Development/Plasticity/Repair

Ubiquitous and Temperature-Dependent Neural Plasticity in Hibernators

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Hibernating mammals are remarkable for surviving near-freezing brain temperatures and near cessation of neural activity for a week or more at a time. This extreme physiological state is associated with dendritic and synaptic changes in hippocampal neurons. Here, we investigate whether these changes are a ubiquitous phenomenon throughout the brain that is driven by temperature. We iontophoretically injected Lucifer yellow into several types of neurons in fixed slices from hibernating ground squirrels. We analyzed neuronal microstructure from animals at several stages of torpor at two different ambient temperatures, and during the summer. We show that neuronal cell bodies, dendrites, and spines from several cell types in hibernating ground squirrels retract on entry into torpor, change little over the course of several days, and then regrow during the 2 h return to euthermia. Similar structural changes take place in neurons from the hippocampus, cortex, and thalamus, suggesting a global phenomenon. Investigation of neural microstructure from groups of animals hibernating at different ambient temperatures revealed that there is a linear relationship between neural retraction and minimum body temperature. Despite significant temperature-dependent differences in extent of retraction during torpor, recovery reaches the same final values of cell body area, dendritic arbor complexity, and spine density. This study demonstrates large-scale and seemingly ubiquitous neural plasticity in the ground squirrel brain during torpor. It also defines a temperature-driven model of dramatic neural plasticity, which provides a unique opportunity to explore mechanisms of large-scale regrowth in adult mammals, and the effects of remodeling on learning and memory.

Key words: **dendrite; spine; hibernation; torpor; temperature; plasticity**

Introduction

Neurons are known to alter microstructure in response to electrical activity and neurotrophins (Murphy and Segal, 1997; Maletic-Savatic et al., 1999), learning (Greenough et al., 1986), and injury (Schauwecker and McNeill, 1996), and during development (Duffy and Rakic, 1983). However, large-scale changes in neural microstructure are not commonly observed after animals reach maturity, which may limit the capacity of adult mammals to recover from trauma or disease (for review, see Bower, 1990). An exception is the adult hibernator, which exhibits repeated large-scale plasticity of dendritic microstructures and synapses during torpor, as revealed by Golgi stain (Popov et al., 1992), electron microscopy (Popov and Bocharova, 1992), and immunohistochemical studies (Arendt et al., 2003) of the hippocampus of hibernating ground squirrels. These studies highlight the phenomenon within apical branches from hippocampal neurons, but do not address the extent to which it occurs across the brain, nor what the driving mechanisms may be. Answering these questions should advance the potential use of this model system for learning about mechanisms behind large-scale neural restructuring in adult mammals, and processes including hypothermia and learning and memory.

Hibernating mammals survive extended periods of cold ambient temperatures and low food supply by lowering their body temperature and metabolic rate. The hibernation season is characterized by repeated bouts of torpor, each of which can last up to 2 weeks. During torpor, both core and brain temperatures drop to within 0.5−3°C of ambient temperature (Strumwasser, 1959), often reaching near-freezing and even below-freezing temperatures in the wild (Barnes, 1989). Concomitant with this periodic drop in core body temperature, there is a virtual cessation of neural activity (Strumwasser, 1959; Walker et al., 1977), a dramatic drop in metabolic rate (Buck and Barnes, 2000), and a halting of many cell maintenance processes, including transcription and translation (Frerichs et al., 1998; van Breukelen and Martin, 2002). In between torpor bouts, hibernators briefly arouse to euthermic temperatures, during which time measurable neural activity resumes.

Here, we advance the understanding of the dynamics of neural remodeling during hibernation by addressing the following objectives. To understand the scope of structural change across the brain, we compared neural retraction in three different brain regions, as well as in structurally distinct regions within neurons. We investigated the time course of these structural changes in

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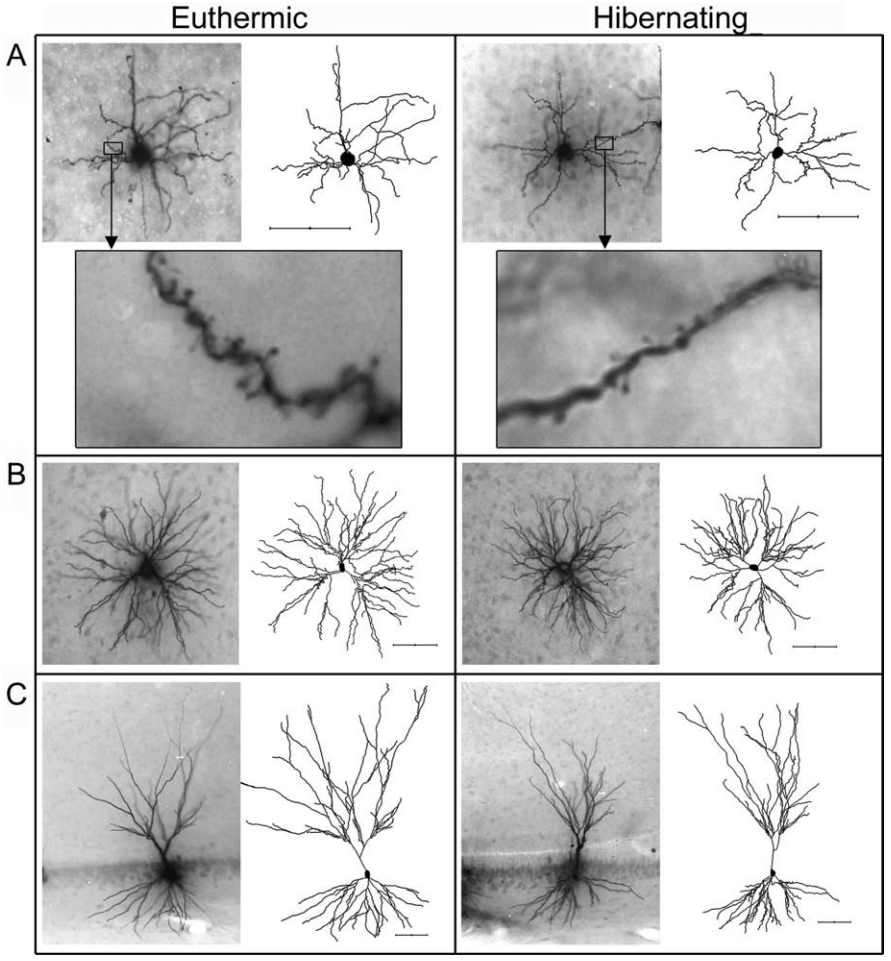

