Research Report

Temperature modifies potentiation but not depotentiation in bidirectional hippocampal plasticity of Syrian hamsters (Mesocricetus auratus)

Nathan W. Bronson, John B. Piro, Jock S. Hamilton, John M. Horowitz*, Barbara A. Horwitz

Section of Neurobiology, Physiology, and Behavior, University of California, Davis, 1 Shields Ave, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Accepted 30 March 2006
Available online 15 June 2006

Keywords:
Limbic system
Long-term depression
CA1 pyramidal cells
Hibernators
Brain temperature

ABSTRACT

Previous studies have shown that one form of neuroplasticity, population spike (PS) potentiation, can be established in the hamster hippocampus at temperatures above 20 °C. Here, we tested three related hypotheses; namely, that in Syrian hamsters: (1) PS potentiation can be elicited below 20 °C and that at any constant temperature, potentiation can be described by a pair of sigmoidal functions matched to input/output curves; (2) potentiation can be partially reversed by depotentiation (a second and distinctive form of neuroplasticity); and (3) tetanus evokes long-term potentiation in slices from animals housed under conditions corresponding to various stages of the annual hibernation cycle. To test these hypotheses, we measured PS amplitudes and fEPSP slopes in CA1 pyramidal cells in hippocampal slices. We found that sigmoidal functions before and after tetanus showed PS enhancement at 18 °C and a larger enhancement at 28 °C, thereby supporting hypothesis 1. We also found that low-frequency stimulation reduced the amplitude of the potentiated PS by ∼29% at both 18 °C and 28 °C, consistent with hypothesis 2; and that slices from nonhibernating hamsters on long and short photoperiods and from hamsters in hibernation all showed at least 40% increases in fEPSP slope following tetanus at a slice temperature of 23 °C, supporting hypothesis 3. Thus, bidirectional plasticity is present in hamsters. That is, both potentiation and depotentiation were readily evoked at 28 °C; potentiation was muted, while depotentiation (the reversal of the potentiation) remained robust at 18 °C. Moreover, potentiated responses could be elicited in slices from animals housed under diverse conditions.

1. Introduction

While nonhibernating mammals regulate brain temperature near 37 °C throughout their lifetime, the brain temperature of hibernators such as the Syrian hamster varies from 37 °C in the euthermic state to about 6 °C when the animal is in hibernation. Neural activity in hibernators is markedly slowed as brain temperature falls (Hamill et al., 1989; Heller, 1979; Chatfield and Lyman, 1954). Yet even at 6 °C, where neocortical EEG activity is attenuated (Chatfield and Lyman, 1954), sensory and homeostatic systems in the central nervous system of the hibernating hamster continue to function appropriately so that, for example, loud sounds arouse the hamster: and respiration and core temperature continue to be well regulated, albeit at altered set points (Beckman and Stanton, 1976; Heller, 1979; Horowitz and Horrigan, 1996).
Nonetheless, hibernation affects neural systems as shown by the findings that memories formed by active squirrels during the summer are partially degraded by hibernation (Millesi et al., 2001). More generally, differences in brain activity in nonhibernators and hibernators are seen not only at 6 °C, but also at much warmer temperatures (Gabriel et al., 1998; Spangenberger et al., 1995a). A striking adenosine-mediated depression of hippocampal synaptic transmission is seen in rats but not in hamsters at increasing temperatures corresponding to the rise in body temperature in hamsters during arousal from hibernation (Gabriel et al., 1998; Spangenberger et al., 1995a). This lower sensitivity to adenosine in hamsters was proposed to be a prerequisite for safe arousal from hibernation by avoiding a temperature-induced disturbance in neuronal communication. In addition, these studies (Gabriel et al., 1998; Spangenberger et al., 1995a) showed that cellular mechanisms in the hippocampus of hibernators may be quite different than those of nonhibernators.

Cellular mechanisms for several forms of neural plasticity have been intensively studied in the hippocampus of nonhibernating mammals (Bear, 2003; Kemp and Bashir, 2001; Luthi et al., 2004; Malenka and Nicoll, 1999) as they likely underlie memory and learning (Tang et al., 2001). In contrast, the only form of neuroplasticity that has been studied in hibernating species is long-term potentiation (LTP) (Krelstein and Horowitz, 1990; Krelstein et al., 1990; Spangenberger et al., 1995b); i.e., hamster studies show an increased population spike (PS) amplitude (the evoked response of a population of CA1 pyramidal cells) following tetanus of Schaffer collateral/commissural fibers.

