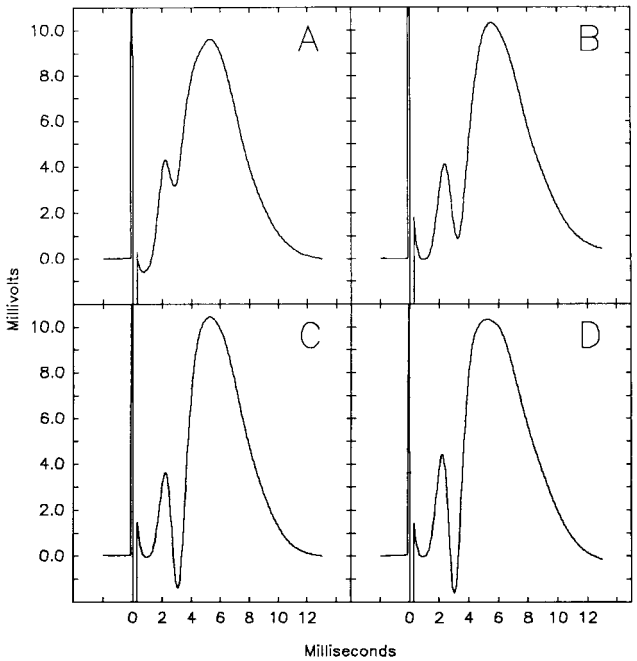

Fig. 3. Field potentials evoked from a rat during a Sleep recording session by a low intensity stimulus showing the Baseline response (A), the Sleep/Closed response (B), the Sleep/Open response (C), and the Awake response (D).

A previous ANOVA had indicated that for the EPSP slope high intensity data all measures were significantly below baseline. A further repeated measures ANOVA on the Four-Measure Control and Two-Measure Control data indicated that there was no change in EPSP slope over time ($P > 0.50$).

3.2.3. Population spike

The data for the PS collapsed across both Sleep sessions (Fig. 4, bottom) were subjected to repeated measures ANOVA, which yielded a significant main

Table 2
Baseline electrophysiological results for sleep and sleep replication sessions

Stimulation intensity	Test pulse Intensity (μA)	Sleep session		Sleep Replication Session	
		EPSP slope (mV/ms)	Population spike amplitude (mV)	EPSP slope (mV/ms)	Population spike amplitude (mV)
Low	85.7 +12.9	2.04 +0.49	2.43 +0.46	2.06 +0.49	2.01 +0.34
Medium	200.0 +36.2	3.72 +0.98	6.44 +1.01	3.56 +0.94	6.42 +0.95
High	528.6 +56.6	5.58 +1.63	11.43 +0.93	5.47 +1.53	10.93 +0.84

Values represent mean and S.E.M.

effect of behavioral condition [$F_{4,24} = 14.22$; $P < 0.001$]. The main effect of stimulation intensity approached but did not reach significance [$F_{2,12} = 2.65$; $P = 0.10$]. Thus PS amplitude differed across behavioral condition, and there was a non-significant trend for the effect to vary with test pulse intensity. Post-hoc contrasts showed that the following measures were significantly ($P < 0.05$) above baseline: low intensity: Sleep/Closed, Sleep/Open, Awake; medium intensity: Sleep/Open, Awake; high intensity: Sleep/Open.

Because there was a trend for the greatest change in PS amplitude to occur at the lowest stimulation intensity these data were examined further. Results obtained with the low intensity test pulses were plotted as difference scores relative to the Sleep session Baseline (Fig. 5, bottom). To examine whether the baselines remained stable across recording sessions, mean baseline measures were also plotted as difference scores relative to the Sleep session Baseline (Fig. 5, bottom). Repeated measures ANOVA indicated that the four Baseline measures did not differ ($P > 0.50$).