Figure 1. Sample images and Neurolucida reconstructions. ***A–C***, Examples of layer 4 cortical spiny stellate neurons (***A***), ventral posterior thalamic somatosensory relay neurons (***B***), and hippocampal CA3 neurons (***C***) from euthermic (12 h after arousal induction) and torpid (6 d after torpor entrance) ground squirrels. Higher-magnification images from cortical dendritic arbors show dendritic spines. Images are from 100-μm-thick sections, whereas reconstructions also include dendrite branches from adjoining sections. The scale is the same for images and tracings within a cell type. Scale bars, 100 μm.

relation to the torpor bout, and also compared aroused winter animals to euthermic summer animals to understand the completeness of the recovery from torpor. To test how these changes are driven by temperature, we compared the extent of retraction and the extent of recovery in groups of animals hibernating at different ambient temperatures. Our approach was to visualize whole neurons with all of their dendritic processes from male and female golden-mantled ground squirrels hibernating at 5 and 15°C using iontophoretic Lucifer yellow injections into neurons in fixed slices (Buhl and Lubke, 1989). Our results show that hibernators experience a temperature-dependent retraction of neural microstructure across all brain regions investigated, and that recovery of microstructure to fixed parameters is rapid and complete with each return to euthermia.

Materials and Methods

Tissue collection. All procedures were performed in accordance with Stanford University's Institutional Animal Care and Use Committee. Golden-mantled ground squirrels (*Spermophilus lateralis*) were trapped in autumn 2003 in the Sierra Nevada, California (permit from California Department of Fish and Game). To precisely monitor torpor bouts, animals were implanted with abdominal temperature telemeters (model VM-FH-LT; Mini Mitter, Bend, OR). These 20 × 7 mm devices did not interfere with the behavior and health of the squirrels in any discernable way.

In late October 2003, the ambient temperature was decreased to 5°C, and the lighting was changed to constant dim red light. This permitted hibernation in all animals. The ambient temperature was raised to 15°C for the month of February 2004 to analyze neural microstructure at a different minimum body temperature, after which it was lowered to 5°C again. Animals allocated to the 15°C groups were killed in February, and those allocated to the 5°C were killed in January and March. Comparison of neural structure from animals killed in January and March demonstrated no difference (at $\alpha = 0.05$), suggesting that any temperature-related differences were not attributable to differences in the frequency or number of torpor bouts between the groups.

Body temperature of animals was remotely monitored to assess torpor bout length, and was used as an estimate of brain temperature because the brain is in thermal equilibrium with the rest of the body during torpor (Lyman and Chatfield, 1950). Eight experimental groups were used, each made up of six to seven animals. Five of these groups hibernated at 5°C, two of these groups hibernated at 15°C, and one group was killed in the summer. For animals hibernating at 5°C, the groups were as follows: 1, 3, and 6 d after torpor entrance, and 2 and 12 h after arousal induction. For animals hibernating at 15°C, the groups were as follows: 3 d after torpor entrance, and 12 h after arousal induction. Entry into torpor was defined as a drop of body temperature <34°C. Arousal from torpor was initiated by 2 min of gentle handling, a technique that induced complete arousal to >34°C in ~2 h. For the two euthermic groups of animals at 5°C, arousal was induced at 6 d of torpor to ensure that all animals in the euthermic groups spent the same amount of time in torpor during that cycle. For the 15°C euthermic group, arousal was induced at 3 d of torpor, which is the approximate torpor bout length at that temperature.

After a minimum of two complete torpor bouts, brain tissue was collected at specific times in the torpor bout ($N = 6–7$ animals per group). Animals were removed from their cages, deeply anesthetized with halothane for 1 min, and killed by perfusion through the heart with heparinized PBS followed by 4% paraformaldehyde in phosphate buffer (pH 7.40–7.45). Brains were removed and postfixed for 6–7 h in the same fixative and then transferred to PBS. Sequential 400 μm coronal slices were cut on a vibratome in cold PBS. Tissue was stored in 0.1% sodium azide in PBS at 4°C until cell filling, 1–3 d later. A group of six summer animals was killed in June in the middle of the light cycle using the same methods.

Lucifer yellow injection. Intracellular injection micropipettes were back-filled with 3–4% Lucifer yellow (Sigma, St. Louis, MO). The 400-μm-thick slices containing the target brain region were placed in cold PBS under a Zeiss (Oberkochen, Germany) fluorescent microscope. The pipette was advanced through the target region blindly until a cell body was punctured. Lucifer yellow was then iontophoretically injected into the cell for 2–3 min. This amount of time was determined to be sufficient to fill all target cell types in slices from both euthermic and hibernating squirrels by measuring thickness of terminal branches of both groups. The target neurons analyzed were: somatosensory layer 4 spiny stellate neurons, ventral posterior thalamic somatosensory projection neurons,

and hippocampal CA3 neurons. Cells were labeled in the same region and from similar slices across animals, to decrease unnecessary variability. The investigator was blind to the experimental group of the animal. Approximately three to six cells in each slice were labeled, of which up to three cells were analyzed. After injection, slices were placed back in buffer and stored at 4°C until recutting, 1–3 d later.

Immunohistochemistry. Slices were recut to 100 μm on a freezing microtome. Slices were then processed immunohistochemically to produce a semipermanent label, using rabbit anti-Lucifer yellow (A-5750; Invitrogen, Eugene, OR), biotinylated goat anti-rabbit (BA-1000; Vector Laboratories, Burlingame, CA), and avidin–biotin peroxidase reagents (PK-4000 Vectastain ABC Peroxidase kit; Vector Laboratories). Slices were mounted under 0.5% gelatin, dried overnight, dehydrated in ascending alcohols, cleared with Xylene Substitute (Electron Microscopy Sciences, Fort Washington, PA), and coverslipped under DPX (Electron Microscopy Sciences).