In nonhibernating species, other distinctive forms of neuroplasticity that appear to be important in rapidly shaping memories include depotentiation, a partial reversal of LTP by low-frequency stimulation (LFS), and long-term depression (LTD), a suppression in response amplitude that is also generated by LFS but that does not require prior potentiation and is most easily observed in rats less than 3 weeks old (Bear and Malenka, 1994; Dudek and Bear, 1993; Kemp et al., 2000; Milner et al., 2004). Because induction of hibernation in Syrian hamsters requires several weeks of photoperiod accommodation followed by exposure to a cold environment, animals are usually older than 16 weeks of age before they hibernate. They are thus at an age where depotentiation, but not LTD, can be readily evoked in rats. Depotentiation partially reverses LTP, but even without depotentiation, LTP only lasts for hours in vivo preparations and days in vitro preparations at one stimulus intensity are sufficient to detect depotentiation.

Studies examining hippocampal anatomy show a reduction of neural connectivity during hibernation (Popov and Bocharova, 1992; Popov et al., 1992), indicating that the hippocampus of a hibernating species has a form of morphological plasticity occurring over a much longer period of time than the rapid induction of LTP driven by changes in electrical activity over afferent fibers. Whether the reduction in connectivity reduces induction of LTP during hibernation and for a period of time following arousal has yet to be determined.

In this study, we tested the hypotheses that (1) I/O data (matched by sigmoidal curves) show long-lasting bidirectional plasticity (potentiation/depotentiation) in the hamster hippocampus over a range of temperatures from 33 °C to a low temperature threshold (near 18 °C); (2) potentiation and depotentiation have different temperature dependencies; and (3) hippocampal slices obtained from hibernating hamsters and from euthermic hamsters on a long or short photoperiod all have cellular mechanisms supporting the generation of LTP.

2. Results

2.1. Effects of temperature on tetanus evoked potentiation

With temperature held constant, input-output relationships were determined before and after tetanization of the input pathway by measuring PS amplitudes over a range of stimulus intensities. An example of a pair of sigmoidal curves fitted to the two I/O data sets (a control set recorded before tetanus and a second set recorded 30 min after tetanus) is
shown in Fig. 1. For this slice, stimulus intensities were varied from 2 to 23 V, and bath temperature was held constant at 28 °C throughout the experiment. Point a denotes the response at a stimulus intensity (in this case, 9 V) that evoked a half-maximal response. Following tetanus, changes in three parameters were seen (Fig. 1): (1) the maximum response increased; (2) the slope of the tangent to the sigmoidal curve at point a increased; and (3) the magnitude of the response at a midpoint stimulus increased (as shown by the vertical line from point a on the control curve to point b on the post-tetanus curve). This line corresponds to potentiation observed in PS amplitude in hamster experiments (Krelstein and Horowitz, 1990; Krelstein et al., 1990; Spangenberger et al., 1995b).

To compare potentiation in 28 slices (from 11 animals, each slice at a constant bath temperature of either 18, 23, or 28 °C), parameters of the post-tetanus curve were expressed as a percentage of those for the control (pre-tetanus) curve in each slice (Table 1). Comparing responses measured at the warmest and coolest temperatures (28 and 18 °C), the effect of the lower temperature was to shift sigmoidal curves to the right (Fig. 2). Tetanus resulted in potentiation at all temperatures, an enhancement that persisted throughout the remainder of the experiment (at least 30 min). Finally, while the response was potentiated at every temperature, the increase of the midpoint response ($O_v$ for input $I_{mid}$) was significantly less at 18 °C than at 23 °C and 28 °C.

A sigmoidal curve (Eq. (1)) was fitted to each I/O curve obtained in a slice (data for one I/O curve obtained just before tetanus, and data for a second I/O curve obtained 30 min after tetanus). Values in the table, expressed as mean ± SEM for n = number of slices, show the % increase of post-tetanus parameters of the sigmoidal curve over pre-tetanus (control) parameters. Values for each parameter were compared among groups by a one-way ANOVA. The P values (bottom row) show a significant difference in sigmoidal response where the stimulus intensity was fixed at the value evoking a half-maximal response on the control curve. A post hoc test (Newman-Keuls) indicated that the source of this difference was the response of the 18 °C group (a) compared to both the 23 and 28 °C groups (b). No statistical differences in potentiation changes were found among the other groups. When the 23 °C data were excluded, analysis via a paired t test also indicated that the only significant difference between the 18 and 28 °C groups was in the midpoint response ($P = 0.0015$).

