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Short Communications

Influence of brown adipose tissue on deep cervical temperature during sleep in the young rabbit[1]

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Summary. In young rabbits the slope of the temperature in the deep cervical region close to brown adipose tissue increased during desynchronized sleep at low ambient temperature. No increase occurred at neutral ambient temperature. In control rabbits (after disappearance of brown adipose tissue), the slope of deep cervical temperature did not increase during desynchronized sleep at low or neutral ambient temperatures.
Key words. Sleep; brown adipose tissue; rabbit.

Central control of body temperature is markedly altered during desynchronized sleep (DS), i.e. rapid eye movement or paradoxical sleep, as compared with synchronized sleep (SS), i.e. slow wave sleep. In particular, it has been shown that the sympathetic control of vasomotion in cutaneous heat exchangers ceases during DS (see ref. 4 for a review of temperature regulation during sleep). Sympathetic efferent activity also controls brown adipose tissue (BAT), a main effector of nonshivering thermogenesis. The present study was undertaken for the following reasons:
a) a repatterning of sympathetic outflow occurs during DS[5];
b) the control of a metabolic thermoregulatory effector (BAT) might differ from that of a motor thermoregulatory effector, e.g. cutaneous vasculature;
c) the BAT thermogenic role is especially well established at a very young age, when
d) the sleep cycle exhibits a high DS content[6].

The experiments were performed on young New Zealand white rabbits implanted, under general anesthesia (sodium pentobarbitone 40 mg/kg s.c. 45 min after premedication with Flunitrazepam 0.5 mg/kg i.m.), with electrodes for EEG recordings. Thermistors were also placed in the hypothalamus, and deep in the cervical region, located bilaterally between lateral and posterior BAT cervical lobes[7]. Since white fat replaces the BAT deposits in the adult rabbit, ontogenetic timing of the recording sessions was critical. The rabbits were implanted just after weaning at 4 weeks of age, and the recording sessions carried out during the 5th to 6th week of age (weight 600–700 g), depending on the speed of post-operative recovery. At this age the cervical lobes consist mainly of brown fat. Preliminary recordings of nuchal EMG were also carried out. As previously shown in the adult[8], EMG activity was attenuated in the young rabbit during SS and strongly depressed during DS at the ambient temperatures (T_a's) considered. Therefore, EMG recording was discontinued in sub-

Table 1. Slope changes in deep cervical temperature during sleep

CNT animals			BAT animals	
	0 °C			0 °C
C1	− 0.0032 ± 0.0019		B1	0.0089 ± 0.0022
C2	− 0.0047 ± 0.0020		B2	0.0377 ± 0.0050
C3	− 0.0059 ± 0.0036		B3	0.0150 ± 0.0043
C4	0.0003 ± 0.0036		B4	0.0292 ± 0.0060
			B5	0.0060 ± 0.0023
Group mean	− 0.0033 ± 0.0013	t = 3.27		0.0193 ± 0.0060
	t = − 2.53	df = 7		t = 3.21
	df = 3	p < 0.02		df = 4
	NS			p < 0.05
				t = 3.26
				df = 8
				p < 0.02
	24 °C			24 °C
C1	− 0.0201 ± 0.0145		B1	0.0011 ± 0.0010
C2	0.0021 ± 0.0027		B2	0.0021 ± 0.0030
C3	− 0.0079 ± 0.0040		B3	0.0029 ± 0.0045
C4	− 0.0069 ± 0.0061		B4	− 0.0094 ± 0.0049
			B5	− 0.0047 ± 0.0032
Group mean	− 0.0082 ± 0.0045			− 0.0016 ± 0.0023
	t = − 1.82			t = − 0.69
	df = 3			df = 4
	NS			NS

Mean values (± SE) of slope changes in deep cervical temperature during the transition from synchronized to desynchronized sleep are presented for each animal of the control (CNT) and brown adipose tissue (BAT) groups at 0 °C and 24 °C ambient temperature, respectively. The statistical significance of group means is indicated. Significant differences between group means are also indicated (arrows). Slopes in °C min^{-1}.

sequent experiments, thus avoiding unnecessary mechanical trauma in the region of thermistor implantation. The recording sessions were carried out in a thermoregulated box, on 4 successive days, at 24 °C, 0 °C, 0 °C, and 24 °C T_a's respectively. An example of polygraphic recording at low T_a is shown in the figure. Sessions were sometimes repeated in order to obtain sufficient data. Good tolerance of the experimental situation was indicated by the steady increase in body weight throughout the recording sessions in all but two animals (see below).

At the end of the recording sessions the animals were reanesthetized with sodium pentobarbitone, and the BAT calorigenic response to noradrenaline was tested before and after injection of the β-blocking agent propranolol. Solutions of noradrenaline HCl (Sigma; 5 µg/ml saline) and DL propranolol HCl (Sigma; 0.5 mg/ml saline) were infused into the ear vein at a dose of 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively (injection rate 0.2 ml/min). The animals were then sacrificed with a sodium pentobarbitone overdose. BAT lobes were excised, fixed in 10%

Table 2. Correlation coefficients between slope changes in hypothalamic and deep cervical temperature during sleep