Analysis. Neurons were traced and analyzed in three dimensions at a resolution of 100× using Neurolucida software (MicroBrightField, Williston, VT) connected to a Zeiss light microscope. Spines were marked on randomly selected tertiary or quaternary branch segments in both apical and basal dendritic arbors. Parameters analyzed included cell body area, length and width of the dendritic field, path length of each dendrite, numbers of nodes per dendrite, maximum branch order, numbers of intersections with concentric circles 10 μm apart (a modified Scholl analysis), spine density, and density and length of thalamic hair-like processes. All reconstructions and analyses were performed by an investigator who was blind to the identity of the experimental group. Normality of means was tested using the Shapiro–Wilk test. Interanimal variability within groups was not significant at $\alpha = 0.05$, so means from the 25–35 neurons per group were pooled. Comparison of group means was performed with ANOVA, and a *post hoc* correction for multiple comparisons was made using Fisher's least significant difference method.

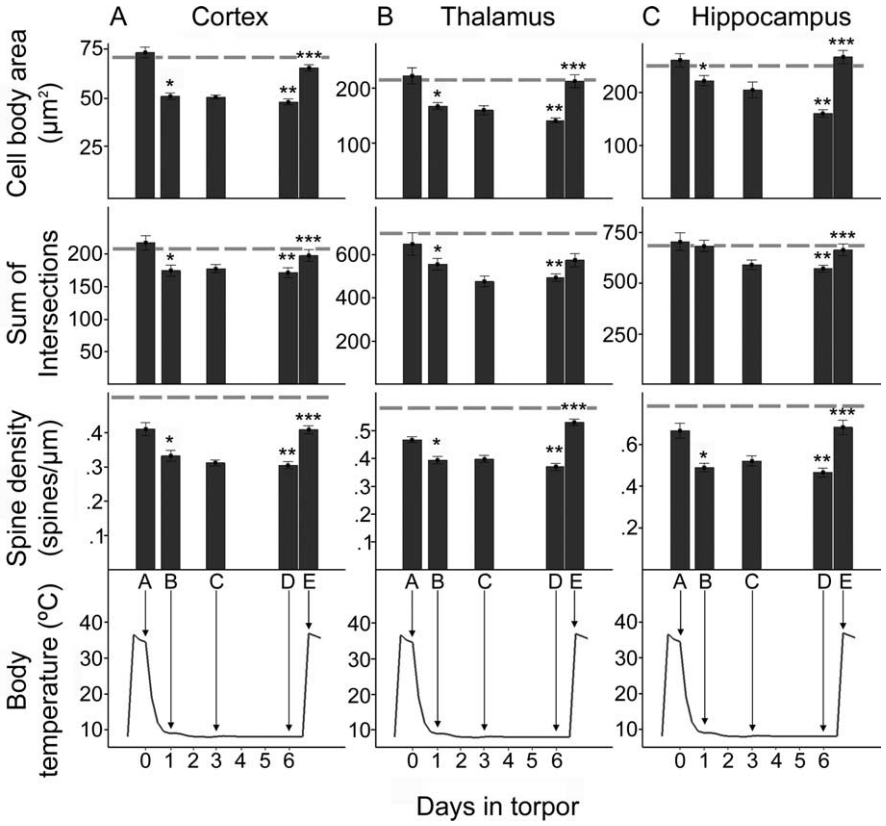

Figure 2. Torpor-related changes in neural microstructure. ***A–C***, Structural parameters for cortical spiny stellate cells (***A***), ventral posterior thalamic relay cells (***B***), and hippocampal CA3 neurons (***C***) at specific time points in a torpor bout, as indicated in the body temperature graph below. Parameters shown are mean ± SEM: cell body area (in square micrometers), sum of intersections of the entire dendritic arbor with concentrically drawn circles 10 μm apart starting at the cell body (a modified Scholl analysis), and spine density (number of spines per micrometer of dendrite) on tertiary and quaternary branch segments. Group means comprise data from 25–35 cells from six to seven animals per group. Means from a group of ground squirrels in the summer provide a comparison with winter euthermic values, and are represented by a dashed line through each graph. Statistically significant comparisons (at $\alpha = 0.05$) are marked with asterisks above bars as follows: *A > B; **A > D; ***E > D. Not all significant comparisons are shown here.

Results

Neural structural retraction is similar in different brain regions

To investigate whether previously observed torpor-related changes in neural microstructure are specific to the hippocampus (Popov et al., 1992), or part of a more global phenomenon, we investigated changes in cell body area, dendritic branching, and spine density in three regions: cortical layer 4 spiny stellate cells from somatosensory cortex, ventral posterior thalamic somatosensory relay neurons, and hippocampal CA3 pyramidal cells. Neural structural properties of these cells were compared at two time points in animals hibernating at 5°C: 6 d into a torpor bout, and 12 h into interbout euthermia. Sample images and tracings of the three cell types are shown in Figure 1.

All three cell types exhibited statistically significant decreases in the parameters measured, as can be seen by comparing groups A and D in Figure 2 ($p < 0.001$ for all comparisons). The percentage decrease of individual structural parameters by the end of torpor was similar across cell types (Fig. 3), suggesting that torpor-related structural changes may be taking place generally across the brain.

This finding allows us to provide a general characterization of the extent of neural retraction associated with a brain temperature during torpor of ~5°C. Cell body area demonstrated the greatest percentage change, with a 35–40% decrease in torpor (Fig. 3*A*) ($p < 0.001$ for each cell type). Complexity of branching decreased 20–25%, as indicated by a modified Scholl analysis (Fig. 3*B*) ($p < 0.001$ for each cell type) and by changes in maximum branch order and numbers of nodes per dendrite (supplemental Fig. 1, available at www.jneurosci.org as supplemental material) ($p = 0.014$ for maximum branch order in thalamic cells, and $p < 0.001$ for all other comparisons). Spine density decreased 20–30% (Fig. 3*C*) ($p < 0.001$ for each cell type).

