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[^{14}C]2-DEOXYGLUCOSE UPTAKE IN GROUND SQUIRREL BRAIN DURING HIBERNATION[1]

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Abstract

Autoradiographic patterns of [^{14}C]2-deoxyglucose uptake are described throughout the brains of hibernating and euthermic ground squirrels. Autoradiographs of the brains of hibernating animals are generally homogeneous in comparison to euthermic animals; hence, the relative 2-deoxyglucose uptake (R2DGU) of gray to white matter for the majority of the 85 neural structures examined decreases during hibernation. Two categories of structures are identified as potentially important in hibernation: (*1*) structures that have the highest R2DGU during hibernation (cochlear nucleus, paratrigeminal nucleus, and superior colliculus) and (*2*) structures that undergo the least reduction in R2DGU in the transition from euthermia to hibernation (suprachiasmatic nucleus and lateral septal nucleus).

The percentage of reduction in R2DGU that a structure undergoes in the transition from euthermia to hibernation is proportional to the R2DGU of that structure during euthermia. The suprachiasmatic, paratrigeminal, and cochlear nuclei undergo less of a reduction than would be predicted from this relationship and may be particularly important during hibernation. Sensory nuclei that receive primary afferent projections are among the structures with the highest R2DGU during hibernation. These metabolically active structures may be responsible for the sensitivity of the hibernator to environmental stimuli.

The phenomenon of hibernation involves a complex suite of physiological adaptations. As body temperature falls from euthermic levels of 37°C to as low as 2°C in the ground squirrel, heart rate decreases from 250 to 300 beats/min to 7 to 10 beats/min and respiration slows from 100 to 150 breaths/min to 2 to 3 breaths/min (Twente and Twente, 1978). During hibernation, animals assume a stereotyped curled-up posture with the head tucked beneath the tail, indicating maintained activity in some motor control pathways. A hibernating animal retains muscle tonus and postural control, whereas the hypothermic animal at the same body temperature does not (Musacchia, 1976). Hibernating animals also remain responsive to auditory, vibratory, tactile, and thermal stimuli. Hibernators will deflect their ear pinnae to the source of auditory stimulation, orient to the stimulus, and in extreme cases, undergo a complete rewarming (arousal) to euthermia (Strumwasser, 1959).

Hibernation also involves distinct changes in the nervous and endocrine systems. Action potentials are conducted in peripheral nerves of hibernating ground squirrels below 5°C, but conduction is blocked in the nonhibernating (euthermic) state at 5° to 6°C (Kehl and Morrison, 1960). Involution of the endocrine system may be necessary for entrance into hibernation (Lyman and Chatfield, 1955) and the hypothalamohypophyseal neurosecretory system reaches its lowest activity during the hibernation season. Lower rates of synthesis, transport, and release of neurosecretory material from the axons of the paraventricular and supraoptic nuclei of the hypothalamus are observed in a deeply hibernating species than in a species which undergoes a shallower hibernation (Polenov and Yurisova, 1975).

Hypothalamic thermosensitivity studies reveal that the decline in body temperature during entrance into hibernation is a regulated process (Heller et al., 1977). Hypothalamic regulation of body temperature remains functional throughout hibernation, enabling animals to

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maintain a body temperature above ambient temperature (Heller and Colliver, 1974; Florant and Heller, 1977). Temperature-sensitive neurons within the hypothalamus of the euthermic golden hamster, a hibernating species, continue to discharge at 10°C, whereas thermosensitive cells in the non-hibernating guinea pig cease firing below a hypothalamic temperature of 28°C (Wünnenberg et al., 1976).

Current knowledge of neural activity underlying the entrance into, maintenance of, and arousal from hibernation is primarily limited to subcortical EEG studies from a limited number of brain sites (Lyman and Chatfield, 1955; Shtark, 1972; Heller, 1979). The autoradiographic [^{14}C]2-deoxyglucose method (Sokoloff et al., 1977) measures glucose utilization in localized brain areas and provides a pictorial representation of metabolic activity throughout the brain. We have used this method to investigate the 2-deoxyglucose uptake of brain structures during hibernation and believe that this reflects the underlying neural activity during this state. Preliminary reports of this work have appeared (Kilduff et al., 1980, 1981).

Materials and Methods

Golden mantled ground squirrels (*Citellus lateralis*) were housed in individual cages, each containing a nest box and cotton nesting material. Food (sunflower seeds and dry dog food supplemented occasionally by vegetables) and water were supplied *ad libitum*. The colony room was maintained at 5 ± 1°C in constant darkness (DD) to facilitate entry of the animals into hibernation.

Under pentobarbital anesthesia, the animals were prepared with a catheter in the external jugular vein and a subcutaneous thermocouple re-entrant tube. The catheter was externalized to a latex-covered Plexiglas button sutured into the middle of the animal's back which allowed easy access to the catheter. The latex cap enabled repeated injections of heparinized saline to ensure catheter patency. The polyethylene (PE) thermocouple re-entrant tube was sutured subcutaneously rostral to the Plexiglas button.

At least 1 week was allowed for recovery after the catheterization. For an experiment, an animal was placed into a 1-liter metabolism chamber inside of a larger environmental chamber maintained at 5°C in DD. A thermocouple was introduced into the re-entrant tube to monitor body temperature and a 23 gauge needle, connected to a syringe by a 40-cm length of PE-50 tubing, was placed in the latex-covered button. Since the syringe was outside of the environmental chamber, it was possible to inject without disturbing the animal. During all experiments, ambient temperature (T_A), body temperature (T_b), and metabolism were monitored continuously as previously described (Heller et al., 1974). Euthermic animals were allowed at least 1 hr to acclimate to the experimental chamber before the [^{14}C]2-deoxyglucose (2DG) injection. Removal of hibernating animals from the colony room and placement in the metabolism chamber usually induced a partial arousal. No animal of this group was injected until the animal had a regular oxygen extraction record and a constant T_b below 8°C, near passive thermal equilibrium with the environment.

The [^{14}C]2-deoxy-D-glucose (54 Ci/mol, Amersham), evaporated and reconstituted in sterile saline, was injected at a concentration of 150 μCi/kg via the catheter and was followed by an injection of 0.40 ml of saline. For euthermic animals, 45 min elapsed between the time of [^{14}C]2DG injection and a lethal injection of 1.0 ml of sodium pentobarbital (50 mg/ml). Blood samples (0.3 ml) were obtained at 1 min after 2DG injection and subsequently, every 4 min during the 45-min incubation period. Blood samples were spun in a Beckman Microfuge for 1 min and duplicate 20-μl aliquots of plasma were counted in a Beckman LS 7500 scintillation counter. The concentration of [^{14}C]2DG in the plasma was calculated for the 45-min incubation period (Fig. 1). Red blood cells were returned to the animal in a volume of 0.3 ml within 2 min after each sample was withdrawn.

The metabolism of ground squirrels hibernating at a 5°C ambient temperature is approximately 1/30 that of euthermic metabolic levels (Snapp and Heller, 1981). Therefore, a much longer [^{14}C]2DG incubation time than the 45-min period used for euthermic animals was required. From these metabolic considerations alone, an incubation period as long as 22.5 hr (30 × 45 min) seemed reasonable. We opted for a series of injections in six different animals with 2, 4, 8, 12, 18, and 24 hr elapsing between the time of [^{14}C]2DG injection and the time of sodium pentobarbital overdose. Blood glucose is regulated in the hibernating animal at the same level as during euthermia (Musacchia and Deavers, 1981); hence,

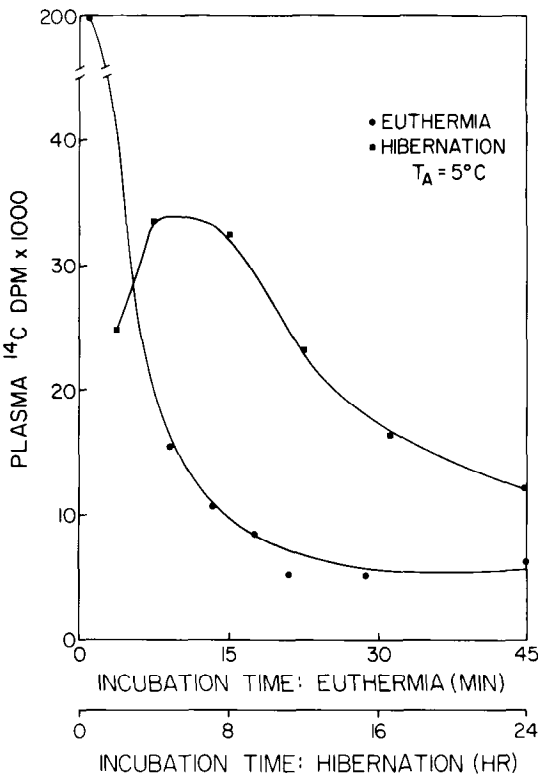

Figure 1. Concentration of plasma [^{14}C]2-deoxyglucose during the euthermia and hibernation experiments. The euthermia curve was derived from timed blood samples taken from a single animal. The hibernation curve is a composite derived from single blood samples taken from each of six animals.

the tracer requirement for the 2DG technique (Sokoloff et al., 1977) was not violated in the hibernation experiments. Because of the long time period (2 weeks minimum) between the catheterization of an animal, its entrance into hibernation, and subsequent experimentation, it was not possible to withdraw blood samples from an individual animal during a [^{14}C]2DG incubation. At the time of sacrifice, however, duplicate blood samples from each of the six hibernating animals were obtained and treated as above to construct the composite curve for hibernators shown in Figure 1.

At the time of sacrifice for each animal, its brain and spinal cord were removed and frozen rapidly in 2-methylbutane cooled to −40°C in dry ice. The tissues then were embedded in Lipshaw embedding matrix and sectioned immediately or stored at −70°C. All tissues were sectioned at −20°C in an American Optical Cryo-Cut II cryostat. Two or three consecutive 20-μm sections were picked up on coverslips every 100 μm throughout the brain, except in the diencephalon between the mammillary bodies and just rostral to the anterior commissure where every section was taken. The sections were dried rapidly on a hot plate at 60°C and then exposed to Kodak SB-5 x-ray film for 7 days at room temperature. Subsequently, selected sections were stained with cresyl violet and the structures represented on the autoradiographs were identified according to rat and ground squirrel brain atlases (Joseph et al., 1966; Koenig and Klippel, 1967; Palkovits and Jacobowitz, 1974; Pellegrino et al., 1979).