Fig. 1 – Sigmoidal curves matched to I/O data obtained before and after tetanus show potentiation. One sigmoidal curve (heavy line) is matched to I/O data recorded before tetanus (open circles), a second curve (light line) is matched to I/O data (open squares) recorded after tetanus. Parameters measured in comparing sigmoidal curves include the change in the maximum response, the change in the linear slope at the midpoint of each curve, and the change in the response amplitude (the voltage change of the vertical line drawn from point a, the midpoint of the pre-tetanus sigmoidal curve, to point b, the intersection of the vertical line with the post-tetanus curve).

Fig. 2 – Tetanus-induced shifts in I/O curves at both 18 °C and 28 °C show potentiation. Each pair (black/grey) of curves shows average sigmoidal fits to I/O data measured before (black) and after (grey) tetanus at 18 °C (solid curves, n = 14) and at 28 °C (dotted curves, n = 11). All fits had correlation coefficients of $r > 0.98$. At both temperatures, the pre-tetanus control curves had smaller slopes and smaller responses at the control midpoint than did the corresponding post-tetanus curves (Table 1).

Table 1 – Effect of temperature on potentiation

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>% Increase of maximum ($O_b$)</th>
<th>% Increase of slope ($S$)</th>
<th>% Increase of $O_v$ for input $I_{mid}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 (n = 14)</td>
<td>3.2 ± 3.3</td>
<td>51.8 ± 30.8</td>
<td>37.3 ± 14.2 a</td>
</tr>
<tr>
<td>23 (n = 3)</td>
<td>11.5 ± 5.5</td>
<td>103.6 ± 40.4</td>
<td>115.3 ± 15.7 b</td>
</tr>
<tr>
<td>28 (n = 11)</td>
<td>5.6 ± 3.9</td>
<td>122.2 ± 29.4</td>
<td>98.8 ± 7.6 b</td>
</tr>
<tr>
<td>P value</td>
<td>0.5840</td>
<td>0.2500</td>
<td>0.0019</td>
</tr>
</tbody>
</table>

A sigmoidal curve (Eq. (1)) was fitted to each I/O curve obtained in a slice (data for one I/O curve obtained just before tetanus, and data for a second I/O curve obtained 30 min after tetanus). Values in the table, expressed as mean ± SEM for n = number of slices, show the % increase of post-tetanus parameters of the sigmoidal curve over pre-tetanus (control) parameters. Values for each parameter were compared among groups by a one-way ANOVA. The P values (bottom row) show a significant difference in sigmoidal response where the stimulus intensity was fixed at the value evoking a half-maximal response on the control curve. A post hoc test (Newman-Keuls) indicated that the source of this difference was the response of the 18 °C group (a) compared to both the 23 and 28 °C groups (b). No statistical differences in potentiation changes were found among the other groups. When the 23 °C data were excluded, analysis via a paired t test also indicated that the only significant difference between the 18 and 28 °C groups was in the midpoint response ($P = 0.0015$).
2.2. Effects of lowering temperature on input–output relations

Although the experiments in Section 2.1 demonstrate the effect of temperature on the magnitude of tetanus-induced potentiation, they do not provide information on effects of temperature on input–output relations when no tetanus is applied. That is, in experiments described in the previous section, each slice was held at a single fixed temperature throughout the recording period. To directly assess the effects of temperature on a nontetanized slice, we obtained data for I/O curves at 18 °C and 28 °C in each slice.

Sigmoidal curves fitted to the I/O curves at these two temperatures showed three changes (Table 2, Fig. 3A): (1) curves at 18 °C had a significantly smaller slope at their midpoint than did curves at 28 °C; (2) the maximum amplitude decreased at 18 °C; and (3) the midpoint response amplitude at 18 °C was smaller than that at 28 °C. (In addition, records of evoked responses showed that decreases in amplitude were accompanied by a progressive increase in the latency to the peak of the population response.)