Several results indicate that the torpor-related decrease in dendritic complexity is a result of retraction, and that this process is occurring generally throughout the cell. Analysis of the location of changes in dendritic arborization revealed that the majority of torpor-related decrease occurred in the number and length of higher order dendrites (Fig. 4). This finding points to a mechanism involving retraction of dendrites, rather than severing of branches throughout the arbor. Furthermore, there were no differences in arbor complexity between apical and basal dendrites at any point in the torpor bout (at $\alpha = 0.05$), suggesting that dendritic retraction occurs generally across the cell. Spine density

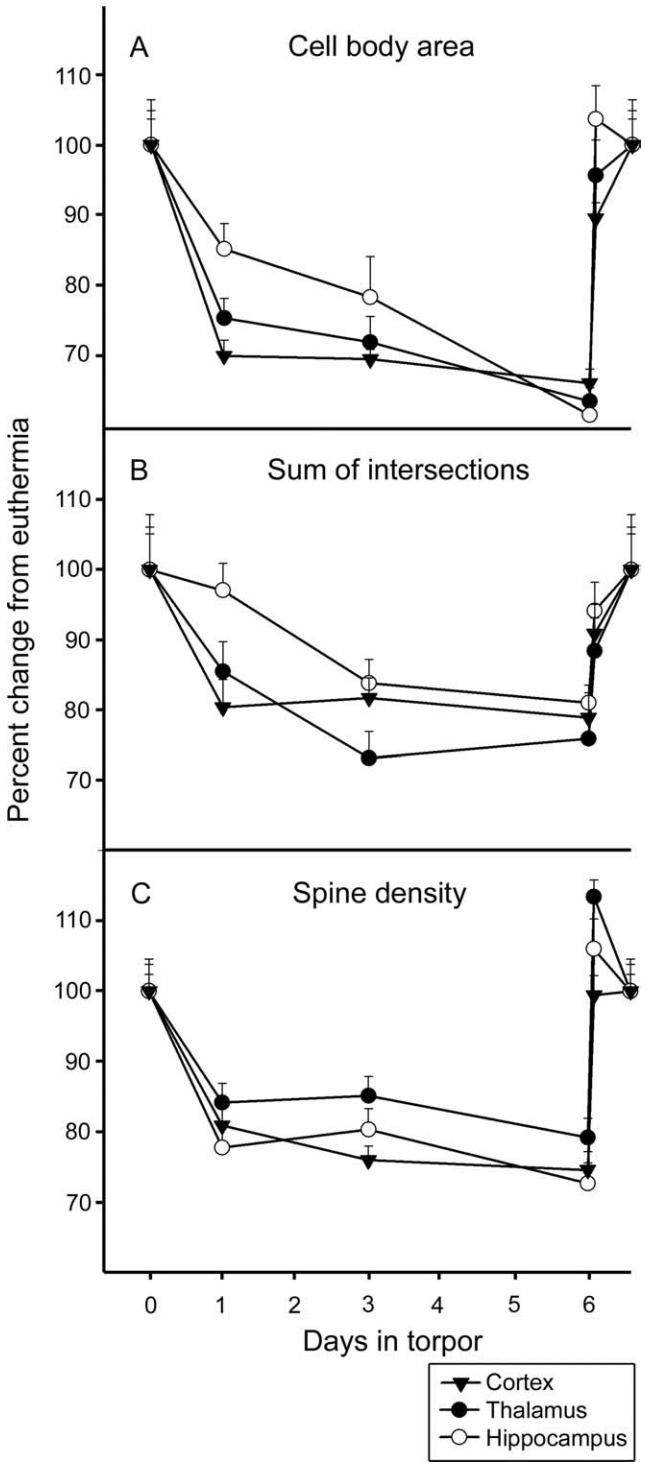

Figure 3. Time course of neural microstructural change in torpor. ***A–C***, Percentage change of cell body area (in square micrometers) (***A***), sum of intersections (***B***), and spine density (number of spines per micrometer of dendrite) (***C***) from interbout euthermic values. Means + SEM from the cortex, thalamus, and hippocampus are marked with different symbols (see key at bottom). By the end of torpor, all cell types exhibit the same percentage decrease of all three structural parameters. Greatest percentage changes are seen in cell body area. Spine density changes occur most rapidly, with all of the decrease taking place by day 1, and all of the recovery taking place in 2 h.

decreases also occurred similarly on apical and basal dendrites, as well as on proximal and distal branches, as indicated by the lack of significant difference in spine density decrease during torpor between these regions of the arbor (at $\alpha = 0.05$). This suggests that spine retraction also takes place generally across the cell.

Torpor-related changes in neural microstructure track body temperature

We analyzed the time course of torpor-related structural decrease by examining neurons at five different time points in the torpor cycle from animals hibernating at 5°C: 1, 3, and 6 d after torpor entrance, and 2 and 12 h after arousal induction. These time points correspond to the start and end of both torpor and interbout euthermia. The torpor cycle of a golden-mantled ground squirrel hibernating at 5°C consists of a 1 d entry phase, a 5–7 d interval of deep torpor at a body temperature of ~7°C, a 2 h spontaneous return to euthermia, and an 8–12 h interbout euthermic interval, followed by torpor reentry. A sample body temperature graph from a squirrel hibernating at 5°C is shown in Figure 2.

The majority of decrease in the thalamus and cortex took place by day 1 (Fig. 2; supplemental Fig. 1, available at www.jneurosci.org as supplemental material), demonstrating that the retraction occurs as the animals enter torpor, rather than over several days at low temperature. The decrease in hippocampal cell body area and dendritic complexity occurred more gradually (Fig. 3) (hippocampus vs thalamus and cortex: cell body area, $p = 0.005$; intersections, $p = 0.003$), although spine density decreased as quickly as in the other cell types.

The majority of the recovery took place during the 2 h arousal window, a consistent result among the three brain regions. The extent of recovery in 2 h was impressive, with the length of dendritic fields of cortical neurons increasing an average of 61 μm, and path length of apical branches increasing 141 μm (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). We conclude that torpor-related changes occur quickly, principally during the entry and exit phases of torpor.

Recovery of neural microstructure is complete with each arousal

To understand the completeness of the recovery between torpor bouts, we compared aroused winter animals to euthermic summer animals. Spine density was the only parameter that changed significantly between winter and summer euthermia, increasing 20–30% ($p < 0.001$ for each cell type), as shown in Figure 2. Dendritic spines are the sites of the majority of excitatory synaptic input in the mature mammalian brain (for review, see Hering and Sheng, 2001). Augmentation of spines from winter to summer indicates that significant synaptic restructuring, and possibly a net increase in synaptic communication potential, takes place after the hibernation season.