Based on the histological sections, 85 neural structures were delineated on the autoradiographs and the optical density (O.D.) of each was measured using a Tobias TBX densitometer (Tobias Associates, Ivyland, PA) equipped with a 0.33-mm aperture. On each autoradiograph, the densitometer was zeroed over an unexposed portion of film and the O.D. of the densest portion of each structure was measured in at least five sections to obtain a mean O.D. for each structure within an animal. The curves of plasma [^{14}C]2DG for euthermia and hibernation in Figure 1 and the O.D. value would allow calculation of regional cerebral glucose utilization for each structure according to Sokoloff's equation, if the kinetic constants for transport and phosphorylation for these two states in this species were known. Since these values are not known, a relative measure of glucose utilization of euthermic and hibernating brain was calculated. The relative 2-deoxyglucose uptake (R2DGU) for each structure within each animal was calculated as the ratio of the O.D. of the structure to the O.D. of a white matter structure (in our case, the denominator was the O.D. of the optic tract):

$$\text{relative 2DG uptake (R2DGU)} = \frac{\text{O.D. structure}}{\text{O.D. optic tract}}$$

Optical density ratios have been used previously to control for slight variations in the dose of the tracer, section thickness, and film development (Plum et al., 1976; Sharp, 1976; Schwartz and Gainer, 1977; Collins, 1978; Meibach et al., 1980). White matter was chosen as the reference because of its low metabolic activity. White matter structures were the least active neural structures in the brain of the euthermic ground squirrel, as also has been found in the rat, cat, and monkey (Sokoloff, 1978). White matter structures also had the lowest metabolic activity in the brain of the hibernating ground squirrel. The optical density of all of the structures examined in both groups remained within the linear range of the exposure-density relation for the x-ray film, as determined by plotting the optical density of the [^{14}C]methyl methacrylate standards against their radioactive content.

Our procedure for the euthermic animals follows the [^{14}C]2DG method as described by Sokoloff et al. (1977), but the incubation periods utilized for the hibernating animals were much longer. This departure from the previous methodology was based on four considerations: (*1*) since the body temperature of hibernating animals used in this experiment was 7 to 8°C, the enzymatic reactions involved in the transport of [^{14}C]2DG into cells and its subsequent phosphorylation would be expected to be much lower from Q_{10} considerations alone; (*2*) the circulation time of the injected label would be considerably lengthened since heart rate decreases from 250 to 300 beats/min in euthermic ground squirrels to 7 to 10 beats/min in hibernators (Twente and Twente, 1978); (*3*) as mentioned above, overall metabolism of *C. lateralis* hibernating at 5°C is approximately 1/30 that of euthermic animals (Snapp and Heller, 1981); and (*4*) the plasma [^{14}C]2DG curve (Fig. 1) indicates that hibernating animals still had higher plasma [^{14}C]2DG levels after 24 hr than euthermic animals after 45 min. Inspection of the autoradiographs of brains of hibernators revealed that the most reproducible autoradiographs were obtained with incubation periods of 8 hr or longer. Also, the optical density of various structures increased with longer incubation times, indicating that 2DG was still being taken up and 2DG-6-phosphatase was still accumulating after 24 hr. Thus, the data presented below are based on the 8-, 12-, 18-, and 24-hr incubation animals.

The mean relative 2-deoxyglucose uptake (mean R2DGU) for each structure in the two groups (hibernating and euthermic) was calculated as the mean of the R2DGU values of the three euthermic animals and the four longest bouts of hibernation (8, 12, 18, and 24 hr). These means for the two groups were subjected to two-tailed *t* tests to determine the differences between the groups after ascertaining that the variances for any structure did not differ between the two groups (Sokal and Rohlf, 1969). The percentage of change between hibernation and euthermia was calculated as follows for each structure:

$$\%\ \text{change} = \frac{\text{hibernating R2DGU} - \text{euthermic R2DGU}}{\text{euthermic R2DGU}} \times 100$$

The complete list of neural structures examined as well as the euthermic and hibernating R2DGU values are given in the Appendix.

Results

Autoradiographs of brains of hibernating ground squirrels were remarkably homogeneous in comparison to those of euthermic animals. Figure 2 illustrates the marked reduction in R2DGU during hibernation in the inferior colliculus, superior olivary nucleus, and vestibu-

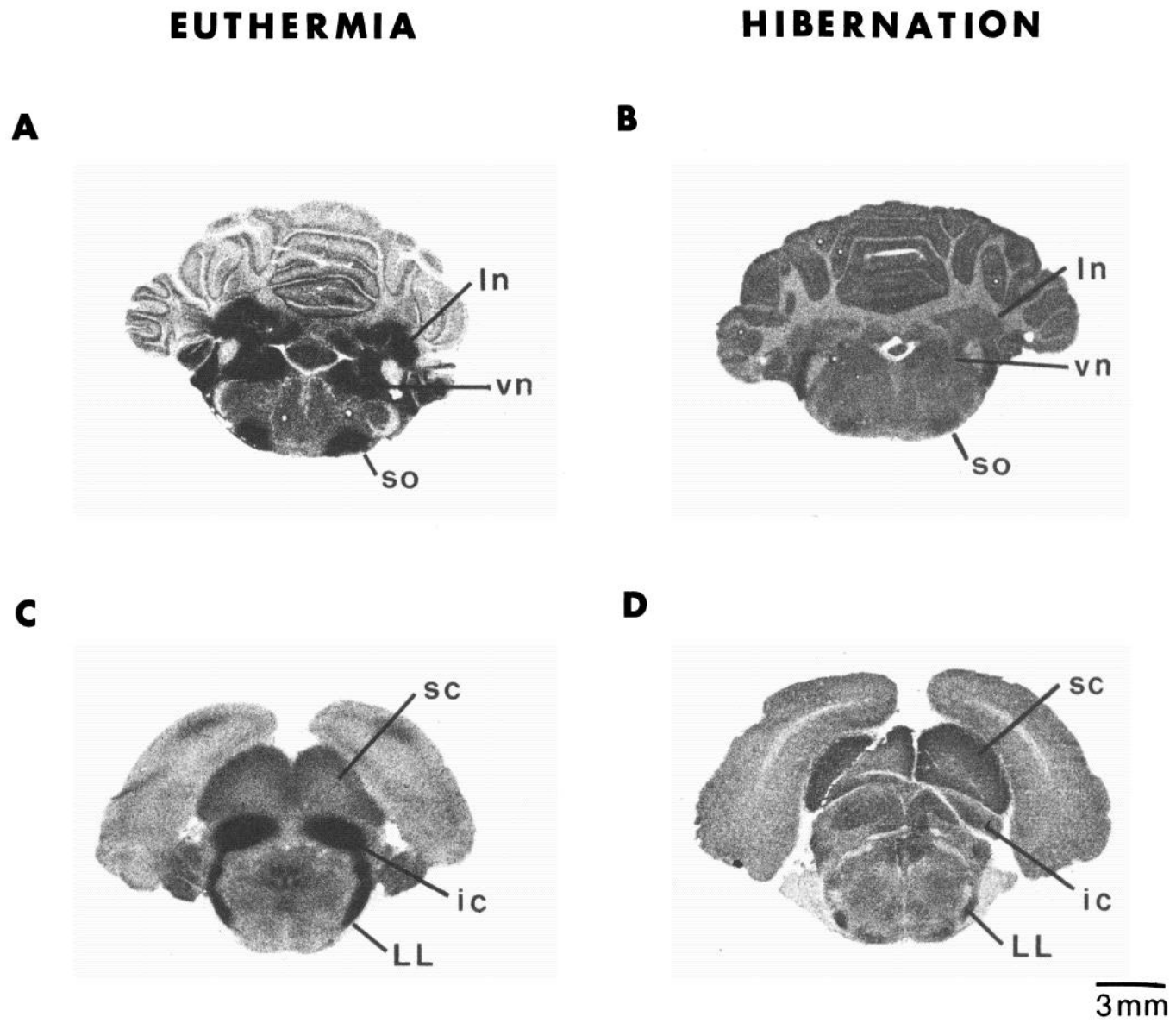

Figure 2. [^{14}C]2-Deoxyglucose autoradiographs of sections through the pons (*A* and *B*) and midbrain (*C* and *D*) of euthermic and hibernating animals. Note the overall homogeneity of the autoradiographs of hibernating (*B* and *D*) compared to euthermic (*A* and *C*) animals and the reduction in metabolic activity apparent in the lateral cerebellar nucleus (*ln*), vestibular nuclei (*vn*), and superior olivary nucleus (*so*) in the hibernator (*B*) relative to the euthermic animal (*A*). Similar decreases in metabolic activity are evident in the inferior colliculus (*ic*) and lateral lemniscus (*LL*) in *D* versus *C* but are not apparent in the superior colliculus (*sc*).

lar and cerebellar nuclei. The homogeneity of the hibernating brain evident in this figure is reflected in the distribution of the R2DGU values for the 85 neural structures examined (Fig. 3). Note that the histogram of R2DGU values for the hibernators is characterized by a clustering around a mean value and a narrower range of R2DGU values (1.02 to 2.34) relative to euthermia (0.89 to 5.28). The shift of this distribution to lower values in hibernators indicates that the average 2DG uptake in gray matter structures compared to that in optic tract was reduced during hibernation relative to euthermia.

This reduction in R2DGU is shown in Table I for structures that had the greatest R2DGU values during euthermia, as indicated by the column entitled "Ordinal Rank: Euthermia." The structures with the highest R2DGU values during euthermia are primarily sensory, specifically auditory, as has been found in the rat, cat, and monkey (Sokoloff, 1978). Note that all of these structures undergo a large (>35%) and significant reduction in their R2DGU during hibernation. In most cases, these reductions result in a marked decrease of the ordinal rank of activity of the structure during hibernation.

Some structures do stand out in the rather homogeneous autoradiographs of hibernating brain and thus have the largest R2DGU values during hibernation. These structures comprise the first category of structures that may be important during hibernation and, in many cases, are sensory nuclei that receive primary afferents. Figure 4 illustrates the cochlear nucleus and the superior

colliculus in the two states and Figure 5 presents the dorsal horn of the spinal cord and the nucleus of the lateral olfactory tract. Table II lists the structures that have the highest R2DGU values during hibernation. The columns labeled "Ordinal Rank: Hibernation, Euthermia" reveal that a structure can become among the most active during hibernation in one of two ways: (*1*) it can have such a large R2DGU value during euthermia that, even after a relatively large reduction, it still remains a highly active structure in hibernation or (*2*) the R2DGU value during hibernation can remain about the same as it is during euthermia, the net effect being that the ordinal ranking of the structure rises. The former case is exemplified by the cochlear nucleus which has the second highest R2DGU during euthermia and undergoes a 38% reduction during the transition to hibernation but still has the highest R2DGU value during hibernation. On the other hand, the suprachiasmatic nucleus shows little change in its R2DGU value in hibernation compared to euthermia; consequently, its ordinal ranking rises from 71 in euthermia to 7 during hibernation.