Because of the similarity of results from the collapsed Sleep and Sleep Replication sessions and the Four-Measure control sessions these data were subjected to repeated measures ANOVA. There was no effect of recording session ($P > 0.50$), but there was a significant effect of behavioral condition [$F_{4,24} = 18.56$; $P < 0.001$], indicating that the changes due to behavioral condition occurred equally during both Sleep sessions and the Four-Measure control session. A previous ANOVA had indicated that for the PS amplitude low intensity data the Sleep/Closed, Sleep/Open and Awake measures were significantly above baseline.

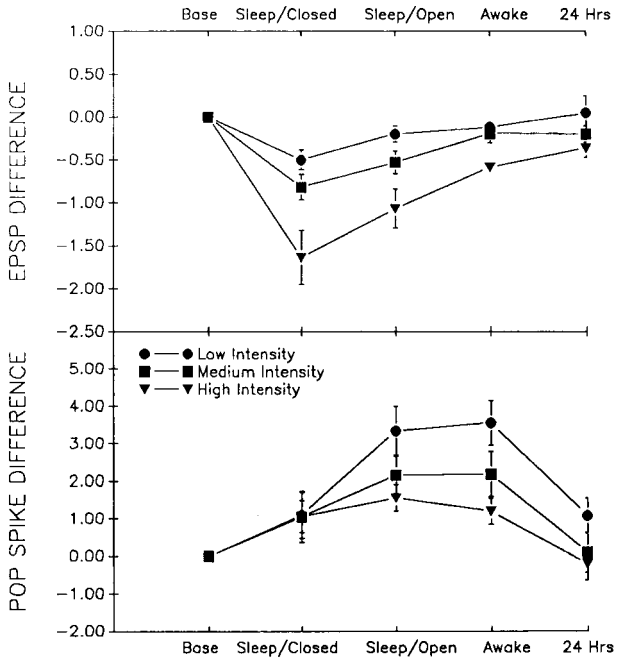

Fig. 4. Top: EPSP difference scores relative to the Sleep session Baseline for low, medium, and high intensity pulses. Bottom: population spike difference scores relative to the Sleep session Baseline for low, medium, and high intensity pulses.

Figs. 4 and 5 based on data from 7 rats. The ordinate represents difference scores, in mV, relative to the Baseline measure taken at the beginning of the original Sleep session. Data points represent the mean and S.E.M.

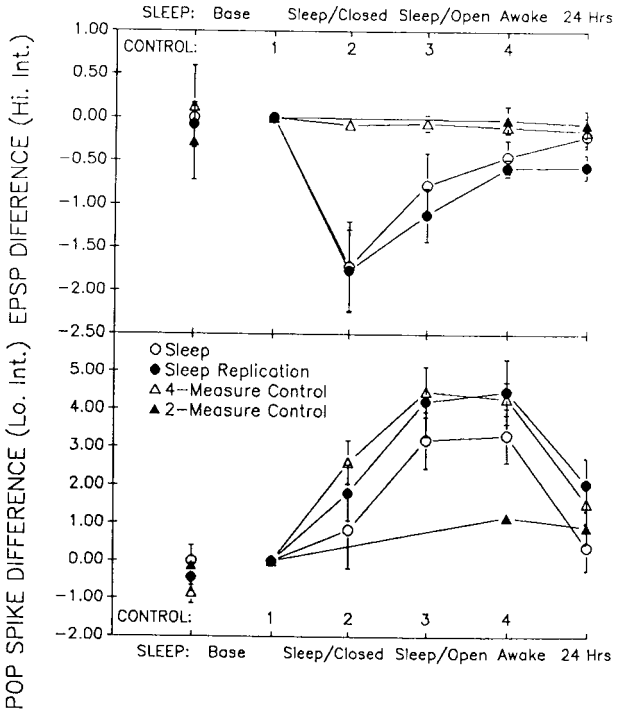

Fig. 5. Top: EPSP difference scores for high intensity pulses obtained during the Sleep, Sleep Replication, 4-Measure Control, and 2-Measure Control sessions. To the left of the main plot on this and the PS plot below are the Baseline measures for each of these sessions, plotted relative to the Baseline from the Sleep sessions, which is taken as zero. Bottom: population spike difference scores for low intensity pulses obtained during the Sleep, Sleep Replication, 4-Measure Control, and 2-Measure Control sessions.