The winter animals used for the analysis above were killed at various times throughout the hibernation season. We analyzed whether the time spent hibernating, and thus the number of torpor bouts experienced, affects neural microstructure. We compared neural microstructure from interbout euthermic squirrels hibernating at 5°C in early January and late March ($N = 12$ cells per group). There were no statistically significant differences between these two groups of animals ($p > 0.05$ for all comparisons), despite a greater number of torpor bouts experienced by the latter group. We conclude that neural microstructural recovery is complete with each arousal from torpor.

Torpor-related changes in thalamic hair-like processes

We observed hair-like processes on thalamic dendrites that were present only in tissue from euthermic animals. Neurons with these unique hair-like processes have been documented in several thalamic nuclei, including the ventral posterior nucleus (Yen et al., 1985) and pulvinar (Darian-Smith et al., 1999) of the thalamus, but their role has not yet been established. We quantified

the torpor-related changes in number and length of these processes. The changes, shown in Figure 5, were more dramatic than any of the other parameters measured in this study. The density of hairs per micrometer of dendrite decreased by ~95% in torpor ($p < 0.001$), with 80% of that decrease occurring in the entry phase, and 45% of the recovery taking place during the 2 h return to euthermia. Length of these hairs decreased by ~70% in torpor ($p < 0.001$), with 90% of that decrease occurring in the entry phase and 90% of the recovery taking place during the return to euthermia. To assess whether this finding was an artifact of the filling process, we measured the width of these hairs at their base and found no difference between torpid and euthermic animals ($p = 0.40$). These findings indicate that thalamic hair-like processes are extremely sensitive to torpor.

The density of hair-like processes continued to increase 45% from winter to summer euthermia ($p < 0.001$) resulting in a maximum hair density of 4.91 ± 0.44 hairs per 100 μm of dendrite (mean ± SEM). The length of these hairs, however, did not change significantly from winter to summer euthermia ($p = 0.64$). These results suggest that the length of thalamic hair-like processes recovers rapidly, but that growth of new hairs is a longer process.

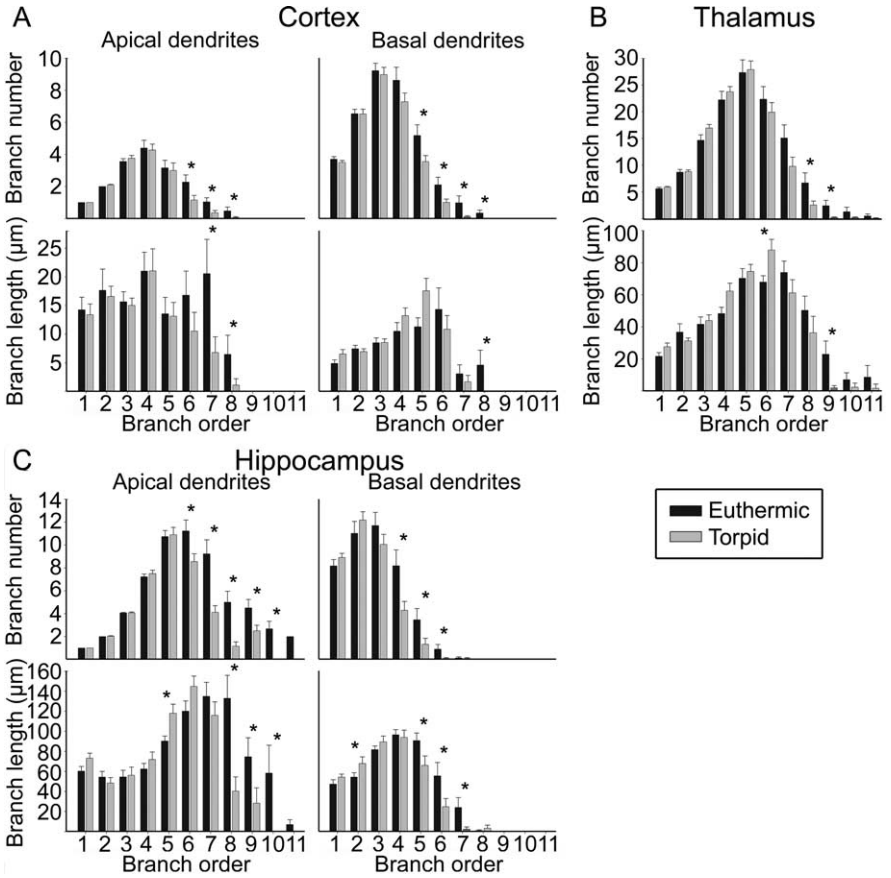

Figure 4. Torpor-related changes occur in higher-order branches. ***A–C***, Number and length of dendritic branches by branch order for cortical spiny stellate neurons (***A***), ventral posterior thalamic relay cells (***B***), and hippocampal CA3 neurons (***C***). Apical and basal dendrites are shown separately. Means + SEM are from euthermic (12 h after arousal induction) and torpid (6 d after torpor entrance) animals. Significant comparisons at $\alpha = 0.05$ are marked with asterisks above bars. For all three cell types, torpor-related changes occur in the higher-order branches.

Neural microstructure retracts linearly with minimum torpor body temperature

The ambient temperature during hibernation determines minimum body temperature (Strumwasser, 1959). Minimum body temperature, in turn, determines many physiological and behavioral parameters, including torpor bout length (Buck and Barnes, 2000), neural activity during torpor (Krilowicz et al., 1988), and electroencephalographic (EEG) power spectrum on return to euthermia (Larkin and Heller, 1996). However, it was previously unknown whether ambient temperature also influences the extent of neural plasticity during torpor.

Analyses of neural cell body size, degree of dendritic arborization, and dendritic spine density were made from cells in the cortex, thalamus, and hippocampus of animals 3 d into a torpor bout at 5 and 15°C. In all three brain regions, there was a temperature-dependent trend in structural parameters, with mean structural parameters from animals hibernating at 5°C consistently lower than those from animals hibernating at 15°C (Fig. 6). The difference in neural microstructure between the two temperatures reached statistical significance for cell body area in all three cell types ($p < 0.005$ for cortex and thalamus; $p = 0.014$ for hippocampus), and for spine density in the cortex ($p = 0.005$) and degree of arbor complexity in the thalamus ($p = 0.041$) (Fig. 7).