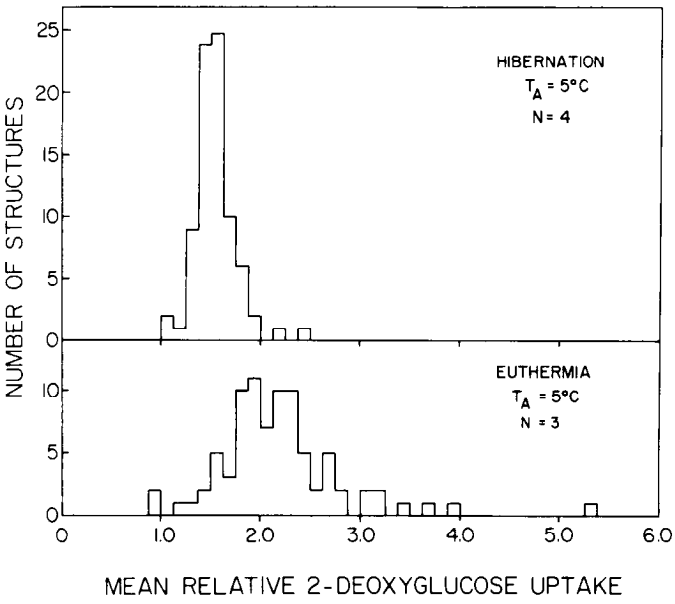

Figure 3. Distribution of the mean relative 2-deoxyglucose uptake of the 85 neural structures examined in hibernation and euthermia. The homogeneity of the cerebral metabolic activity evident in the autoradiographs in Figure 2 is represented here by a clumped distribution in the hibernation values relative to the euthermia values.

A second category of important structures includes those that undergo the smallest reduction in R2DGU in hibernation compared to euthermia (Table III). Several white matter structures in this table increase their R2DGU values during hibernation, but no change in ordinal rank of these structures results. In contrast, some gray matter structures, such as the pontine (Fig. 2), suprachiasmatic, and lateral septal nuclei (Fig. 6), markedly increase in ordinal rank as a result of a small percentage of change in R2DGU values between euthermia and hibernation.

The cerebellar hemispheres also change greatly in ordinal rank as a result of a relatively small difference in R2DGU. These data reflect a qualitative change in the pattern of 2DG uptake seen in the cerebellum: the laminar distribution of 2DG uptake characteristic of the euthermic brain is absent in the hibernator (Figs. 2 and 4). Curiously, most cerebellar structures have small to moderate reductions of relative 2DG uptake during hibernation, but the cerebellar nuclei have large, significant reductions in their R2DGU values (as is evident from Figs. 2 and 4).

A laminar distribution of 2DG uptake in euthermic cerebral cortex is also absent from the cerebral cortex of the hibernator (Figs. 2, 4, 5, and 6). All cortical areas examined undergo significant reductions of R2DGU greater than 30% (see "Appendix"). The frontal cortex is an exception; its 35% reduction is not significant due primarily to the large variability in the R2DGU of this structure during euthermia.

From the data presented in Table I, it is evident that all of the structures that had the highest R2DGU values during euthermia undergo large and significant reductions in R2DGU upon entrance into hibernation. This suggests that there may be a relationship between the R2DGU of a structure during euthermia and the percentage of reduction in R2DGU that that structure undergoes during the transition to hibernation. Figure 7 plots the R2DGU during euthermia against the percentage of reduction in R2DGU for all 85 structures. A fairly linear relationship emerges: the greater the euthermic R2DGU of a structure, the greater the percentage of reduction in R2DGU that that structure undergoes dur-

TABLE I

Neural structures exhibiting the greatest relative 2-deoxyglucose uptake during euthermia and changes upon hibernation

Structure	Euthermic R2DGU[a] ± SEM	Ordinal Rank		Percent Change in R2DGU[a] upon Hibernation	Significance Level
		Euthermia	Hibernation		
Inferior colliculus	5.28 ± 0.39	1	4	−65.0	0.01
Cochlear nucleus	3.83 ± 0.18	2	1	−38.9	0.001
Superior olivary nucleus	3.66 ± 0.26	3	9	−52.0	0.001
Nucleus of lateral lemniscus	3.36 ± 0.18	4	18	−50.3	0.01
Vestibular nucleus	3.23 ± 0.21	5	21	−50.0	0.001
Mammillary body	3.22 ± 0.24	6	17	−47.9	0.001
Nucleus interpositus	3.08 ± 0.24	7	42	−51.1	0.01
Fastigial nucleus	3.06 ± 0.25	8	39	−50.4	0.01
Dentate nucleus	2.79 ± 0.25	9	25	−42.9	0.02
Dorsal raphe nucleus	2.74 ± 0.26	10	20	−39.7	0.01

[a] Relative 2-deoxyglucose uptake = O.D. structure/O.D. optic tract.

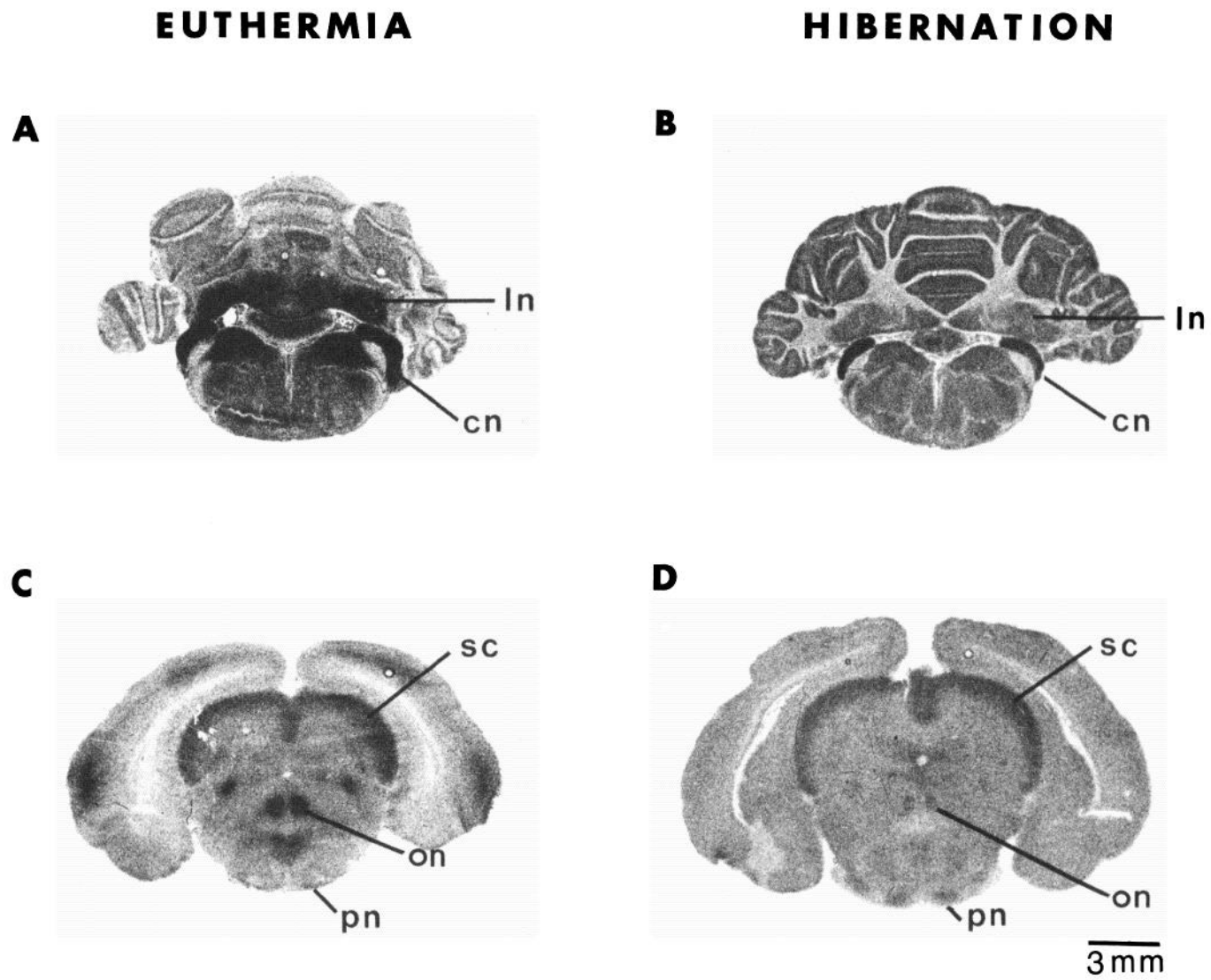

Figure 4. Autoradiographs through the cochlear nuclei (*cn*) reveal a relatively high level of 2DG uptake in this structure during hibernation (*B*), whereas surrounding structures, such as the lateral cerebellar nucleus (*ln*), are more reduced in 2DG uptake when compared to euthermia (*A*). Similarly, the superior colliculus (*sc*) and pontine nuclei (*pn*) remain highly labeled during hibernation (*D* versus *C*), whereas the oculomotor nuclei (*on*) are reduced in 2DG uptake. Note also the lack of distinct laminar activity in the visual cortex of the hibernator (*D*) in comparison to the euthermic condition (*C*).

ing hibernation. An interesting aspect of this relationship is that several structures lie above the others. These structures undergo *less* of a reduction than would be predicted on the basis of their euthermic R2DGU and include several structures that have been identified as important during hibernation in Tables II and III: the cochlear nucleus, the suprachiasmatic nucleus, and paratrigeminal nucleus.

There were also some regional changes in R2DGU values that may be of interest in regard to the control of hibernation. During euthermia, the lateral preoptic area had a greater R2DGU value than the medial preoptic area (Table IV). The medial septal area had a greater R2DGU than the lateral septal area (local ratio, 1.08 during euthermia). These patterns were reversed during hibernation in all animals: the medial preoptic area had a greater R2DGU than the lateral preoptic, and the lateral septal area had a greater R2DGU than the medial septal nucleus (cf., Fig. 6). Note that, in both cases, the reversal occurred as a result of the general relationship depicted in Figure 7: the area with the greater euthermic R2DGU underwent the larger percentage of reduction of R2DGU in hibernation.

Discussion

The [^{14}C]2DG method has been used to demonstrate metabolic changes in the nervous system during sensory, pharmacological, and physiological stimulation (Kennedy et al., 1975; Plum et al., 1976; Sharp et al., 1977; Hubel et al., 1978). Although metabolic changes in the visual system as a function of the sleep state have been described with this method (Livingstone and Hubel, 1980), this is the first description of the changes in 2DG uptake in brain during hibernation.