4. Discussion

4.1. Effects of brain temperature change on field potentials

Recently Moser et al. [19] showed that changes in brain temperature have profound effects on DG field potentials. Increased brain temperature caused by radiant heat or muscular effort led to reliable increases in EPSP slope and decreases in PS amplitude. Swimming in cool water led to the reverse changes. Our results from the Radiant Heat and Cool Water Immer-

sion sessions confirm those of Moser et al. We obtained highly significant positive correlations between brain temperature and EPSP slope in every case, accounting for up to 77% of the variance, and highly significant negative correlations between brain temperature and PS amplitude in most cases, accounting for up to 76% of the variance. All but one of the failures to obtain a significant correlation between temperature and PS amplitude occurred for data from the SWS session, or for PS measures obtained using low intensity pulses, or both (Table 1). Failure to obtain statistically significant correlations in these cases may have resulted in part from the greater variability in the PS amplitude measure that occurs during SWS compared to waking (Fig. 1A), and when using low intensity test pulses compared to high intensity test pulses (see [15]). Increased variability of this measure would be expected to decrease the likelihood of obtaining statistical significance in the correlations that were calculated here.

The experimental design caused brain temperature changes to reverse direction at least once, and the field potential measures changed accordingly. This rules out the possibility that the correlations were an artifact of the coincidental drift of both brain temperature and field potential measures, and suggests a causal relation between changes in brain temperature and field potentials, a conclusion consistent with earlier research [3,4,21,24]. It is interesting that the amplitude and frequency of hippocampal rhythmical slow (theta) activity is highly correlated with core temperature in curarized [27] and behaving rats [28].

Measures taken during the Control session also suggest a causal relation between brain temperature and field potential changes. In the rats that showed a small drop in brain temperature there was an associated slight reduction in EPSP slope (Fig. 2B and Table 1). In the rat that had stable brain temperatures throughout, field potential measures also remained stable (Fig. 2A and Table 1). The temperature changes during the Control condition were the smallest observed during the experiment, and were generally associated with the smallest correlations with the field potential measures.

Our results extend the observation of a relation between brain temperature and field potential changes to the SWS and urethane anesthetized states. During both of these sessions changes in brain temperature caused by SWS onset and subsequent awakening, and by anesthetization and subsequent heating, led to field potential changes that were similar to those caused by cooling and heating respectively, in the other sessions. Every correlation between brain temperature and EPSP slope was positive and highly significant, and most correlations between brain temperature and population spike amplitude were negative and highly significant. The field potential changes that occurred as a result of urethane may be the result, in part, of the reduction in brain temperature rather than a direct effect of the anesthetic since they were reversed by heating. Previously we and others have reported that urethane anesthesia attenuates long-term potentiation in chronically prepared animals [9,13]. The present findings suggest that this effect might be due in part to a secondary effect of urethane on brain temperature.

These results suggest that a portion of the field potential change that occurs during SWS relative to awake immobility is likely due to the reduction in brain temperature that accompanies SWS. However, it is not possible to determine from these data whether this accounts for all of the change that occurs during SWS. This is perhaps especially true for the PS, which showed much greater variability during the temperature reduction that accompanies SWS than during comparable temperature reductions that accompany the Cool Water Immersion and Urethane conditions (see Figs. 1 and 2). Thus, endogenous hippocampal EEG patterns may interact with the changes due to temperature fluctuations to determine the size of the field potential.