Because sigmoidal curves closely fitted the data at all temperatures (Figs. 1, 2, 3A), a second protocol was used to determine the effect of a sequence of bath temperatures on PS amplitude in a single slice. First, the stimulus intensity that evoked a half-maximal response was found by constructing an I/O curve at 28 °C. This half-maximal stimulus intensity was then used throughout the remainder of the experiment, while temperature was slowly decreased to the point where evoked responses could no longer be detected (Fig. 3B). We found no appreciable decline in PS amplitude until the temperature decreased below 24 °C. [Raising the temperature in a stepwise fashion gave the same evoked response amplitudes as lowering the temperature in a stepwise fashion. In addition, repeated measurements for the construction of two or more I/O curves at the same temperature showed that the I/O curves were stable over time (data not shown)]. Fig. 3B illustrates the typical shape of the decline in PS amplitude as bath temperature was lowered, although in most slices, population spikes could be recorded at temperatures at and below 15 °C.

Even when the recording chamber temperature was several degrees above the threshold for recording population spikes, it was not always possible to induce potentiation. The scatter plot for 30 slices (Fig. 4) shows that when bath temperature was set below 16 °C, no increase in PS amplitude following tetanus was detected. In contrast, potentiation was seen in 11 of the 17 slices with bath temperatures between 17 and 19 °C; and at temperatures higher than 19 °C, potentiation was established in 10 of 10 slices. (To confirm that a potentiation was stable at temperatures near threshold, PS amplitudes in Fig. 4 were measured 60 min following tetanus.) Thus, PS potentiation

---

Table 2 – Effect of temperature on parameters of sigmoidal curves fitted to I/O data

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Maximum (mV)</th>
<th>Slope (mV/V)</th>
<th>Response at the midpoint stimulus (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 (n = 4)</td>
<td>2.07 ± 0.30</td>
<td>0.25 ± 0.05</td>
<td>0.56 ± 14.2</td>
</tr>
<tr>
<td>28 (n = 4)</td>
<td>2.87 ± 0.43</td>
<td>0.46 ± 0.09</td>
<td>1.44 ± 0.21</td>
</tr>
<tr>
<td>% of 28 °C response</td>
<td>73.4 ± 7.8%</td>
<td>56.3 ± 10.8%</td>
<td>73.3 ± 7.8%</td>
</tr>
<tr>
<td>P value</td>
<td>0.023</td>
<td>0.033</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n = number of slices. Changes of values were compared by a one-tailed paired t test. The “% of 28 °C response” is the ratio (18 °C response/28 °C response) × 100.

---

Fig. 3 – Temperature dependence of PS amplitudes. (A) In recordings from a single slice, the bath temperature was held constant at 28 °C, while PS amplitudes were recorded (open triangles) for an initial I/O data set; then the bath temperature was decreased to 18 °C and maintained constant at this lower temperature while PS amplitudes (filled triangles) were recorded for a second I/O data set. For each I/O data set, PS amplitudes were measured over a 20 V range of stimulus intensities. Sigmoidal fits were made to both data sets (dashed and solid curves). Both slope and maximum were smaller at 18 °C. (B) In recordings from a single slice, the stimulus intensity was held constant at 9V, while the temperature was slowly lowered from 28 °C to 18 °C, and PS amplitudes were measured every 4 min. Points a and b indicate the starting and ending PS amplitudes, respectively. A progressive decrease in PS amplitude with decreasing temperature was evident below 24 °C.
could be detected below 20 °C (Fig. 4), with population spikes being evoked at still lower temperatures, albeit with low amplitudes.

2.3. The effects of incubation temperature on subsequent PS enhancement

Two additional slice preparation paradigms were used to determine if incubation chamber temperature had a persisting effect on slice properties after slices were transferred to the recording chamber. Both incubation protocols followed the same preparation procedure described in Experimental procedures, except that alternate slices were placed in different incubation chambers so that half of the slices were immersed in an incubation chamber at 22 ± 2 °C (the warm group), while the other half were immersed in an incubation chamber at 18 °C (the cold group). Thus, slices in the warm group recovered under different conditions than those in the cold group (Watson et al., 1997; Weiner et al., 1997). Slices from the incubation chambers were subsequently transferred to a recording chamber (with temperature clamped at either 18 °C or 29 °C).

The fraction of slices that showed PS enhancement was dependent on prior incubation temperature as shown by the number of slices that showed at least modest enhancement, an increase in PS amplitude of at least 20% above the pre-tetanus PS amplitude (a less stringent requirement than the 25% increase, denoted potentiation in other experiments in this study). When recording chamber temperature was set to 18 °C, tetanus evoked an increased enhancement of ≥20% in 6 of 6 slices from the warm-incubation group but in only 4 of 8 slices from the cold-incubation group. That is, only half the slices from the cold group showed even a modest PS enhancement, indicating that for this group, the temperature threshold for consistent enhancement had shifted to a temperature above 18 °C. Thus, effects of the cold incubation persisted, affecting the responses of slices in the recording chamber. Interestingly, if a slice from the cold-incubation group did show enhancement, it was often fairly robust (greater than 40% in 4 out of 8 slices, Fig. 5A).