Since the rate constants for transport of 2DG into cells and its subsequent phosphorylation have not been cal-

culated for the ground squirrel during euthermia and hibernation, we used a relative measure of 2DG uptake. It is likely that changes in neural discharge rates affect cellular glucose metabolism during hibernation (and, hence, the transport of glucose and 2DG into cells) and that changes in neural activity also result in changes in 2DG uptake. Therefore, relative 2DG uptake probably reflects neural activity of the brain during hibernation.

The observation that the brain is remarkably homogeneous during hibernation may not seem surprising at first glance, since cerebral metabolic activity would be expected to decrease during hibernation due to the reduction in tissue temperature. Although this prediction could readily be made for absolute glucose utilization, it need not apply to relative glucose utilization. If the temperature sensitivity of the metabolism of all neural tissues were the same, then the percentage of reduction observed would be identical in all structures and the R2DGU values would be unchanged. The autoradiographic images of the brain structures would be similar to the euthermic condition only lighter; with longer exposures of the x-ray film to the sections of hibernating brains, it would be possible to approximate the autoradiographic appearance of the euthermic brain. This is clearly not the case: the autoradiographic appearance of the hibernating brain is qualitatively different from that of the euthermic brain. The fact that the reductions in R2DGU calculated from these autoradiographs do vary suggests that these reductions are not due solely to temperature (Q_{10}) effects and that the metabolic activity of some structures is indeed decreased less than others during hibernation.

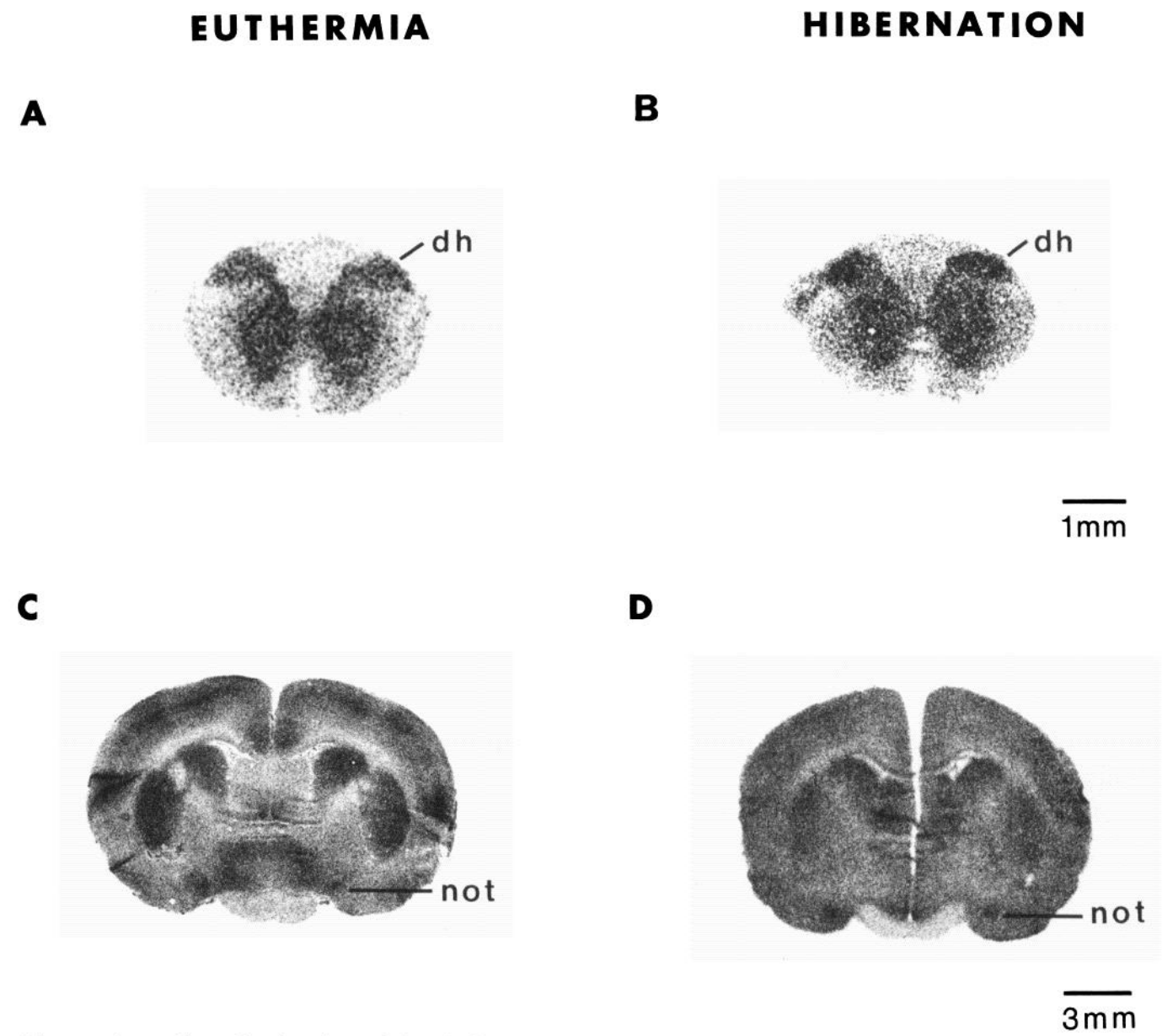

Figure 5. Autoradiographs of sections of the spinal cord (*A* and *B*) and forebrain (*C* and *D*) of the ground squirrel. Structures that maintain high 2DG uptake during hibernation include the dorsal horn of the spinal cord (*dh*) and the nucleus of the lateral olfactory tract (*not*).

TABLE II

Neural structures exhibiting the greatest relative 2-deoxyglucose uptake during hibernation and their changes relative to euthermia

Structure	Hibernation R2DGU[a] ± SEM	Ordinal Rank		Percent Change in R2DGU[a] from Euthermic Values	Significance Level
		Hibernation	Euthermia		
Cochlear nucleus	2.34 ± 0.08	1	2	−38.9	0.001
Paratrigeminal nucleus	2.18 ± 0.22	2	79	50.3	0.05
Dorsal tegmental nucleus	1.92 ± 0.03	3	23	−18.6	0.01
Inferior colliculus	1.85 ± 0.04	4	1	−65.0	0.01
Superior colliculus, superficial layer	1.80 ± 0.04	5	25	−20.5	0.01
Locus coeruleus	1.80 ± 0.11	6	49	−10.2	ns[b]
Suprachiasmatic nucleus	1.79 ± 0.03	7	71	4.6	ns
Inferior olivary nucleus	1.76 ± 0.03	8	18	−27.6	0.05
Superior olivary nucleus	1.75 ± 0.07	9	3	−52.0	0.001
Habenular nuclei	1.74 ± 0.07	10	14	−34.4	0.01

[a] Relative 2-deoxyglucose uptake = O.D. structure/O.D. optic tract.
[b] ns, not significant.

TABLE III

Structures undergoing the smallest reduction in relative 2-deoxyglucose uptake during hibernation in comparison to euthermia

Structure	Percent Change	Ordinal Rank during	
		Euthermia	Hibernation
Gray matter			
Paratrigeminal nucleus	50.3	79	2
Suprachiasmatic nucleus	4.6	71	9
Lateral septal nucleus	4.2	78	28
Anterior cerebellar hemisphere	3.8	80	32
Pontine nuclei	3.7	79	31
Posterior cerebellar hemisphere	−1.4	76	29
Flocculus	−6.7	65	14
Dorsal horn	−10.1	69	27
Locus coeruleus	−10.2	49	7
Pontine gray	−13.0	72	53
Ventral hippocampus	−13.6	77	75
Uvula	−16.2	51	19
Medial preoptic area	−18.0	68	57
White matter			
Cerebellar white matter	18.8	84	83
Spinal white matter	15.6	85	84
Corpus callosum	8.7	82	82
Internal capsule	2.9	81	78

The results suggest two ways of identifying structures which may have significant functional roles during hibernation. One way is to identify structures which have the highest relative 2DG uptake during hibernation (Table II). Among these structures, there are several "lower order" sensory nuclei, such as the cochlear nucleus and superior colliculus. Other sensory structures that stand out in the autoradiographs of hibernating brain but do not appear in this table include the nucleus of the olfactory tract (Fig. 5), dorsal horn of the spinal cord (Fig. 5), and ventrobasal thalamus. The high 2DG uptake in sensory structures may be due to two causes: (*1*) the uptake could reflect the sensitivity of the hibernator to environmental stimuli mentioned in the introduction or (*2*) since it has not been established whether the areas of 2DG uptake correspond to cell bodies or to neuropil, it is possible that the areas of uptake reflect inhibitory or excitatory synaptic input to lower order sensory nuclei from "higher" structures.

We would call particular attention to other structures in this category that undergo large increases in ordinal rank during hibernation, specifically, the paratrigeminal nucleus, dorsal tegmental nucleus, locus coeruleus, and suprachiasmatic nucleus. The paratrigeminal nucleus is a poorly characterized medullary cell group lying between the inferior cerebellar peduncle and tract of the spinal trigeminal nucleus (Chan-Palay, 1978a, b). Its connections are unknown and its possible role in hibernation will be discussed elsewhere (T. S. Kilduff, F. R. Sharp, and H. C. Heller, manuscript in preparation). The dorsal tegmental nucleus receives afferents from the lateral habenula and from the interpeduncular nucleus (Smaha and Kaelber, 1973). Efferent pathways from the dorsal tegmental nucleus include a reciprocal projection to the interpeduncular nucleus (Briggs and Kaelber, 1971; Marchand et al., 1980) and projections to the lateral hypothalamus, preoptic area, and mammillary nuclei (Briggs and Kaelber, 1971). The connections of this nucleus suggest a relationship with the limbic system, but its functional role is unknown. In the hibernating animal, this structure and one of its afferent sources, the lateral habenula (a limbic structure), are among the structures exhibiting the greatest relative 2DG uptake (Table II). It is through such correlations that the 2DG method may help establish the function of such poorly understood structures.

