ELSEVIER

Brain Research 699 (1995) 214–220

BRAIN RESEARCH

Research report

Pontine and basal forebrain transections disinhibit brown fat thermogenesis in neonatal rats

Mark S. Blumberg *, Susan L. Schalk, Greta Sokoloff

Department of Psychology, The University of Iowa, Iowa City, IA 52242, USA

Accepted 6 July 1995

Abstract

Bignall, Heggeness and Palmer (1975) were the first to demonstrate increases in metabolic heat production following midpontine transection in neonatal rats. Subsequent work in adult rats has shown that this procedure disinhibits thermogenesis by brown adipose tissue (BAT). Bignall and his colleagues also found that hypothalamic ablation did not result in increased thermogenesis in 5-day-olds, leading them to conclude that thermoregulation depends on more caudal structures at that age. We have also found that midpontine transection disinhibits BAT thermogenesis and, furthermore, have extended that finding to newborn pups. When transections were made in the basal forebrain, however, we also found profound and rapid increases in brown fat thermogenesis. These results suggest the presence of at least two sources of inhibition of BAT thermogenesis in newborn rats: one located in the rostral pons-caudal midbrain and one located in the basal forebrain.

Keywords: Brown adipose tissue; Non-shivering thermogenesis; Thermoregulation; Decerebration; Neonate; Hypothalamus

1. Introduction

In 1975, Bignall et al. [3] reported on the effects of midpontine transection on the metabolic responses of neonatal rats. These investigators found that transected 5- and 10-day-old rat pups significantly increased metabolic heat production even when the pups were maintained in a thermoneutral environment, and even in pups that had been starved for as long as 10 h. Thus, Bignall et al. concluded that midpontine transection releases the tonic inhibition of neural sites caudal to the transection.

This finding in neonatal rats is similar to that found in adult animals. For example, Rothwell et al. [13] and Shibata et al. [14] performed pre-pontine transections on anesthetized adult rats and reported significant increases in metabolic heat production due to increases in thermogenesis by brown adipose tissue (BAT). Moreover, these investigators found that more rostral transections did not elicit non-shivering thermogenesis. Shibata et al. [14] concluded from their data that there is an inhibitory system "located somewhere between the lower midbrain and the upper pons" (p. 273).

In addition to pontine transection, Bignall et al. [3] ablated and aspirated the hypothalamus of 5-day-olds in order to determine the importance of this brain structure for thermoregulation at that age. Based on their results, they concluded that "the hypothalamus seems to play little role in cold-induced thermogenesis in the infant rat, since removing it neither increased metabolism as in the case of midpontine transection, or abolished it (in the cold)" (p. 184).

It is clear from numerous experiments on adult rats that destruction of the medial preoptic area of hypothalamus results in a profound increase in brain and body temperature [7,9]. Thus, if the findings of Bignall et al. [3] are accurate then inhibitory influences on metabolic heat production from the anterior hypothalamus must develop after the age of 5 days. Alternatively, it is possible that their failure to demonstrate a role for the hypothalamus arose from the use of a different methodological procedure (i.e. hypothalamic ablation and aspiration) than that used to demonstrate disinhibition in the midpontine region (i.e. transection).

In the present study, we first replicated and extended the Bignall et al. [3] study by demonstrating that 1- to 3-day-olds, like older pups, increase non-shivering thermogenesis after pontine transection. In addition, contrary to

* Corresponding author. Fax: (1) (319) 335-0191; e-mail: mark-blumberg@blue.weeg.uiowa.edu

SSDI 0006-8993(95)00908-6

Bignall et al., we report that basal forebrain transection disinhibits heat production by brown adipose tissue. Thus, there appear to be at least two systems that tonically inhibit BAT thermogenesis in the neonatal rat: one located in the rostral pons-caudal midbrain and one located in the basal forebrain.

2. Materials and methods

2.1. Experimental subjects

A total of 30 1- to 3-day-old and 30 7- to 8-day-old rat pups of both sexes were used. All pups were born to Harlan Sprague–Dawley females in the animal colony at the University of Iowa. The pups were raised in litters that were culled to 8 pups within 3 days after birth (day of birth = Day 0). Litters and mothers were raised in standard laboratory cages ($48 \times 20 \times 26$ cm) in which food and water were available ad libitum. All animals were maintained on a 12:12 h light/dark schedule with lights on at 06.00 h.