In examining the effects of incubation chamber temperature on subsequent recordings, we found that slices from the cold-incubation group were generally less responsive than slices from the warm-incubation group. For example, PS enhancement following tetanus was significantly lower with the recording chamber temperature set to 18 °C than when it was set to 29 °C for slices from the cold-incubation group, whereas PS enhancement at 18 °C and at 29 °C did not statistically differ in slices from the warm-incubation group (Fig. 5B). The stimulus intensity required to evoke a half-maximal response at 18 °C in slices from the cold-incubation group was significantly higher than that required to evoke a half-maximal response at 29 °C in slices from the warm-incubation group (Fig. 5C). However, some slice properties were not dependent on incubation temperature — notably, the amplitude of the half-maximal response (Fig. 5D) of cold and warm groups did not statistically differ at either recording chamber temperature.

Increased PS amplitudes were associated with increases in the slope of a line matching the initial portion of the evoked response, a rising field potential (fEPSP) between the shock artifact and the population spike (Jeffery, 1995). This increase in slope is illustrated in Fig. 5A at 18 °C for responses measured before and after tetanus.

2.4. The effects of temperature on depotentiation

After PS potentiation was established in CA1 pyramidal cells (section 2.1), LFS was applied (1 Hz for 15 min at the half-maximal response level) to determine if depotentiation, a partial reversal of the potentiation, could be induced (Figs. 6A–C). Since depotentiation had not been previously described in hibernating species at any temperature, we used several bath temperatures (33 °C, 28 °C, and 18 °C) to assess its presence and its temperature dependence. Because at low temperatures, population spikes could be measured while fEPSP slopes were more difficult to assess, the responses of slices in the recording chamber. At warmer temperatures, following the establishment of LTP, a decrease in fEPSP slope was noted in responses recorded after LFS compared with responses just preceding LFS.) Plots of PS amplitudes versus time (Figs. 6A–C) show that, after establishment of a stable baseline (point a), tetanus evoked an enhancement of PS amplitude that persisted for at least 30 min (b) and subsequent LFS depressed this response (the period between c and d). Thus, at all three temperatures, the application of LFS induced depotentiation.

The effect of temperature on the magnitude of this depotentiation, expressed as percent of the measured amplitude just prior to LFS, was greatest at 18 °C and
least at 33 °C (Table 3). Taken together, the data in Tables 1 and 3 showed that mechanisms supporting bidirectional plasticity (potentiation/depotentiation) were present in the hamster hippocampus and could be evoked significantly below euthermic temperatures.

2.5. Potentiation in slices from euthermic hamsters on long and short photoperiods and from hamsters in hibernation

Although at low temperatures population spikes had small amplitudes and increased durations, their parameters could be relatively easily measured because the spike-like shape of the waveform allowed identification of peak values (Fig. 7). In contrast, it was more difficult to measure fEPSP slopes at low temperatures due to the gradual change in their waveforms and their lower amplitudes (because stimulus intensity, while sufficient to evoke synaptic activity, was below the threshold value needed to evoke population spikes). However, at 23 °C, fEPSP slopes were readily measured (Fig. 7); therefore, this temperature was chosen as a standard to which responses in hamsters housed under the three different combinations of light–dark and temperature (described in Experimental procedures) were compared.

At 23 °C, the slope of the fEPSP in all three hamster groups increased significantly (P < 0.05) following tetanus, directly demonstrating the establishment of LTP. Specifically, 30 min after tetanus, the fEPSP slope for slices in hamsters from group 1 (long photoperiod) had increased 50.6 ± 11.4% (mean ± SEM); that for group 2 (short photoperiod) had increased 54.0 ± 17.2%; and that for group 3 (where each hamster was in hibernation at the time of sacrifice) had increased 42.8 ± 7.6%.

3. Discussion

This study appears to be the first report of cellular mechanisms supporting depotentiation being present in a hibernating species. Additionally, we found that mechanisms generating depotentiation remain stable and robust between 28 °C and 18 °C (Table 3). In contrast, there is a marked decrease in potentiation as temperature falls from 28 °C to 18 °C (Table 1). Below a threshold temperature of 18 °C, potentiation cannot
be reliably induced (Fig. 4) and hence cannot be reversed. Nonetheless, in each slice studied, population spikes could always be evoked at temperatures below 18 °C.