The locus coeruleus has noradrenergic connections throughout the diencephalon and forebrain (Amaral and Sinnamon, 1977). Because of the widespread nature of these connections, an extensive body of literature has arisen on the putative role of the locus coeruleus in the control of the arousal state (Hobson et al., 1974; Ramm, 1979). This structure also has been suggested as possibly being involved in the control of cerebral blood flow (Sharp and Schwartz, 1977; Schwartz, 1978). Either or both functions would be compatible with the altered physiological state of hibernation. With regard to the latter proposed function, a scintillation-based [^{3}H]2DG study demonstrated that locus coeruleus stimulation decreased deoxyglucose uptake in the ipsilateral cortex (Abraham et al., 1979). Perhaps activity in the locus coeruleus during deep hibernation results in the disappearance of the laminar pattern of 2DG uptake characteristic of the cerebral cortex in euthermia (Figs. 2, 4, 5,

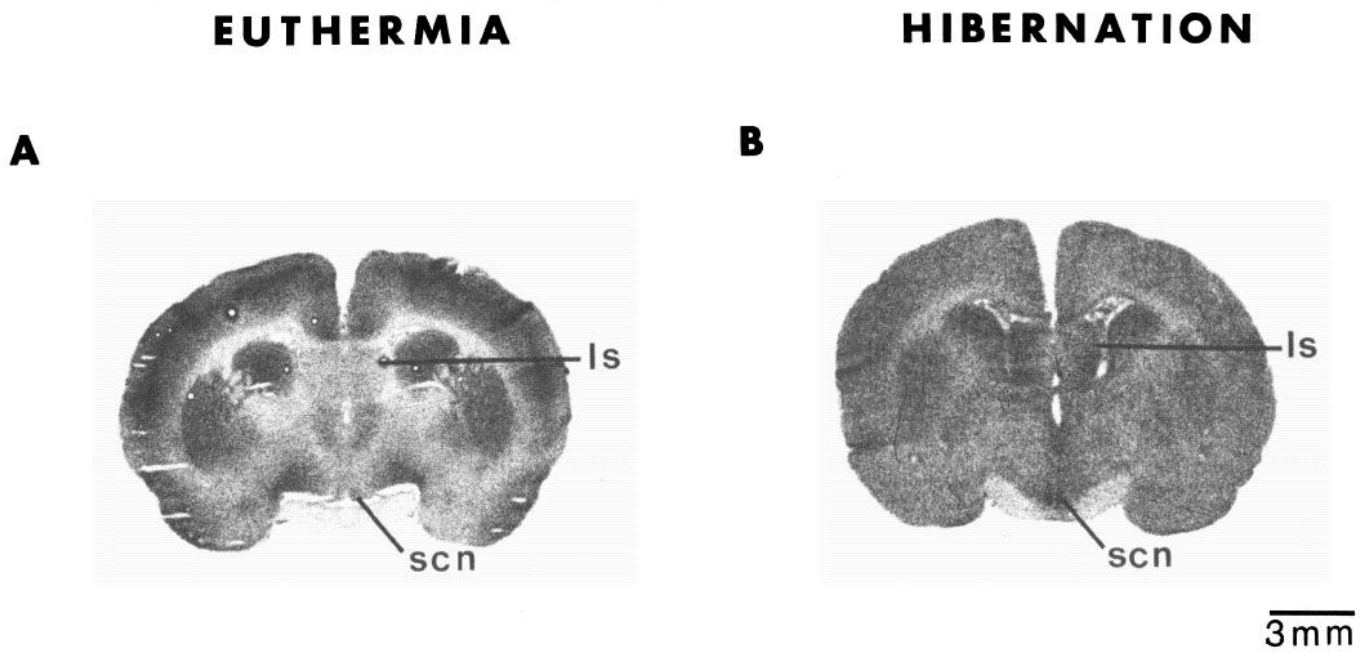

Figure 6. Autoradiographs through the diencephalon of the ground squirrel showing a relatively high 2DG uptake in the lateral septal (*ls*) and suprachiasmatic (*scn*) nuclei during hibernation.

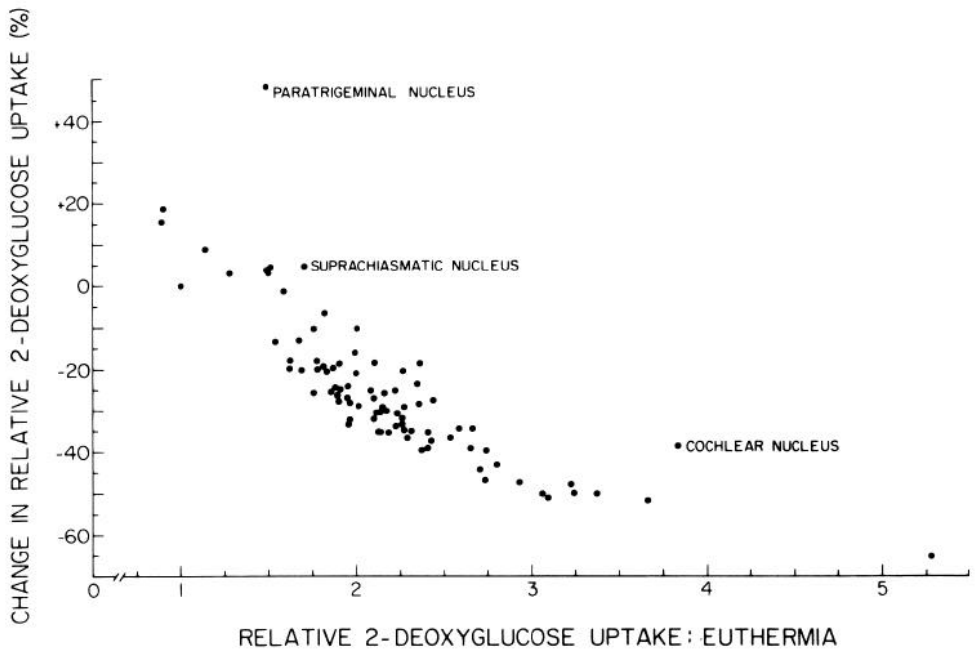

Figure 7. The relationship between the mean relative 2DG uptake of a neural structure during euthermia and the percentage of reduction in mean R2DGU that that structure undergoes in the transition from euthermia to hibernation. Each *point* represents one of the 85 neural structures examined. The labeled structures deviate from this relationship to the greatest extent; these structures undergo less of a reduction in mean R2DGU during hibernation than would be predicted from their R2DGU during euthermia.

and 6) and in the disappearance of the laminar pattern in the cerebellar cortex. This laminar pattern corresponds to cortical layer IV and is thought to represent specific sensory afferents from the thalamus (Schwartz and Sharp, 1978). The absence of this pattern during deep hibernation is consistent with EEG studies that report an absence of cortical EEG activity below a body temperature of 15°C (Shtark, 1972; Heller, 1979).

Increasing evidence suggests that the suprachiasmatic nucleus (SCN) is involved in the control of circadian rhythmicity in mammals (Stephan and Zucker, 1972; Rusak and Zucker, 1979; Inouye and Kawamura, 1979). This structure undergoes a circadian rhythm of [^{14}C]2DG uptake in the rat (Schwartz and Gainer, 1977; Schwartz et al., 1980). Bouts of shallow hibernation or torpor in some species of rodents appear to have an underlying

TABLE IV
Local changes in relative 2-deoxyglucose uptake in preoptic and septal areas

Structure	Euthermia	Hibernation	Percent Change	*p*
Septal area R2DGU[a]				
Medial	1.62 ± 0.26	1.30 ± 0.04	−19.5	ns[b]
Lateral	1.50 ± 0.15	1.57 ± 0.06	4.2	ns
Local ratio Medial/ Lateral	1.08	0.83		
Preoptic area R2DGU				
Medial	1.77 ± 0.12	1.45 ± 0.01	−18.0	ns
Lateral	1.95 ± 0.07	1.30 ± 0.03	−33.3	0.001
Local ratio Medial/ Lateral	0.91	1.12		

[a] Relative 2-deoxyglucose uptake = O.D. structure/O.D. optic tract.
[b] ns, not significant.

circadian rhythmicity, since the intervals between successive arousals are multiples of 24 hr (French, 1977; Cranford, 1981). This rhythmicity persists when the animals are placed in constant darkness. A similar timing of the arousals from torpor for a ground squirrel is evident in the data of Walker et al. (1979). Thus, the metabolic activity evident in the SCN in the present study (Fig. 6) may reflect time-keeping demands during hibernation.

The SCN may have its effect via a neurohumoral output. This nucleus has parvocellular neurons containing neurophysin in association with vasopressin, as occurs in the magnocellular neurons of the paraventricular and supraoptic nuclei (Sofroniew and Weindl, 1978). The SCN receives direct projections from the retina (Moore and Lenn, 1972; Moore, 1973), ventral lateral geniculate nucleus (Swanson et al., 1974), and dorsal and median raphe nuclei (Aghajanian et al., 1969). Projections from this area go to regions of the brain associated with the release of neurohumoral substances: the median eminence, periventricular hypothalamus (Swanson and Cowan, 1975), and periaqueductal gray (Kucera and Favrod, 1979). Of particular interest to our results is the existence of a vasopressin-containing fiber pathway which projects to the lateral septal nucleus.

A second way of identifying structures possibly important during hibernation is to look for small reductions in the R2DGU during hibernation in comparison to euthermia (Table III). Structures of interest in this category include the suprachiasmatic nucleus, pontine nuclei, lateral septal nucleus, paratrigeminal nucleus, cerebellar cortex, dorsal horn of the spinal cord, and the locus coeruleus. The suprachiasmatic nucleus, paratrigeminal nucleus, and locus coeruleus have been discussed above.

The pontine nuclei give rise to a crossed pontocerebellar pathway terminating in the cerebellar hemispheres (Brodal, 1954) and receive reciprocal connections from the dentate and interpositus nuclei (Brodal et al., 1972). The decreased 2DG uptake evident in these cerebellar nuclei during hibernation may reflect a change in neural activity in these structures and their projections to the pontine nuclei. A change in activity of the pontine nuclei may, in turn, affect 2DG uptake in the cerebellar hemispheres, causing the disappearance of the laminar distribution of 2DG uptake characteristic of euthermia.

The lateral septal nucleus is a limbic structure that receives afferents from the hippocampal formation and projects primarily to the medial septal/diagonal band complex but also has a heavy projection to the medial preoptic and suprachiasmatic nuclei (Swanson and Cowan, 1979; Garris, 1979). Thus, a reciprocal pathway exists to link the lateral septal nucleus with another structure that is among the most active during deep hibernation, the suprachiasmatic nucleus. The connection of the lateral septal nucleus with the medial preoptic area is of some interest considering the parallel changes in the metabolic activity of these structures observed in this study (Table IV). During euthermia, the medial preoptic area has a lower relative 2DG uptake than the lateral preoptic area; this pattern is reversed during hibernation. Since the lateral preoptic area has a sparse cell population relative to the medial preoptic and is comprised primarily of fibers from the medial forebrain bundle, the highly significant ($p < 0.001$) decrease in relative 2DG uptake in the lateral preoptic area may reflect decreased ascending input from lower brainstem centers. In contrast, the insignificant change in R2DGU in the medial preoptic area during hibernation may reflect the thermosensitivity of the hibernator: as in euthermia, cooling the preoptic area during hibernation results in increases in oxygen consumption and body temperature, whereas heating this area has the opposite effects (Williams and Heath, 1970; Heller and Colliver, 1974). A greater proportion of thermosensitive neurons is found in the medial rather than lateral preoptic area; thermosensitive cells also are found in the septal area (Eisenman and Jackson, 1967). The medial preoptic area also has been reported to project to the lateral septal area (Conrad and Pfaff, 1976; Anderson and Shen, 1980); hence, reciprocal pathways connect the lateral septal area to both the medial preoptic area and SCN.