2.2. Pontine transections

Eighteen 1- to 3-day-old and 16 7- to 8-day-old pups were assigned to one of two groups, transected ($n = 12$ and $n = 10$, respectively) and control ($n = 6$ at each age). The control pups were further divided into two groups, defined by whether they experienced a sham surgical procedure or were not manipulated.

Transections were performed under ether or hypothermia anesthesia. All of the 7- to 8-day-old pups were anesthetized with ether. Among the 1- to 3-day-olds, all pups were anesthetized using hypothermia [12] except for two decerebrate pups and one sham pup that were given ether in order to control for its use in the older pups (the type of anesthesia used did not appear to affect the results in these pups). Once a pup was anesthetized, its skull was exposed and a small hole was drilled a variable distance caudal to lambda; this distance varied from 0 to 3 mm. After the hole was drilled, a hypodermic needle was inserted into the hole and was dragged from side to side to transect the brainstem. After removing the needle, the overlying skin was closed and the pup was placed inside an incubator to recover at a thermoneutral temperature (35–36°C). Sham decerebrations were identical except the needle was not inserted through the hole in the skull.

2.3. Basal forebrain transections

Twelve 1- to 2-day-olds from 6 litters and 14 8- to 9-day-olds from 7 litters were used. Two pups were tested from each litter; one pup was assigned to the transected group and the other to the sham control group. Decerebrations were performed under hypothermia (1- to 2-day-olds) or ether (8- to 9-day-olds) anesthesia. The skull was exposed and a small hole was drilled just off the midline (to avoid the sagittal sinus) 4–5 mm rostral to lambda. A blunted, 25 gauge hypodermic needle was inserted into the hole and was dragged from side to side to transect the brainstem. After removing the needle, the overlying skin was closed and the pup was placed inside an incubator to recover at a thermoneutral temperature (35–36°C). Sham decerebrations were identical except the needle was inserted only a short distance (approximately 1 mm) through the hole in the skull.

2.4. Procedure

While a pup was recovering from anesthesia, chromel-constantan thermocouples (Omega, Stamford, CT) were attached. One thermocouple was attached to the skin on the midline overlying the interscapular brown fat pad and the other was attached on the midline in the lumbar region. Collodion was used to glue the thermocouples in place. All thermocouples were calibrated before use against a mercury thermometer accurate to within 0.1°C.

For the pontine transection group, after a period of recovery (mean ± S.E.M.: 52 ± 2 min; range: 29–68 min with one outlier at 107 min) the pup was transported to a polyethylene mesh cage ($7 \times 3.5 \times 4.5$ cm) which was then placed inside one of two double-walled glass metabolic chambers (1- to 3-day-olds: height = 16 cm; inside diameter = 8 cm; 7- to 8-day-olds: height = 17 cm; inside diameter = 12.5 cm). Air temperature within the chamber was controlled within a thermoneutral range using a temperature-controlled water circulator; temperature-controlled water passed between the two walls of the glass chamber and, in turn, air temperature within the chamber was regulated. Humidified air was circulated through the chamber at 300 or 350 ml/min and the oxygen concentration of the exhaust air was dried and then analyzed using a dual-channel electrochemical oxygen analyzer (Ametek, Pittsburgh, PA). All oxygen consumption values are presented as ml O_2/min/100 g.

For the basal forebrain transection group, after 8–25 min of recovery (transected: 14 ± 2 min; sham: 14 ± 2 min) pups at both ages were transported to the larger metabolic chamber (height = 17 cm; inside diameter = 12.5 cm). Because we found the older pups to be particularly active after transection, only they were placed in the small polyethylene mesh cage ($7 \times 3.5 \times 4.5$ cm); the 1- to 2-day-olds were allowed to move freely on a polyethylene mesh platform. Both pups from a litter were tested on the same day. Pairs were matched for sex and the ordering of tests of transected and sham pups was counterbalanced.

Data from the oxygen analyzer, two air temperature thermocouples, and two physiological temperature thermocouples were fed into a computerized data acquisition system (National Instruments, Austin, TX). After the recovered pup was placed in the metabolic chamber, data

recording began. Data were collected and stored on disk at least once every 5 min.

2.5. Histology

At the conclusion of each test, the pup was sacrificed and its head was fixed in formalin until histology was performed. Brains were frozen and sliced mid-sagittally and the levels of the decerebrations were determined. Estimations of transection level were made using the atlas of Altman and Bayer [1] for the E22 rat fetus. In addition, the atlas of Paxinos and Watson [10] for the adult rat was used to confirm gross relations among neural structures.