3.1. Tetanus-induced potentiation at all temperatures above threshold

Sigmoidal curves fitted to I/O data obtained before and after tetanus showed that responses evoked by a half-maximal stimulus provided a good one-point assessment of plasticity in the hamster at all three temperatures evaluated. As a result, stimulation at other intensities was not required to optimally detect potentiation. PS amplitudes at half-maximal stimulus showed robust potentiation in slices at 23 °C, frequently exceeding 90% above control values, and were similar to those reported in previous hamster studies (Krelstein and Horowitz, 1990; Krelstein et al., 1990; Spangenberg et al., 1995b).

At 23 °C, sigmoidal curves for population spike I/O data were consistent with fEPSP slope increases showing LTP. The increased PS amplitude following tetanus indicates network plasticity, including possible alterations at sites between the dendrites and the axon hillock (E–S coupling) such as altered GABAergic inputs to synapses located on the soma (Chavez-Noriega et al., 1989). However, the measurements showing increased fEPSP slopes at 23 °C in the hamster hippocampus indicate that plasticity occurred on a more restricted portion of the circuit—namely, the synapse between CA3 and CA1 pyramidal cells (Malenka and Nicoll, 1999). This is consistent with the work of Spangenberg et al. showing that at 22 °C, PS enhancement in Turkish hamsters was also associated with increased fEPSP slope (Spangenberg et al., 1995b). In addition, at 23 °C increased fEPSP slopes following tetanus in slices obtained from hibernating hamsters and from hamsters on a long or on a short photoperiod clearly indicated that all groups maintained the ability to generate LTP. In particular, the increase in fEPSP slope seen at 23 °C in slices from hibernating hamsters implies that during arousal from hibernation, mnemonic mechanisms can immediately store new information despite the reduction of neural connectivity that occurs in the hibernating state (Popov and Bocharova, 1992; Popov et al., 1992). Finally, evidence that PS potentiation reflects LTP at even lower temperatures is provided by the increased fEPSP slopes following tetanus observed at 18 °C (Fig. 5A), where the fEPSP is measured on the portion of the

![Fig. 6](image)

**Table 3 – Effect of temperature on depotentiation**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>% Depotentiation</th>
<th>Slope (mV/V) for at midpoint response</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 (n = 9)</td>
<td>30.4 ± 5.1</td>
<td>0.1 ± 0.03</td>
<td>0.010</td>
</tr>
<tr>
<td>28 (n = 12)</td>
<td>28.0 ± 4.6</td>
<td>0.4 ± 0.10</td>
<td>0.008</td>
</tr>
<tr>
<td>33 (n = 6)</td>
<td>15.2 ± 2.8</td>
<td>0.3 ± 0.10</td>
<td>0.005</td>
</tr>
</tbody>
</table>

For each slice, a sigmoidal curve was fitted to each of three I/O curves: one before and one after tetanus (to ensure potentiation was established) and a third 30 min following LFS (to detect depotentiation). Values are means ± SEM of characteristics calculated from the sigmoidal fit of each slice’s I/O curve. The % depotentiation in population spike amplitude is for half-maximal stimulus intensity. Paired t test comparisons of PS amplitude 30 min after tetanus and just prior to LFS compared with PS amplitude 30 min after LFS (listed as P values in column 4) show significant depotentiation at all three temperatures. (Values = mean ± SEM for n = number of slices.)
response preceding the population spike (a method described in Jeffery, 1995).

In this study, the temperature threshold for establishing potentiation was 18 °C (Fig. 4), 2 °C lower than previously reported (Krelstein et al., 1990). This difference may reflect the different protocols used in tissue preparation and incubation. To partially test this possibility, slices were incubated under cold (harsh) and warm (mild) conditions (Watson et al., 1997; Weiner et al., 1997). The fraction of slices from the cold-incubation group that then showed PS spike enhancement following tetanus at 18 °C was less than that of slices from the warm group. Nonetheless, a 2 °C variation in reported thresholds is relatively small. A more significant finding consistently reported in hamster studies and confirmed in this study is that a threshold for potentiation exists such that at temperatures several degrees below threshold, single shock stimulation continues to excite CA1 pyramidal cells to generate population spikes even though tetanus fails to evoke potentiation. This inability to induce PS potentiation at low temperatures (e.g., at 15 °C in Fig. 4) is likely due to insufficient calcium entry through NMDA channels as suggested by experiments where calcium concentration was altered (Krelstein and Horowitz, 1990).