The relatively high levels of 2DG uptake in and the known connections between the lateral septal nucleus, medial preoptic area, and SCN may represent a functional "circuit" during hibernation. The lateral septal nucleus could receive circadian information from the SCN and thermal input from the medial preoptic area throughout this state. Since the lateral septal area provides efferents to limbic system structures, such as the mammillary bodies and the medial septal area, this structure could effect an arousal from hibernation if warranted by afferent input from the SCN and medial preoptic area. This suggestion bears some similarity to a model recently proposed to account for the arousal from hibernation (Heller, 1979). Unlike this model, however, our results do not implicate the hippocampus as playing a role during deep hibernation, since this structure undergoes a 35% reduction in R2DGU during hibernation ($p < 0.01$).

The final structure which falls into the second category of structures deemed important during hibernation is the dorsal horn of the spinal cord. The dorsal horn is the site of termination of thermal, nociceptive, and large diameter proprioceptive afferents from the periphery. Ambient temperature is perhaps the most important environmen-

tal parameter that a hibernator has to monitor to prevent its freezing to death. Thus, it is tempting to speculate that the metabolic activity in the dorsal horn may reflect processing of thermal information. This structure, perhaps along with the paratrigeminal nucleus (T. S. Kilduff, F. R. Sharp, and H. C. Heller, manuscript in preparation), could relay thermal information to the medial preoptic area.

A recent technique has enabled the localization of u-like opiate receptors in the brain of the rat (Herkenham and Pert, 1980). With respect to our hibernation results, it is of considerable interest that such receptors have been described in several "primary" sensory nuclei. Wherever such nuclei have a laminar organization, u-like receptors are found preferentially in the most superficial layers. Sensory nuclei in which such receptors have been described are the primary terminal zones in the dorsal horn of the spinal cord and spinal trigeminal nucleus (somatosensory input), the superficial layer of the superior colliculus (visual input), and the dorsal portion of the dorsal cochlear nucleus (auditory input) (Herkenham and Pert, 1980). The coincidence of the locations of these receptors and the sensory structures found metabolically active in the brain of the hibernator in our study is intriguing. High circulating levels of endogenous opiates during hibernation is suggested by the fact that ground squirrels administered morphine during hibernation fail to develop the abstinence syndrome when subsequently injected with naloxone during euthermia (Beckman and Llados-Eckman, 1980). However, both nicotinic (Hunt and Schmidt, 1978) and muscarinic (Wamsley et al., 1981) cholinergic receptors also occur in brain areas in direct receipt of sensory inputs and substance P is found in large amounts in many of these same areas (Nicoll et al., 1980).

An intriguing result of this study was the discovery of a general relationship between euthermic R2DGU and the percentage of reduction that a structure incurred during hibernation (Fig. 7). This orderly relationship between euthermic R2DGU and the percentage of reduction depicted in Figure 7 was unexpected. Figure 8 presents similar data from an experiment in which rats were anesthetized with sodium thiopental and the [^{14}C]2DG uptake was measured in 25 brain areas (Sokoloff et al., 1977). Unlike Figure 7, there is no clear relationship between the R2DGU of a structure in the unanesthetized condition and the percentage of reduction that the structure underwent when the rats were anesthetized. Our interpretation of the data in Figure 7 is that (*1*) the reduction in R2DGU of a given neural structure is not due solely to the direct effect of temperature on the metabolic functions of that tissue; (*2*) there is, however, a general principle governing the decrease in R2DGU of neural structures during hibernation; and (*3*) deviations from this general principle may be due to the maintenance of specific functions significant in the process of hibernation. We have already addressed the last point but can we speculate on what might be the underlying cause of this "general principle" governing the decrease in R2DGU during hibernation if it is not a Q_{10} phenomenon?

Despite our conclusion that the changes in R2DGU in areas of the brain cannot be explained simply by a consideration of temperature effects, obviously, Q_{10} effects will apply to single cells. The general relationship shown in Figure 7 must be an emergent property of networks of cells, each of which is affected by temperature. The effect of temperature on a population of cells may depend, then, on their pattern of interactions. For example, if the activity of a population of cells were largely influenced by excitatory afferent input which was temperature sensitive, the effect of temperature on that population of cells would be compounded by the temperature sensitivity of the afferent inputs. Hence, the larger

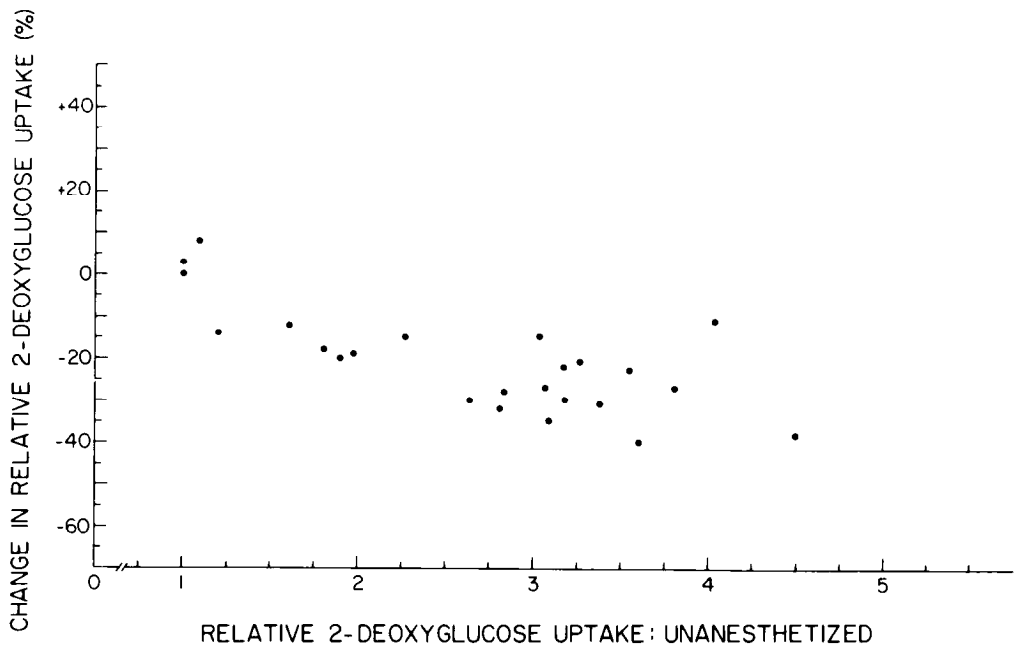

Figure 8. The relationship between the mean R2DGU in the unanesthetized state and the percentage of reduction in mean R2DGU during anesthesia in rats anesthetized with sodium thiopental. Data are derived from Sokoloff et al. (1977).

the number of such afferents that a structure received, the greater will be its apparent temperature sensitivity as the temperature of the whole nervous system changes. Therefore, we feel that the direct proportionality shown in Figure 7 between level of euthermic activity and the percentage of reduction during hibernation strongly suggests that the 2DG uptake that a given structure shows during euthermia is largely a reflection of the number of functionally active afferents that it receives.

This work has been an initial study of neural metabolic activity during hibernation. Having described the basal activity of various neural structures during euthermia and deep hibernation, we now look forward to applying the [^{14}C]2DG technique to the dynamic phases of this state, such as the entrance and arousal processes, which should reveal more about the control systems involved in this phenomenon. We also are undertaking parallel studies on animals in forced hypothermia to compare with hibernation as a means of exploring the adaptive versus the temperature-induced changes in neural function involved in hibernation. These approaches to the study of this fascinating naturally occurring state may contribute much to our understanding of the functional activity of the nervous system.