CNT animals			BAT animals	
	0 °C			0 °C
C1	0.0475		B1	0.3501
C2	− 0.0180		B2	0.1890
C3	0.0780		B3	0.5102
C4	− 0.0535		B4	0.5948
			B5	0.2070
Group mean	0.0135 ± 0.0300	t = 3.75		0.3702 ± 0.0806
	t = 0.45	df = 7		t = 4.59
	df = 3	p < 0.01		df = 4
	NS			p < 0.02
				t = 3.46
				df = 8
				p < 0.01
	24 °C			24 °C
C1	− 0.2173		B1	− 0.2133
C2	− 0.3252		B2	− 0.2357
C3	− 0.4015		B3	− 0.6470
C4	0.1780		B4	0.1360
			B5	− 0.1291
Group mean	− 0.1915 ± 0.1280			− 0.2178 ± 0.1260
	t = − 1.49			t = − 1.72
	df = 3			df = 4
	NS			NS

Correlation coefficients between slope changes in hypothalamic and deep cervical temperature during the transition from synchronized to desynchronized sleep are presented for each animal of the control (CNT) and brown adipose tissue (BAT) groups at 0 °C and 24 °C ambient temperature, respectively. The statistical significance of group means is indicated. Significant differences between group means are also indicated (arrows).

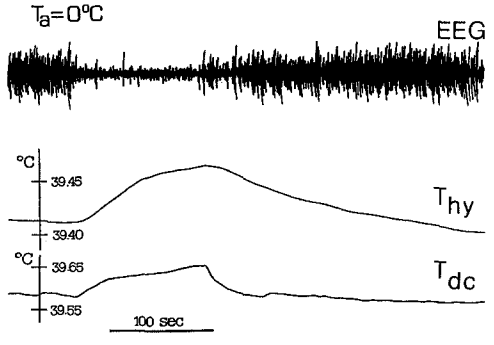
formalin saline and stained with hematoxylin and eosin for histological examination.

Of the 9 rabbits used in this study, 4 failed to show any rise in deep cervical temperature (T_{dc}) after noradrenaline infusion. Post-mortem macroscopic and histological examination showed that in two of these the cervical lobes had almost disappeared (these were the animals that had not gained weight during the experimental sessions). In the other two animals (which were slightly older) the fat deposits appeared macroscopically white and microscopically unilocular. Since no significant inter-animal differences were found, these 4 animals were used as a 'control' group.

The change in slope of T_{dc} from SS to DS is shown in table 1. In the control group no significant slope changes occurred in T_{dc} from SS to DS, either at low T_a, or at T_a close to thermal neutrality. In the remaining 5 animals of this study, BAT was both histologically present and functionally responsive (noradrenaline-propranolol test). These animals were considered as the 'BAT group'. In this group slope changes in T_{dc} from SS to DS are positive and are statistically significant at low T_a, but not significantly different from zero at neutral T_a. Similarly, slope changes in T_{dc} and hypothalamic temperature (T_{hy}) from SS to DS are positively and significantly correlated with each other only in the BAT group at low T_a (table 2).

To our knowledge, the only previous measurements of BAT temperature (T_{BAT}) during DS were obtained in the golden hamster *(Mesocricetus auratus)*[9]. In this species both T_{hy} and T_{BAT} decreased during DS in a cool environment. Since T_{hy} increases during DS in most species, the parallel drop of T_{hy} and T_{BAT} in the golden hamster suggests that in an animal of small mass the impairment of thermoregulation during DS causes an overall body cooling at T_a below neutrality. A similar decrease in T_{hy} during DS below thermoneutrality has been recorded in the pocket mouse *(Perognathus longimembris)*[10]. This overall body cooling might mask specific temperature changes occurring in BAT during DS. In our experimental conditions the choice of animals with a relatively large body mass and good thermal insulation allowed reliable recordings of temperature changes in the deep cervical region close to BAT.

No data exist on BAT blood flow during sleep. Total O_2 consumption during DS increases in man, both in newborns[11,12] and adults[13], but decreases in other species[14–17]. Species differences are probably due to the relative contribution of brain metabolism (brain metabolism increases in DS[18]). These differences underline the difficulty of inferring local metabolic changes from measurements of total O_2 consumption during DS. In the absence of direct circulatory or metabolic data, one could interpret T_{dc} slope changes as secondary to passive hemodynamic adjustments occurring in this sleep stage at a low T_a[19,20]. Moreover, random bursts of sympathetic activity, which accompany the phasic events in the somatic sphere during DS, might involve both vascular and parenchymal innervation of brown fat, causing an increase in T_{dc}. If this were the case, temperature changes in BAT would represent further examples of the variability characterizing effector functions in DS[21]. On the other hand, an increase in BAT thermogenesis during DS at low T_a cannot be completely ruled out. While hypothalamic thermoregulatory integration is markedly altered during DS[22], the possibility exists of a spinal control of BAT thermogenesis in early life. The limited extent of T_{dc} slope change (table 1) would not necessarily refute the hypothesis of a tonic activation of BAT in DS, since the strong coupling between flow and metabolism in the tissue[23] ensures that an increased thermal clearance meets an increased heat production. The positive correlation between the slope changes in T_{dc} and T_{hy} occurring at a low T_a (table 2) is consistent with this hypothesis.



Polygraphic recording showing a desynchronized sleep episode at low ambient temperature (T_a). Note the increase in hypothalamic (T_{hy}) and deep cervical (T_{dc}) temperatures associated with electroencephalographic (EEG) desynchronization.

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