### 3.2. Bidirectional plasticity

Sigmoidal curves fitted to I/O data provided evidence that responses evoked by a half-maximal stimulus were a good one-point assessment of depotentiation at all temperatures. Depotentiation was robust in slices at 28 °C, averaging 28%; and even at slice temperatures only a few degrees greater than 18 °C, LFS consistently reversed the potentiation by approximately 30%, showing that mechanisms supporting depotentiation remained operational (Table 3).

Thus, a major finding of this study was that bidirectional plasticity was present at all temperatures above threshold, albeit its two components (potentiation and depotentiation) had different temperature sensitivities. Because the calcium current required to elicit depotentiation is less than that required to elicit potentiation (Bear and Malenka, 1994), low calcium influx could result in smaller potentiation yet remain sufficient to fully activate depotentiation at temperatures just above threshold.

### 3.3. The role of the hippocampus in hibernation

While the hippocampus plays a supportive role in the formation of new memory traces, it is also part of several other functional systems involving diverse brain areas (cf., Holscher, 2003). In hibernators, the hippocampus is part of a distinctive functional system that reorders brain activity over the hibernation cycle (Beckman and Stanton, 1976; Heller, 1979; Horowitz and Horrigan, 1996; Mihailovic, 1972). It is one of the last cortical regions to show decreased EEG as hamsters enter deep hibernation and is one of the first to regain activity as animals arouse (Mihailovic, 1972). Intrahippocampal infusion of histamine at a dose of 3 fmol/h significantly delays arousal in golden-mantled ground squirrels (Citellus lateralis) (Sallmen et al., 2003), a finding that strongly supports the proposal that the hippocampus is part of the system controlling arousal from hibernation.

Other portions of this arousal system include the midbrain reticular formation, hypothalamus, and septal area (Beckman and Stanton, 1976; Heller and Colliver, 1974; Heller et al., 1977). Medial septal neurons directly drive the hippocampus (and provide the source of the theta-rhythm of hippocampal EEG), and the striking observation that they double their activity in slices from hibernating compared to nonhibernating ground squirrels (C. undulates) implies that there is a state-dependent alteration in hippocampal input (Belousov et al., 1990).

The present study demonstrated that at a tissue temperature of 23 °C, LTP could be readily established for animals housed a variety of photoperiods and ambient temperatures. Moreover, at 23 °C, depotentiation was also readily observed in slices from hamsters housed under conditions (14:10 L:D 22 °C), corresponding to those encountered by summer active hamsters. But as slice temperature fell, activation of bidirectional plasticity was suppressed, implying that below a threshold of 18 °C, activity-induced modulation of the mnemonic functional system is essentially suspended. Yet in deep hibernation (at a brain temperature near 6 °C), hippocampal activity over a second functional system can affect the duration of the hibernation bout. Taken together these two functional systems appropriately support behavior throughout the hibernation cycle.

### 4. Experimental procedures

#### 4.1. Animals

Thirty 4- to 6-week-old Syrian Hamsters (Mesocricetus auratus) were obtained either from a colony at UC Davis or from Simonsen Labs Inc. (Gilroy, CA). Animals were placed in one of three groups: (1) a group housed at 22 °C on long photoperiod (14:10 light:dark); (2) a group acclimated to short photoperiod (10:14 L:D) for 4-6 weeks at 22 °C, and (3) a group acclimated to short photoperiod (10:14 L:D) for 4-6 weeks at 22 °C, and subsequently transferred to a 7 °C facility (10:14 L:D) to induce hibernation. Experiments were conducted on group 1 animals unless otherwise noted. All animals were fed ad libitum.

All animal use procedures were in compliance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the University of California at Davis.