The number of rats studied in this way was small. However, four points suggest that these results are reliable, and are consistent with other work. First, the results were highly consistent across the experimental subjects, showing a similar magnitude and direction of change. Second, the results were highly consistent across experimental conditions, showing similar relationships among the different methods of changing brain temperature. Third, our findings from the Radiant Heat and Cool Water Immersion sessions were similar to those of Moser et al. [19] using similar techniques. Fourth, our findings were consistent with the results of earlier studies using acute anesthetized [3,4] or in vitro preparations that evaluated the effect of temperature changes on field potentials [21] and intracellularly recorded action potentials [24] from the CA1 region in the hippocampal slice preparation. Those studies attributed the changes to a number of possible factors, among them a slowing of ion channel kinetics and decreased hyperpolarization in CA1 pyramidal cells at lowered temperatures.

4.2. Sleep experiment

Winson and Abzug [30] reported that field potentials evoked throughout the trisynaptic circuit by stimulation of the PP changed as a function of behavior; in the DG during SWS, EPSP slope decreased and PS amplitude increased relative to the still-alert state. Still alertness was induced when "the animal is startled by a loud noise" [30, p. 719]. Both Whishaw and Vanderwolf [29] and Leung [18] have reported the occurrence of hippocampal small amplitude irregular activity at this time, in contrast to the large amplitude irregular activity normally present during awake immobility, in-

dicating that ongoing hippocampal activity is different in the two conditions. Despite this difference in underlying hippocampal activity, our results indicate that a comparison of field potentials evoked during normal awake immobility (not caused by startling the rat with a loud noise) and during SWS shows the same general relations as those reported by Winson and Abzug for still alertness and SWS: EPSP slope is reduced and PS amplitude is increased. The magnitude of these changes varies with the intensity of the test pulse, yielding relatively greater effects for the EPSP at high stimulation intensities, and for the PS at low stimulation intensities. The fact that the same changes occurred during the Sleep and the Sleep Replication sessions indicates that these effects are consistent and reproducible.

One of the goals of this study was to examine the Sleep/Open condition, which can be characterized by four observations made here. First, behaviorally it resembles quiet wakefulness preceeding or following SWS, and could be described as drowsiness, involving an immobile resting posture (ventral body surface flat on the chamber floor, with head lowered onto forepaws) with eyes open. It differs from SWS in that the eyes are open rather than closed, and the body is resting flat on the ventral surface rather than curled up. Second, the neocortical EEG contains a preponderance of slow wave bursts, in contrast to the fast low voltage activity that is characteristic of awake immobility. Third, brain temperature is intermediate between that of wakefulness and SWS (see Fig. 1A), a finding that was observed consistently in all three rats. Fourth, field potentials evoked during the Sleep/Open condition resemble those of SWS (see Fig. 1A), as indicated by statistically similar changes in both field potential measures relative to awake immobility during the Sleep/Closed and Sleep/Open conditions. These observations suggest that the Sleep/Open condition behaviorally resembles quiet wakefulness, but is similar to SWS in electrophysiological responsiveness, with brain temperature intermediate between waking and SWS. The obverse dissociation between behavioral and EEG signs of non-REM sleep has also been reported: the occurrence of a low-voltage fast neocortical EEG in the presence of behavioral, electromyographic, and hippocampal EEG signs of sleep [6].

A striking finding from the Sleep experiment is the persistence of the field potential changes beyond the Sleep/Closed and Sleep/Open conditions. One possible explanation for this is the persistence of a SWS-associated brain temperature reduction into the Awake condition, which might be expected to result in field potential measures like those of SWS. The interval between the end of the Sleep measures and the commencement of the Awake measures was less than 5 min. Based on observations made during the Temperature experiment, the change from a low brain temperature of 36.3 °C during SWS to a normal waking temperature of 38.0°C required approximately 10 min after awakening the rat (Fig. 1a). Thus, it is very likely that brain temperature was still somewhat depressed during the Awake measures, and that field potential measures reflected this. The stability of the EPSP slope control measures throughout the testing period (Fig. 5, top) suggests that the persistent changes in field potentials during the Sleep and Sleep Replication sessions were not the result of instability of the field potential measures.