Appendix

Neural structures examined during euthermia and hibernation

Structure	Euthermic R2DGU ± SEM	Hibernation R2DGU ± SEM	Percent Change in R2DGU upon Hibernation	*t* Value	*p*
Spinal cord					
Dorsal horn	1.76 ± 0.10	1.58 ± 0.10	−10.1	−1.28	ns[a]
Ventral horn	1.83 ± 0.12	1.46 ± 0.06	−20.3	−3.09	0.05
White matter	0.89 ± 0.11	1.03 ± 0.04	15.6	1.36	ns
Cerebellum					
Fastigial nucleus	3.06 ± 0.25	1.52 ± 0.04	−50.4	−7.23	0.01
Nucleus interpositus	3.09 ± 0.24	1.51 ± 0.04	−51.1	−7.62	0.01
Lateral nucleus	2.80 ± 0.25	1.59 ± 0.04	−42.9	−5.70	0.02
Paraflocculus	1.90 ± 0.29	1.54 ± 0.06	−18.7	−1.41	ns
Flocculus	1.82 ± 0.48	1.70 ± 0.06	−6.6	−0.03	ns
Nodulus	2.10 ± 0.30	1.71 ± 0.02	−18.5	−1.56	ns
Uvula	1.99 ± 0.21	1.66 ± 0.01	−16.2	−1.81	ns
Medullary layer	0.90 ± 0.08	1.06 ± 0.04	18.8	2.11	ns
Posterior hemisphere	1.58 ± 0.18	1.56 ± 0.01	−1.4	−0.15	ns
Anterior hemisphere	1.49 ± 0.21	1.55 ± 0.05	3.8	0.31	ns
Nucleus gracilis	1.95 ± 0.18	1.48 ± 0.05	−24.0	−2.89	0.05
Nucleus cuneatus	1.90 ± 0.22	1.37 ± 0.02	−27.6	−2.83	ns
Lateral cuneate nucleus	2.27 ± 0.20	1.60 ± 0.02	−29.5	−3.93	0.05
Paratrigeminal nucleus	1.49 ± 0.24	2.24 ± 0.17	50.3	2.67	0.05
Dorsal motor nucleus of vagus	2.00 ± 0.18	1.58 ± 0.02	−21.0	−2.83	ns
Nucleus of spinal trigeminal	2.10 ± 0.31	1.54 ± 0.04	−26.7	−2.10	ns
Inferior olivary nucleus	2.43 ± 0.20	1.76 ± 0.03	−27.6	−3.89	0.05
Lateral reticular nucleus	1.87 ± 0.17	1.41 ± 0.06	−24.6	−2.87	0.05
Cochlear nucleus	3.83 ± 0.18	2.34 ± 0.08	−38.9	−8.42	0.001
Spinal vestibular nucleus	2.92 ± 0.29	1.54 ± 0.04	−47.4	−5.66	0.02
Principal vestibular nucleus	3.23 ± 0.21	1.61 ± 0.08	−50.1	−8.12	0.001
Pontine gray	1.68 ± 0.20	1.46 ± 0.03	−13.0	−1.30	ns
Superior olivary nucleus	3.66 ± 0.26	1.75 ± 0.07	−52.0	−8.15	0.001
Main sensory nucleus of trigeminal	2.08 ± 0.24	1.55 ± 0.04	−25.5	−2.59	ns
Nucleus of lateral lemniscus	3.36 ± 0.18	1.67 ± 0.04	−50.3	−10.85	0.01
Locus coeruleus	2.01 ± 0.15	1.80 ± 0.11	−10.2	−1.13	ns
Inferior colliculus	5.28 ± 0.39	1.85 ± 0.04	−65.0	−10.38	0.01
Dorsal tegmental nucleus	2.35 ± 0.07	1.91 ± 0.03	−18.6	−6.20	0.01
Dorsal raphe nucleus	2.74 ± 0.26	1.65 ± 0.06	−39.7	−4.71	0.01
Median raphe nucleus	2.38 ± 0.24	1.44 ± 0.04	−39.6	−4.66	0.02
Red nucleus	2.40 ± 0.11	1.46 ± 0.05	−39.1	−8.52	0.001
Oculomotor nucleus	2.53 ± 0.16	1.60 ± 0.02	−36.9	−6.92	0.01
Interpeduncular nucleus	2.58 ± 0.32	1.69 ± 0.08	−34.5	−3.10	ns
Substantia nigra	1.91 ± 0.19	1.43 ± 0.03	−24.8	−2.87	ns
Subthalamic nucleus	2.70 ± 0.23	1.50 ± 0.03	−44.2	−6.01	0.01
Periaqueductal gray	1.81 ± 0.17	1.45 ± 0.06	−19.6	−2.20	ns
Pontine nuclei	1.50 ± 0.06	1.55 ± 0.07	3.6	0.57	ns
Pontine tegmental nucleus	1.86 ± 0.19	1.50 ± 0.07	−19.7	−2.04	ns
Zona incerta	2.14 ± 0.18	1.51 ± 0.02	−29.7	−4.10	0.05
Superior colliculus					
Substantia grisea superficialis, lateral	2.35 ± 0.15	1.79 ± 0.03	−23.6	−4.17	0.01
Substantia grisea superficialis, medial	2.27 ± 0.12	1.80 ± 0.04	−20.5	−4.17	0.01
Substantia grisea medialis, lateral	1.96 ± 0.20	1.32 ± 0.03	−32.5	−3.69	0.05
Substantia grisea medialis, medial	1.89 ± 0.17	1.39 ± 0.05	−26.6	−3.31	0.05

Appendix continued

Structure	Euthermic R2DGU ± SEM	Hibernation R2DGU ± SEM	Percent Change in R2DGU upon Hibernation	*t* Value	*p*
Superior colliculus—continued					
Substantia grisea profundus, lateral	2.18 ± 0.20	1.41 ± 0.03	−35.2	−4.48	0.05
Substantia grisea profundus, medial	1.85 ± 0.10	1.38 ± 0.04	−25.2	−4.80	0.01
Thalamus					
Parafascicular nucleus	2.22 ± 0.15	1.47 ± 0.03	−33.9	−5.86	0.01
Posterior nucleus	2.01 ± 0.12	1.43 ± 0.03	−29.0	−5.62	0.02
Lateral posterior nucleus	1.96 ± 0.02	1.41 ± 0.04	−28.1	−11.57	0.001
Medial geniculate nucleus	2.65 ± 0.27	1.61 ± 0.04	−39.1	−4.50	0.05
Lateral geniculate nucleus	2.25 ± 0.10	1.53 ± 0.05	−32.0	−7.48	0.001
Ventrobasal nucleus	2.35 ± 0.30	1.68 ± 0.04	−28.4	−2.65	ns
Ventrolateral nucleus	2.14 ± 0.13	1.38 ± 0.02	−35.4	−7.11	0.01
Lateral habenula	2.66 ± 0.25	1.74 ± 0.07	−34.4	−4.14	0.01
Medial dorsal nucleus	2.42 ± 0.23	1.51 ± 0.04	−37.5	−4.66	0.02
Lateral dorsal nucleus	2.27 ± 0.15	1.48 ± 0.03	−34.7	−5.96	0.01
Anterior nuclei	2.17 ± 0.09	1.47 ± 0.06	−30.6	−6.28	0.01
Paratenial nucleus	2.27 ± 0.18	1.51 ± 0.12	−33.4	−3.71	0.02
Hypothalamus					
Mammillary body	3.22 ± 0.24	1.68 ± 0.14	−47.9	−5.95	0.01
Dorsomedial nucleus	1.95 ± 0.17	1.42 ± 0.05	−26.9	−3.36	0.02
Ventromedial nucleus	1.78 ± 0.10	1.42 ± 0.05	−19.9	−3.49	0.02
Suprachiasmatic nucleus	1.71 ± 0.14	1.79 ± 0.03	4.6	0.64	ns
Posterior nucleus	2.10 ± 0.11	1.42 ± 0.02	−32.1	−6.91	0.01
Dorsal hippocampus	2.14 ± 0.13	1.38 ± 0.09	−35.4	−5.03	0.01
Ventral hippocampus	1.54 ± 0.14	1.33 ± 0.05	−13.6	−1.63	ns
Lateral preoptic area	1.91 ± 0.07	1.30 ± 0.03	−33.3	−9.73	0.001
Medial preoptic area	1.77 ± 0.12	1.45 ± 0.01	−18.0	−3.03	ns
Caudate nucleus	2.16 ± 0.22	1.60 ± 0.06	−25.7	−2.78	0.05
Putamen	2.24 ± 0.25	1.54 ± 0.04	−31.1	−3.19	0.05
Globus pallidus	1.62 ± 0.11	1.33 ± 0.04	−18.0	−2.92	0.05
Medial amygdaloid nucleus	1.68 ± 0.08	1.34 ± 0.04	−20.2	−3.99	0.02
Basal amygdaloid nucleus	1.75 ± 0.25	1.30 ± 0.04	−25.8	−2.13	ns
Medial septal nucleus	1.62 ± 0.26	1.30 ± 0.04	−19.5	−1.44	ns
Lateral septal nucleus	1.50 ± 0.15	1.57 ± 0.06	4.2	0.44	ns
Cingulate cortex	2.16 ± 0.11	1.51 ± 0.05	−30.1	−6.08	0.01
Nucleus of olfactory tract	2.22 ± 0.10	1.66 ± 0.04	−25.1	−5.71	0.01
Visual cortex	2.13 ± 0.22	1.49 ± 0.06	−30.3	−3.27	0.05
Auditory cortex	2.73 ± 0.33	1.45 ± 0.05	−46.8	−4.54	0.05
Somatosensory cortex	2.29 ± 0.16	1.45 ± 0.06	−36.7	−5.41	0.01
Frontal cortex	2.41 ± 0.40	1.55 ± 0.05	−35.5	−2.53	ns
Pyriform cortex	2.31 ± 0.21	1.50 ± 0.02	−35.0	−4.61	0.01
Corpus callosum	1.14 ± 0.11	1.24 ± 0.03	8.7	0.99	ns
Internal capsule	1.28 ± 0.19	1.32 ± 0.08	2.9	0.20	ns
Optic tract	1.00 ± 0.00	1.00 ± 0.00	0.0	0.00	

[a] ns, not significant.

References

Abraham, W. C., R. L. Delanoy, A. J. Dunn, and S. F. Zornetzer (1979) Locus coeruleus stimulation decreases deoxyglucose uptake in ipsilateral mouse cerebral cortex. Brain Res. *172:* 387–392.

Aghajanian, G. K., F. E. Bloom, and M. H. Sheard (1969) Electron microscopy of degeneration within the serotonin pathway of the rat brain. Brain Res. *13:* 266–273.

Amaral, D. G., and H. M. Sinnamon (1977) The locus coeruleus: Neurobiology of a central noradrenergic nucleus. Prog. Neurobiol. *9:* 147–196.

Anderson, C. H., and C. L. Shen (1980) Efferents of the medial preoptic area of the guinea pig: An autoradiographic study. Brain Res. Bull. *5:* 257–265.

Beckman, A. L., and C. Llados-Eckman (1980) Failure of morphine to produce physical dependence following central administration during hibernation. Soc. Neurosci. Abstr. *6:* 37.

Briggs, T. L., and W. W. Kaelber (1971) Efferent fiber connections of the dorsal and deep tegmental nuclei of Gudden. An experimental study in the cat. Brain Res. *29:* 17–29.

Brodal, A. (1954) Afferent cerebellar connections. In *Aspects of Cerebellar Anatomy*, J. Jansen and A. Brodal, eds., pp. 182–188, Grundt Tanum, Oslo, Norway.

Brodal, A., J. Destombes, A. M. Laceredi, and P. Angaut (1972) A cerebellar projection onto the pontine nuclei. An experimental anatomical study in the cat. Exp. Brain Res. *16:* 115–139.

Chan-Palay, V. (1978a) The paratrigeminal nucleus. I. Neurons and synaptic organization. J. Neurocytol. *7:* 405–418.

Chan-Palay, V. (1978b) The paratrigeminal nucleus. II. Identification and inter-relations of catecholamine axons, indoleamine axons, and substance P immunoreactive cells in the neuropil. J. Neurocytol. *7:* 419–442.

Collins, R. C. (1978) Kindling of neuroanatomic pathways during recurrent focal penicillin seizures. Brain Res. *150:* 503–517.

Conrad, L. C. A., and D. W. Pfaff (1976) Efferents from the

medial basal forebrain and hypothalamus in the rat. I. An autoradiographic study of the medial preoptic area. J. Comp. Neurol. *169:* 185–220.

Cranford, J. (1981) Circadian rhythms of body temperature and periodic arousal in hibernating *Zapus princeps.* Cryobiology *18:* 86.

Eisenman, J. S., and D. C. Jackson (1967) Thermal response patterns of septal and preoptic neurons in cats. Exp. Neurol. *19:* 33–45.

Florant, G. L., and H. C. Heller (1977) CNS regulation of body temperature in euthermic and hibernating marmots (*Marmota flaviventris*). Am. J. Physiol. *232:* R203–208.

French, A. R. (1977) Periodicity of recurrent hypothermia during hibernation in the pocket mouse (*Perognathus longimembris*). J. Comp. Physiol. *115:* 87–100.