Analysis of the percentage decrease of neural structural parameters from interbout euthermia as a function of torpor body temperature suggests that neural microstructure decreases linearly with temperature, as shown in Figure 6. Specifically, based on the euthermic temperature and the two measured torpor temperatures, a 10°C drop in body temperature correlates to an 8–10% decrease in most measured structures in the cell. The slope of temperature-dependent decrease did not differ between cell types ($p = 0.24$) or between parameters ($p = 0.34$).

Neural microstructure recovers to a fixed set of parameters

To assess whether neural microstructure recovers to a fixed set of parameters, regardless of the extent of temperature-dependent retraction, we compared neural microstructure in the 5 and 15°C groups 12 h after arousal. There were no significant structural differences between these two groups of animals across all of the three cell types analyzed (at $\alpha = 0.05$) (Fig. 7). This finding suggests that there is a mechanism that determines final arbor complexity, cell body size, and spine density on recovery from torpor.

Discussion

We have shown that neural microstructure is highly plastic during hibernation to a similar degree in three brain regions, and that these changes parallel body temperature over the course of a torpor bout. Furthermore, we have demonstrated that torpor-related neural retraction is dependent on the ambient temperature, and hence the minimum body temperature reached during a bout of torpor. Recovery of microstructure reaches fixed values of arbor complexity and spine density with each return to euthermia.

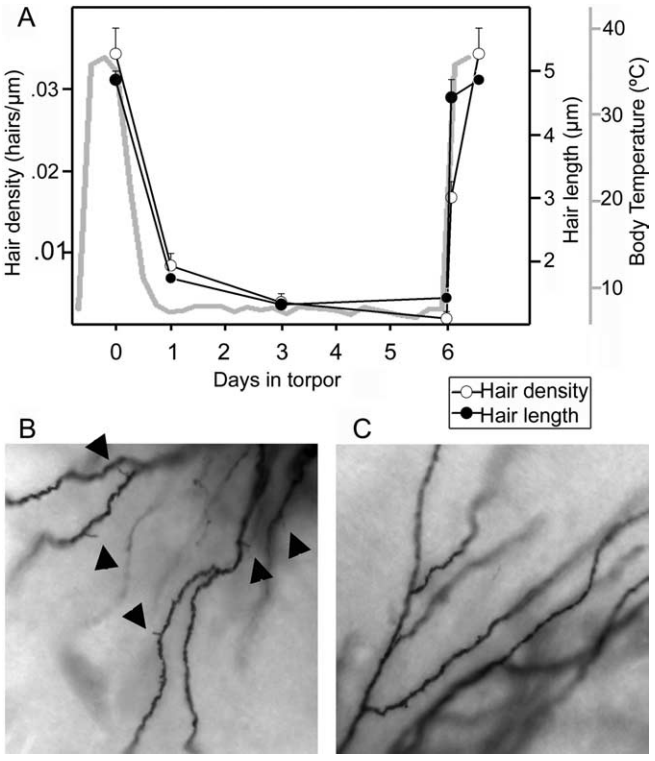

Figure 5. Torpor-related changes in thalamic hair-like processes. ***A***, Time course of torpor-related changes in density and length of thalamic hair-like processes (mean + SEM). A body temperature graph from a hibernating ground squirrel is superimposed. ***B***, ***C***, Images of dendrites from the ventral posterior nucleus of the thalamus from animals 12 h after induced arousal (***B***), and 6 d into a torpor bout (***C***). The arrowheads denote hair-like processes.

The structural changes we have demonstrated in the hibernator brain are among the most dramatic found in nature. Morphological changes similar in character and magnitude have been found in other models of neural plasticity, but never at such a high rate. Whereas dendritic elongation can reach 114 μm per day in the hippocampus of the developing rhesus monkey embryo (Duffy and Rakic, 1983), adult hibernators exhibit similar changes in just 2 h. In adult rats, 4 months of environmental enrichment result in a 17% increase in dendritic intersections (Greenough et al., 1986), similar to the changes seen in a hibernator in 2 h of arousal.

Several findings in this study indicate that neural retraction results from the torpor-related decrease in brain temperature. First, retraction and recovery closely track body temperature changes. Second, the changes are similar in magnitude across distinct regions within cells, as well as across distinct regions of the brain, suggesting that the variable that drives this process acts globally. Last, the extent of retraction is dependent on minimum body temperature during torpor. The hypothesis that temperature drives this process is supported by studies in mice. Chilling hippocampal slices and cultures results in a cessation of dendritic spine motility after a few hours, and overnight incubation at 4°C results in the disappearance of spines (Roelandse and Matus, 2004). In acute hippocampal slice preparations, the loss of spines with exposure to cold appears to be attributable to disrupted ionic and osmotic homeostasis (Kirov et al., 2004). In addition, reduced temperature weakens bonds between tubulin molecules, thereby promoting microtubule disassembly, which can result in rapid dendritic retraction (Kirschner et al., 1974). Dendritic retraction may be enhanced by torpor-related decreases in