4.3. Implications of the findings

The temperature at which tissue is maintained is recognized to be an important variable in studies of hippocampal field potentials in the in vitro slice preparation [21,24]. Our data, together with those of Moser et al. [19], indicate that brain temperature is also an important variable in studies of hippocampal field potentials in intact animals, and that changes in brain temperature can result from changes in the behavioral or drug state of the animal, or from environmental influences. The corresponding temperature-induced changes in field potentials can be large; for example, changes of 50 to 100% or more are not uncommon (Figs. 1 and 2; see also [19]). The reduction in brain temperature and EPSP strength that occurs during SWS may provide a parsimoneous explanation for why LTP of the EPSP is suppressed during SWS [17]. The careful monitoring of behavior, EEG, and brain temperature is advisable in experiments in which hippocampal field potentials are a dependent measure. This is particularly true for the Sleep/Open condition, for which there is a dissociation between behavioral and electrophysiological properties, and into which animals tend to pass during long recording sessions. More generally, the finding that brain temperature in behaving animals varies strongly as a function of behavioral activity level during many different natural behaviors and conditions (e.g. feeding, drinking, grooming, running, estrous) [1,2] suggests that the careful monitoring of behavioral activity levels and brain temperature is advisable in studies involving the recording of field potentials in behaving animals.

Acknowledgements

Supported by a grant to D.P.C. from NSERC, Canada. We thank L. Stan Leung, D. Saucier, and C.H. Vanderwolf for their comments on an earlier draft, and Ed Green for earlier suggestions.

References

[1] Abrams, R., and Hammel, H.T., Hypothalamic temperature in unanesthetized albino rats during feeding and sleeping, *Am. J. Physiol.*, 206 (1964) 641–646.

[2] Abrams, R., and Hammel, H.T., Cyclic variations in hypothalamic temperature in unanesthetized rats, *Am. J. Physiol.*, 208 (1965) 698–702.

[3] Andersen, P., Interhippocampal impulses. II. Apical dendritic activation of CA1 neurons, *Acta Physiol. Scand.*, 48 (1960) 178–208.

[4] Andersen, P., Interhippocampal impulses. III. Basal dendritic activation of CA3 neurons, *Acta Physiol. Scand.*, 48 (1960) 209–230.

[5] Baker, M.A., Brain cooling in endotherms in heat and exercise, *Ann. Rev. Physiol.*, 44 (1982) 85–96.

[6] Bergmann, B., Winter, J., Rosenberg, R. and Rechtschaffen, A., NREM sleep with low-voltage EEG in the rat, *Sleep*, 10 (1987) 1–11.

[7] Brankack, J. and Buzsáki, G., Hippocampal responses evoked by tooth pulp and acoustic stimulation: depth profiles and effect of behavior, *Brain Res.*, 378 (1986) 303–314.

[8] Buzsaki, G., Grastyan, E., Czopf,J., Kellenyi, L. and Prohaska, O., Changes in neuronal transmission in the rat hippocampus during behavior, *Brain Res.*, 225 (1981) 235–247.

[9] Cain, D., Boon, F. and Hargreaves, E., Evidence for different neurochemical contributioins to long-term potentiation and to kindling and kindling-induced potentiation: role of NMDA and urethane-sensitive mechanisms, *Exp. Neurol.*, 116 (1992) 330–338.

[10] Cain, D., Hargreaves, E. and Boon, F., Enduring effects of sleep and pseudosleep states on the evoked potential in the perforant path-dentate gyrus circuit, *Canad. Soc. Brain, Behav. Cog. Sci. Abstr.*, (1992) 27.

[11] Cain, D., Hargreaves, E., Boon, F. and Dennison, Z., An examination of the relations between hippocampal long-term potentiation, kindling, afterdischarge, and place learning in the water maze, *Hippocampus*, 5 (1993) 153–163.

[12] Douglas, R. and Goddard, G., Long-term potentiation of the perforant path–granule cell synapse in the rat hippocampus, *Brain Res.*, 86 (1975) 205–215.