Garris, D. R. (1979) Direct septo-hypothalamic projections in the rat. Neurosci. Lett. *3:* 83–90.

Heller, H. C. (1979) Hibernation: Neural aspects. Annu. Rev. Physiol. *41:* 305–321.

Heller, H. C., and G. W. Colliver (1974) CNS regulation of body temperature during hibernation. Am. J. Physiol. *227:* 583–589.

Heller, H. C., G. W. Colliver, and P. Anand (1974) CNS regulation of body temperature in euthermic hibernators. Am. J. Physiol. *227:* 576–582.

Heller, H. C., G. W. Colliver, and J. Beard (1977) Thermoregulation during entrance into hibernation. Pfluegers Arch. *369:* 55–59.

Herkenham, M., and C. D. Pert (1980) *In vitro* autoradiography of opiate receptors in rat brain suggests loci of "opiategic" pathways. Proc. Natl. Acad. Sci. U. S. A. *77:* 5532–5536.

Hobson, J. A., R. W. McCarley, T. Pivik, and R. Freedman (1974) Selective firing by cat pontine brain stem neurons in desynchronized sleep. J. Neurophysiol. *37:* 497–511.

Hubel, D. H., T. N. Wiesel, and M. P. Stryker (1978) Anatomical demonstration of orientation columns in macaque monkey. J. Comp. Neurol. *177:* 361–380.

Hunt, S., and J. Schmidt (1978) Some observations on the binding patterns of α-bungarotoxin in the central nervous system of the rat. Brain Res. *157:* 213–232.

Inouye, S. T., and H. Kawamura (1979) Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. Proc. Natl. Acad. Sci. U. S. A. *76:* 5962–5966.

Joseph, S. A., K. A. Knigge, L. M. Kalejs, R. A. Hoffman, and P. Reid (1966) *A Stereotaxic Atlas of the Brain of the 13-Line Ground Squirrel (Citellus tridecemlineatus),* Edgewood Arsenal Special Publication No. 100–12, Edgewood Arsenal, Edgewood, MD.

Kehl, T. H., and P. Morrison (1960) Peripheral nerve function and hibernation in the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus.* Bull. Mus. Comp. Zool. *124:* 387–400.

Kennedy, C., M. H. DesRosiers, J. W. Jehle, M. Reivich, F. R. Sharp, and L. Sokoloff (1975) Mapping of functional neural pathways by autoradiographic survey of local metabolic rate with [^{14}C]deoxyglucose. Science *187:* 850–853.

Kilduff, T. S., F. R. Sharp, and H. C. Heller (1980) Neural activity during hibernation: Application of the [^{14}C]deoxyglucose technique. Soc. Neurosci. Abstr. *6:* 769.

Kilduff, T. S., H. C. Heller, and F. R. Sharp (1981) Autoradiographic studies of neural activity during hibernation. Fed. Proc. *40:* 580.

Koenig, J. F. R., and R. A. Klippel (1967) *The Rat Brain,* Robert E. Krieger Publishing Co., Huntington, NY.

Kucera, P., and P. Favrod (1979) Suprachiasmatic nucleus projection to mesencephalic central grey in the woodmouse (*Apodemus sylvaticus L.*). Neuroscience *4:* 1705–1715.

Livingstone, M. S., and D. H. Hubel (1980) Differences between sleeping and waking in visually evoked cortical activity in the cat, measured by a double label 2-deoxyglucose technique. Soc. Neurosci. Abstr. *6:* 314.

Lyman, C. P., and P. O. Chatfield (1955) Physiology of hibernation in mammals. Physiol. Rev. *35:* 403–425.

Marchand, E. R., J. N. Riley, and R. Y. Moore (1980) Interpeduncular nucleus afferents in the rat. Brain Res. *193:* 339–352.

Meibach, R. C., S. D. Glick, D. A. Ross, R. D. Cox, and S. Maayani (1980) Intraperitoneal administration and other modifications of the 2-deoxy-D-glucose technique. Brain Res. *195:* 167–176.

Moore, R. Y. (1973) Retinohypothalamic projection in mammals: A comparative study. Brain Res. *49:* 403–409.

Moore, R. Y., and N. J. Lenn (1972) A retinohypothalamic projection in the rat. J. Comp. Neurol. *146:* 1–14.

Musacchia, X. J. (1976) Helium-cold hypothermia, an approach to depressed metabolism and thermoregulation. In *Regulation of Depressed Metabolism and Thermogenesis,* L. Jansky and X. J. Musacchia, eds., pp. 137–157, Charles C Thomas, Springfield, IL.

Musacchia, X. J., and D. R. Deavers (1981) The regulation of carbohydrate metabolism in hibernation. In *Survival in Cold,* X. J. Musacchia and L. Jansky, eds., pp. 55–76, Elsevier-North Holland, New York.

Nicoll, R. A., C. Schenker, and S. E. Leeman (1980) Substance P as a transmitter candidate. Annu. Rev. Neurosci. *3:* 227–268.

Palkovits, M., and D. M. Jacobowitz (1974) Topographic atlas of catecholamine and acetylcholine-esterase containing neurons in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). J. Comp. Neurol. *157:* 29–42.

Pellegrino, L. J., A. S. Pellegrino, and A. J. Cushman (1979) *A Stereotaxic Atlas of the Rat Brain,* Ed. 2, Plenum Press, New York.

Plum, F., A. Gjedde, and F. E. Samson (1976) Neuroanatomical functional mapping by the radioactive 2-deoxy-glucose method. Neurosci. Res. Program Bull. *14:* 457–518.

Polenov, A. L., and M. N. Yurisova (1975) The hypothalamo-hypophysial system in grounds squirrels, *Citellus erythogenys* Brandt and *Citellus undulatus* Pallas. I. Microanatomy and cytomorphology of the Gomori-positive neurosecretory system with special reference to its state during hibernation. Z. Mikrosk. Anat. Forsch. *89:* 991–1014.

Ramm, P. (1979) The locus coeruleus, catecholamines, and REM sleep: A critical review. Behav. Neural Biol. *25:* 415–448.

Rusak, B., and I. Zucker (1979) Neural regulation of circadian rhythms. Physiol. Rev. *59:* 449–526.

Schwartz, W. J. (1978) 6-Hydroxydopamine lesions of rat locus coeruleus alter brain glucose consumption, as measured by the 2-deoxy-D-[^{14}C]glucose tracer technique. Neurosci. Lett. *7:* 141–150.

Schwartz, W. J., and H. Gainer (1977) Suprachiasmatic nucleus: Use of ^{14}C-labeled deoxyglucose uptake as a functional marker. Science *197:* 1089–1091.

Schwartz, W. J., and F. R. Sharp (1978) Autoradiographic maps of regional brain glucose consumption in resting, awake rats using [^{14}C]2-deoxyglucose. J. Comp. Neurol. *177:* 335–359.

Schwartz, W. J., L. C. Davidson, and C. B. Smith (1980) *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. J. Comp. Neurol. *189:* 157–168.

Sharp, F. R. (1976) Rotation induced increases of glucose uptake in rat vestibular nuclei and vestibulocerebellum. Brain Res. *110:* 141–151.

Sharp, F. R., and W. J. Schwartz (1977) Proposed effects of

brain noradrenaline on neuronal activity and cerebral blood flow during REM sleep. Experientia *33:* 1618–1619.

Sharp, F. R., J. S. Kauer, and G. M. Shepherd (1977) Laminar analysis of 2-deoxyglucose uptake in olfactory bulb and olfactory cortex of rabbit and rat. J. Neurophysiol. *40:* 800–813.

Shtark, M. B. (1972) *The Brain of Hibernating Animals.* NASA Technical Translation TTF-619, National Aeronautics and Space Administration, Washington, DC.

Smaha, L. A., and W. W. Kaelber (1973) Efferent fiber projections of the habenula and interpeduncular nucleus. An experimental study in the opossum and the cat. Exp. Brain Res. *16:* 291–308.

Snapp, B. D., and H. C. Heller (1981) Suppression of metabolism during hibernation in ground squirrels (*Citellus lateralis*). Physiol. Zool. *54:* 297–307.

Sofroniew, M. V., and A. Weindl (1978) Projections from the parvocellular vasopressin- and neurophysin-containing neurons of the suprachiasmatic nucleus. Am. J. Anat. *153:* 391–430.

Sokal, R. R., and F. J. Rohlf (1969) *Biometry,* Freeman, San Francisco.

Sokoloff, L. (1978) Mapping cerebral functional activity with radioactive deoxyglucose. Trends Neurosci. *1:* 75–79.

Sokoloff, L., M. Reivich, C. Kennedy, M. H. DesRosiers, C. S. Patlak, P. D. Pettigrew, O. Sakurada, and M. Shinohara (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. *28:* 13–26.

Stephan, F. K., and I. Zucker (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc. Natl. Acad. Sci. U. S. A. *69:* 1583–1586.

Strumwasser, F. (1959) Regulatory mechanisms, brain activity, and behavior during deep hibernation in the squirrel, *Citellus beecheyi.* Am. J. Physiol. *196:* 23–30.

Swanson, L. W., and W. M. Cowan (1975) The efferent connections of the suprachiasmatic nucleus of the hypothalamus. J. Comp. Neurol. *160:* 1–12.

Swanson, L. W., and W. M. Cowan (1979) The connections of the septal region in the rat. J. Comp. Neurol. *186:* 621–656.

Swanson, L. W., W. M. Cowan, and E. R. Jones (1974) An autoradiographic study of efferent connections of the ventral lateral geniculate nucleus in the albino rat and the cat. J. Comp. Neurol. *156:* 143–163.

Twente, J. W., and J. Twente (1978) Autonomic regulation of hibernation by *Citellus* and *Eptesicus.* In *Strategies in Cold: Natural Torpidity and Thermogenesis,* L. C. H. Wang and J. W. Hudson, eds., pp. 327–373, Academic Press, New York.

Walker, J. M., A. Garber, R. J. Berger, and H. C. Heller (1979) Sleep and estivation (shallow torpor): Continuous processes of energy conservation. Science *204:* 1098–1100.

Wamsley, J. K., M. S. Lewis, W. S. Young, III, and M. J. Kuhar (1981) Autoradiographic localization of muscarinic cholinergic receptors in rat brainstem. J. Neurosci. *1:* 176–191.

Williams, B. A., and J. E. Heath (1970) Responses to preoptic heating and cooling in a hibernator *Citellus tridecemlineatus.* Am. J. Physiol. *218:* 1654–1660.

Wünnenberg, W., G. Merker, and E. Speulda (1976) Thermosensitivity of preoptic neurons in a hibernator (golden hamster) and a non-hibernator (guinea pig). Pfluegers Arch. *363:* 119–123.