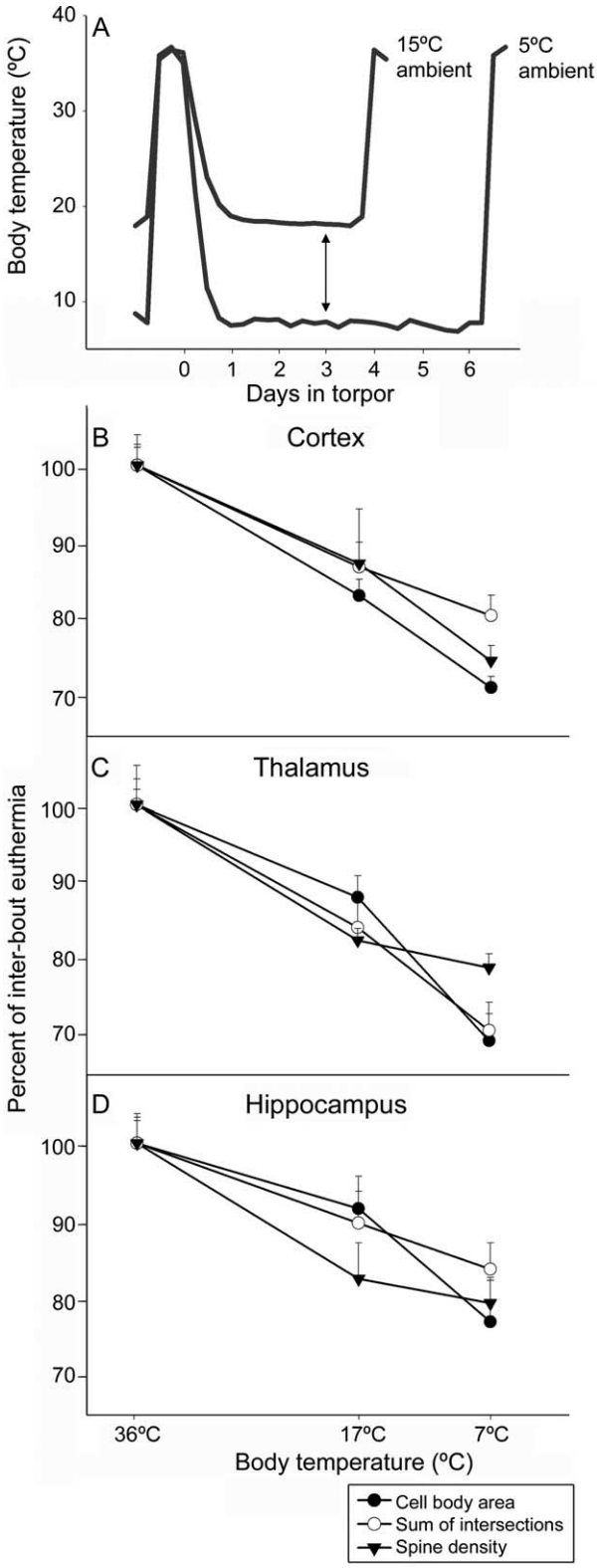

Figure 6. Linear relationship between body temperature and neural microstructure. ***A***, Sample body temperature graphs from animals hibernating at ambient temperatures of 5 and 15°C. Comparison of neural microstructure was made 3 d into torpor at both temperatures, as indicated in the figure. ***B–D***, Percentage decrease of neural structural parameters as a function of body temperature in euthermia (36°C) and body temperature in torpor at the two temperatures (~17 and 7°C) (mean + SEM). Neural cell types depicted are cortical layer 4 spiny stellate neurons (***B***), ventral posterior thalamic somatosensory projection neurons (***C***), and hippocampal CA3 neurons (***D***). The interbout euthermic group comprise data from animals hibernating at both temperatures, because there were no statistically significant differences between them.

microtubule-associated protein 2 density (Arendt et al., 2003), which leads to dynamic instability (Illenberger et al., 1996).

The temperature-dependent decrease in cell body area demonstrated in this study is remarkable because in homeotherms, hypothermia induces cell swelling (Lucas et al., 1990). The rate and extent of cell body shrinkage measured in the hibernator suggests water flux out of the cell, possibly because of changes in ion concentrations at cold temperatures. Although there are no reports on ion concentrations in brain interstitium during hibernation, values from blood plasma indicate that the ionic microenvironment may change (Ferren et al., 1971; Tempel and Musacchia, 1975). The depression of ion pump activity during torpor could contribute to disturbed ionic homeostasis, although hibernators have developed a means to balance this with channel arrest (for review, see Storey and Storey, 2004).

The most dramatic changes observed were those in the thalamic hair-like processes. These structures have been reported in a few other studies of the thalamus (Yen et al., 1985; Lubke, 1993; Darian-Smith et al., 1999). In the hibernator, these processes virtually disappear during entrance into torpor and then recover to near-summer values during rewarming. The torpor-related rates of extension and retraction of these structures are reminiscent of neural filopodia, which are transient precursors to spines (Dailey and Smith, 1996). However, these hairs are probably not filopodia, because their density increased between winter and summer euthermia, suggesting that they are not developing into dendritic spines. Furthermore, in both winter and summer animals, they were unique to the thalamus. Although the function of these hairs is not known, this is the first study to demonstrate that thalamic hairs demonstrate structural plasticity.