[13] Engstrom, D.A., Bennett, M.C., Stevens, K.E., Wilson, R.L., Diamond, D.M., Fleshner, M. and Rose, G.M., Modulation of hippocampal primed burst potentiation by anesthesia, *Brain Res.*, 521 (1990) 148–151.

[14] Hargreaves, E., Boon, F. and Cain, D., Low frequency potentiation of the dentate gyrus field potential as a consequence of input/output curves, *Canad. Soc. Brain, Behav. Cog. Sci. Abstr.*, 3 (1993) 51.

[15] Hargreaves, E. and Cain, D., Variance of perforant-path/dentate-gyrus (PP/DG) evoked potential (EP) measures across the *I/O* curve during behavioral immobility and movement in rats, *Soc. Neurosci. Abstr.*, 17 (1991) 1400.

[16] Hargreaves, E., Cain, D. and Vanderwolf, C., Learning and behavioral long-term potentiation: importance of controlling for motor activity, *J. Neurosci.*, 10 (1990) 1472–1478.

[17] Leonard, B., McNaughton, B. and Barnes, C., Suppression of synaptic plasticity during slow-wave sleep, *Brain Res.*, 425 (1987) 174–177.

[18] Leung, L.S., Behavior-dependent evoked potentials in the hippocampal CA1 region of the rat. I. Correlation with behavior and EEG, *Brain Res.*, 198 (1980) 95–117.

[19] Moser, E., Mathiesen, I. and Andersen, P., Association between brain temperature and dentate field potentials in exploring and swimming rats, *Science*, 259 (1993) 1324–1326.

[20] Racine, R., Tuff, L. and Zaide, J., Kindling, unit discharge patterns and neural plasticity, *Can. J. Neurol. Sci.*, 2 (1975) 395–405.

[21] Schiff, S. and Somjen, G., The effects of temperature on synaptic transmission in hippocampal tissue slices, *Brain Res.*, 345 (1985) 279–284.

[22] Segal, M., A correlation between hippocampal responses to interhemispheric stimulation, hippocampal slow rhythmic activity and behavior, *Electroenceph. Clin. Neurophysiol.*, 45 (1978) 409–411.

[23] Skelton, R., Miller, J. and Phillips, A., Low-frequency stimulation of the perforant path produces long-term potentiation in the dentate gyrus of unanesthetized rats, *Can. J. Physiol. Pharmacol.*, 61 (1983) 1156–1161.

[24] Thompson, S., Masukawa, L. and Prince, D., Temperature dependence of intrinsic membrane properties and synaptic potentials in hippocampal CA1 neurons in vitro, *J. Neurosci.*, 5 (1985) 817–824.

[25] Vanderwolf, C., Harvey, G. and Leung, L.S., Transcallosal evoked potentials in relation to behavior in the rat: Effects of atropine, *p*-chlorophenylalanine, reserpine, scopolamine and trifluoperazine, *Behav. Brain Res.*, 25 (1987) 31–48.

[26] Vanderwolf, C., Kramis, R., Gillespie, L. and Bland, B., Hippocampal rhythmical slow activity and neocortical low voltage fast activity: relations to behavior. In R. Isaacson and K. Pribram (Eds.), *The Hippocampus, Vol. 2, Neurophysiology and Behavior*, Plenum, New York, 1975, pp. 101–128.

[27] Weiss, T., Effect of body temperature on theta rhythm in the rat hippocampus, *Physiol. Bohemoslav.*, 13 (1964) 246–255.

[28] Whishaw, I. and Vanderwolf. C., Hippocampal EEG and behavior: effects of variation in body temperature and relation of EEG to vibrissae movement, swimming and shivering, *Physiol. Behav.*, 6 (1971) 391–397.

[29] Whishaw, I. and Vanderwolf, C., Hippocampal EEG and behavior: changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats, *Behav. Biol.*, 8 (1973) 461–484.

[30] Winson, J. and Abzug, C., Neuronal transmission through hippocampal pathways dependent on behavior, *J. Neurophysiol.*, 41 (1978) 716–732.