#### 4.2. Slice preparation

Animals were killed by decapitation, and the brain was removed and chilled for 2 min in 2 °C artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 3.5 KCl, 2 CaCl₂, 1.25 NaH₂PO₄, 2 MgSO₄, 26 NaHCO₃, and 10 glucose. Hippocampi were removed, cut transversely into 400-μm-thick slices, and placed on filter paper soaked in ACSF in an interface incubation chamber aerated with 95% O₂/5% CO₂ unless otherwise noted. Slices were incubated at room temperature (~22 °C) for at least 30 min before being transferred to an aerated ACSF solution in a Plexiglas recording chamber whose temperature was controlled via a circulating water bath.
4.3. Recording procedures

By adjusting the temperature of a circulating water bath in thermal contact with the recording chamber, the temperature of the recording chamber was set to a particular value (within the range 33 °C to 12 °C) and then held within ±0.5 °C, while a slice was placed in the recording chamber, electrodes positioned, and recordings obtained. In all experiments, except for those shown in Fig. 3, the temperature of each slice was held at a fixed value between 33 °C and 12 °C, while PS amplitudes or fEPSPs were recorded. For the experiments shown in Fig. 3, chamber temperature was reset to a new value between measurements of PS amplitudes. In all experiments, ACSF flow in the recording chamber was maintained at 1.5–2.0 ml/min.

Bipolar tungsten stimulating electrodes were placed in the stratum radiatum of area CA3 to stimulate Schaffer collateral/commissural fibers. Glass microelectrodes filled with 3.0 M NaCl and having a resistance of 2–4 MΩ were positioned either in the stratum pyramidale (near CA1 pyramidal cell somas) to measure PS amplitude or in the stratum radiatum (near CA1 pyramidal cell apical dendrites) to record fEPSPs (Fig. 7). (Note that, while less than optimal, fEPSPs were also measured on the portion of the evoked waveform preceding the PS with the recording electrode placed in the stratum pyramidale, Fig. 7 (Jeffery, 1995)).

Stimulus parameters for single-shock stimulation, tetanus, and LFS were identical in all experiments (unless otherwise noted in results). Shocks delivered to Schaffer collateral/commissural fibers had a pulse duration fixed at 100 μs. Tetanus consisted of two trains (100-Hz pulses for 1 s, with an intertrain interval of 1 min) at a maximal voltage (e.g., 16 V in Fig. 1). LFS consisted of 900 shocks at 1 Hz (stimulation over a 15-min interval). Unless stated otherwise, data for each experiment were obtained for 6 slices from at least 3 animals.

To generate I/O curves (Fig. 1), PS amplitudes were recorded over a range of stimulus strengths. Each trial had three priming pulses and was followed by three stimuli; the latter were averaged. Once a stable response at the half-maximal level was found, an I/O curve was generated. For each trial, the stimulus voltage was lowered until a threshold stimulus was determined. Then the stimulus intensity was raised in steps from the half-maximal level until the maximal response was found. Typically, 7–9 trials at different voltages were used to determine the I/O curve.

4.4. Data analysis

As shown in Fig. 1, input-output data were fitted with a sigmoidal curve, a modified form of the Boltzmann equation:

\[
O_v = O_b + \frac{O_{\text{max}}}{1 + \exp(I_{\text{mid}} - I_v/s)}
\]

where \(O_v\) = output voltage (mV), the amplitude of the PS; \(O_b\) = baseline output (mV), baseline noise as determined as the difference between the maximum and minimum amplitude of the spontaneous activity; \(O_{\text{max}}\) = the maximum output (mV), the maximum PS amplitude; \(I_{\text{mid}}\) = the input voltage (V) at the midpoint of the function; \(I_v\) = the input voltage (V); \(S\) = the slope factor expressed as the change in input voltage for an e-fold; \((e = 2.72)\) change in the output.

The baseline value was fixed as the measured noise levels found at sub-threshold stimulation levels. Other parameters \((O_{\text{max}}, I_{\text{mid}}, s)\) were allowed to float to obtain the best possible fit. Fits were computed and plotted using KaleidaGraph™ from Synergy Software. Comparisons of population I/O curves were made based on the parameters of the fitted sigmoidal functions. To determine if potentiation was established, the amplitude of the PS at the midpoint value of the post-tetanus curve was compared with the response at this same voltage on the pre-tetanus curve. The criterion for potentiation was a persisting post-tetanus increase in response amplitude (at least 25% greater than the pre-tetanus response) measured at 30 to 60 min after tetanus. A midpoint slope [response (mV)/stimulus (V)] for each curve was determined by calculating the slope of a line segment (a line with endpoints on the curve at ±0.5 V of the midpoint stimulus voltage).

Differences between families of curves from each treatment group were analyzed by ANOVA and significant differences were determined using a Student–Newman–Keuls post hoc test, with \(P < 0.05\) considered to be significant.

Acknowledgments

The authors dedicate this study to Dr. Michael Guinan (1957–2004) whose key experimental observations formed the basis for the work. The authors also thank Matthew Lewis for assistance in collecting data.
REFERENCES