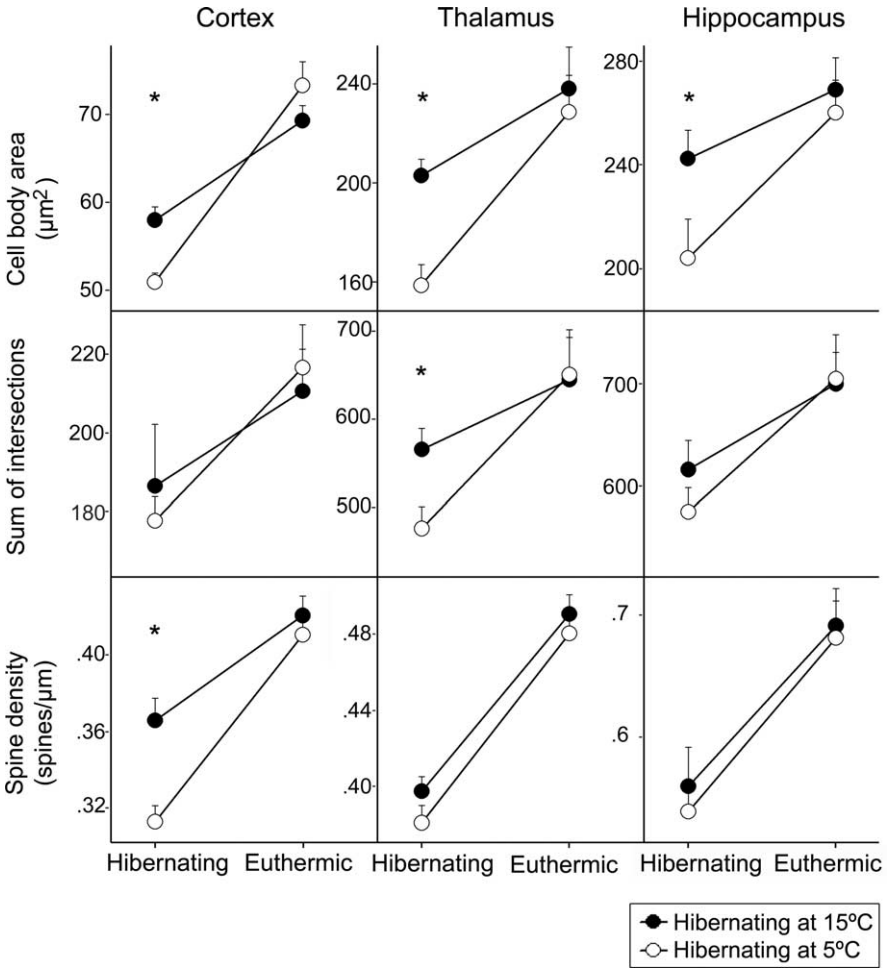

Figure 7. Neural structural recovery reaches fixed parameters. Recovery of neural microstructure in animals hibernating at 5 and 15°C (mean + SEM). Group means are from animals 3 d into a torpor bout and 12 h after induced arousal. Neural cell types shown are as follows: cortical layer 4 spiny stellate neurons, ventral posterior thalamic somatosensory projection neurons, and hippocampal CA3 neurons. Neural structural parameters shown are as follows: cell body area (in square micrometers), sum of intersections, and spine density (number of spines per micrometer of dendrite). Despite significant differences in neural microstructure during torpor at the two temperatures ($p < 0.05$; marked with asterisks), there were no statistically significant differences between recovered structural values.

The three distinct brain regions investigated in this study demonstrated similar magnitudes of torpor-related microstructural loss, suggesting a global phenomenon. These three regions were chosen because they have homogeneous and well characterized dendritic structure, and can be consistently identified in unlabeled slices. It would be interesting to investigate areas that remain highly functional during deep torpor, such as the hypothalamus (Kilduff et al., 1990) and the brainstem respiratory centers (for review, see Milsom et al., 2001). It is possible that these areas are protected from torpor-related microstructural plasticity, possibly by increased concentrations of cytoskeletal stabilizers. Alternately, if these regions are not protected from this form of plasticity, they may possess synaptic redundancy to maintain connectivity. Such future studies would benefit from use of electron microscopy, to corroborate and elaborate on the findings in this study.

The temperature dependence of neural structural loss demonstrated here may shed light on the effect of neural plasticity on EEG power spectra. Larkin and Heller (1996) demonstrated that squirrels hibernating at low ambient temperatures have a greater slow wave activity (SWA) rebound in the hours after arousal than squirrels hibernating at warm ambient temperatures. The elevated SWA with arousal at low temperatures may be related to synaptic loss during hibernation. The temperature-dependent plasticity may result in decreased release or responsiveness to wake-promoting factors, such as acetylcholine from the forebrain or norepinephrine from the locus ceruleus. Alternately, the increase in SWA with arousal may be attributable to faster recovery of inhibitory synapses in the cortex and thalamus. Confirmation of the relationship between loss of neural microstructure and EEG power spectra would significantly advance our understanding of the role of SWA in sleep.

Our finding that neural microstructure recovers to a fixed set of parameters after each torpor bout provides new insights into the process of structural recovery during arousal. These fixed parameters were achieved no matter how many previous torpor bouts the animal had undergone in the hibernation season, or how deep those torpor bouts were, as measured by temperature and extent of retraction. These findings argue for a signaling

mechanism for final structural complexity, or for the return of recovering dendrites, spines, and synapses to their original configuration. There is currently no known mechanism for structural memory in neurons. The hibernator, with its high rate and magnitude of structural recovery, would be an ideal system in which to explore this question.

The time course of microstructural recovery demonstrated here may shed light on recent findings about memory consolidation after torpor. In the Arctic ground squirrel, memory consolidation is more effective 24 h after arousal from torpor than at 3 h or 4 weeks after arousal (Weltzin et al., 2006). In light of the present findings, this would mean that memory consolidation is most efficient after structural recovery has reached full summer values for all parameters of the dendritic arbor, but before dendritic spine density peaks several weeks after arousal. This window of optimal memory consolidation may be the time of maximal synaptic restructuring, including synaptic formation and strengthening or pruning. This peak consolidation time is not likely to provide an adaptive benefit during the hibernation season, because squirrels spend the majority of interbout euthermic intervals sleeping in their hibernaculum (Larkin and Heller, 1996). However, it may be beneficial after the terminal arousal, because it could allow for efficient incorporation of new information with the start of a new season above ground.

The functional significance of neural retraction during torpor is currently unknown. A possible adaptive function for the loss of synapses is neural protection from reperfusion injury with arousal (Ross et al., 2006). However, there are likely costs to this form of plasticity as well, including deficits in some forms of memory that were acquired prehibernation (Mateo and Johnston, 2000; Millesi et al., 2001) and energy costs of repeatedly rebuilding dendritic arbors and spines. In fact, it is possible that hibernators have developed a means to limit temperature-dependent loss of neural microstructure, because neurons from homeotherms have been shown *in vitro* to lose all of their dendritic spines in response to cooling (Kirov et al., 2004; Roelandse and Matus, 2004), in contrast to the hibernator, that *in vivo* only loses ~25% of dendritic spine density.

The findings presented here show that adult mammalian neurons are capable of tremendous structural change in short time periods. For this reason, the hibernator promises to be a valuable model for studying the controls of neural regrowth and the guidance of dendrites and spines to their previous location. These findings also provide evidence that neural microstructure can change dramatically in a temperature-dependent manner. These changes may underlie cognitive impairments in patients recovering from severe hypothermia, like those demonstrated by Walpoth et al. (1997) where body temperature dropped as low as 17°C for several hours. To investigate this process in a homeotherm, it would be interesting to determine whether similar microstructural changes are occurring in a mouse model of torpor (Zhang et al., 2006). More research is needed to further understand the mechanisms driving this highly malleable system, the role that this form of neural plasticity plays in the unique adaptation of hibernators to the cold, and the resulting effects of these repeated neural changes on learning and memory.

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