All transections were verified as complete within the brainstem. Although we were careful to complete the transections at the base of the brain, we made a minimal attempt to complete the transection along its extreme dorsal and lateral aspects. Therefore, the transections were complete only insofar as they traversed the width of the brainstem.

2.6. Data analysis

Data stored on computer disk were imported to Statview 4.0 for the Macintosh for statistical analysis. Mean differences between groups were tested for significance using unpaired *t*-tests. In all cases, means are presented with their standard errors.

3. Results

3.1. Effect of pontine transection on non-shivering thermogenesis

There were no qualitative differences between the data for the 1- to 3- and 7- to 8-day-old pups. Therefore, only the data for the younger pups are presented here. Furthermore, there were no discernible differences between the two control groups for any of the variables measured. Therefore, for the results that follow, we have collapsed both sets of data into one group. This collapsed group is hereafter referred to as the control group.

Air temperature within the chamber varied within a 0.3°C range at the 90, 120, 150, and 180 min time points. Mean air temperature ranged from 35.4–35.6°C. These temperatures are within the 1- to 3-day-old pup's thermoneutral zone, as determined by our observations and those of others [16].

Just as there was wide variability in the level of the transection, so was there variability in the number of animals that exhibited signs of heat production by BAT. Therefore, in order to assess which decerebrations were effective in eliciting BAT thermogenesis and which were not, the pups' individual data are presented below.

First, the data from the control pups were used to generate confidence intervals for the experimental data. Thus, in the top panel of Fig. 1, the dark lines were generated by computing mean interscapular temperature (T_{is}) at 90, 120, 150, and 180 min after the end of sham surgery and either adding or subtracting two standard deviations from the mean at each time point; time 0 for the 3 control animals that did not experience surgery was the time at which they were removed from the nest. In effect, this method provides approximately 95% confidence intervals by which it is possible to judge the thermal responses of the decerebrate animals individually. At least 5 pups contributed to the control values at each time point.

The top panel of Fig. 1 indicates that there were six pups that exhibited significantly elevated interscapular temperatures, five pups that were within the confidence intervals established by the control pups, and one pup that fell significantly below the average interscapular temperature. These 12 pups are numbered for identification.

An increase in interscapular temperature alone is not sufficient to show increased BAT heat production because such an increase in temperature could arise from generalized elevations in metabolic rate, such as that resulting from increased motor activity. Instead, a comparison of interscapular temperature, below which lies a large depot of BAT, with a neutral temperature that does not overlie BAT, provides a relative measure of non-shivering thermogenesis [4]. Thus, the difference between interscapular temperature and back-skin temperature ($T_{is} - T_{back}$) at each time point was used as our measure of heat production by BAT.

The middle panel in Fig. 1 presents the individual $T_{is} - T_{back}$ values for all of the 1- to 3-day-old pups. Again, the dark lines represent the confidence intervals for the control pups (mean $\pm$ 2 standard deviations) and the numbers to the right of the plots identify individual pups. It can be seen that the same pups that exhibited absolute increases in interscapular temperatures (i.e. pups 1–6) also exhibited relative increases in interscapular temperature. These 6 pups exceeded the uppermost confidence interval while the other 6 pups fell within or below the control pups' confidence intervals.

Finally, the bottom panel in Fig. 1 presents the individual oxygen consumption values for all of the 1- to 3-day-old pups. It can be seen that these values are consistent with the values presented in the other two panels. Specifically, pups whose interscapular temperature increased (both absolutely and relatively) consumed more oxygen.

Fig. 2 presents a sagittal view of the brain of a 1-day-old rat on which the transections for each of the 1- to 3-day-old pups are superimposed. The numbers below each transection line correspond to the identification numbers in Fig. 1. First, all of the pups that demonstrated clear BAT heat production (i.e. pups 1–6) were transected in a region that was mid-collicular on its dorsal aspect and pontine on its ventral aspect. (Pup 6 is not presented in Fig. 2 because its

transection traversed the brainstem at an angle. Nonetheless, the cut was confined to an area ranging from the anterior medulla to the anterior pons, that is, within a range delimited by pups 1–5.) The majority of the remainder of the pups (pups 7–11) exhibited transections ranging from the mid-hypothalamus to the rostral pons. The one exception was pup 12, which displayed a significant drop in interscapular temperature (see Fig. 1, top panel); this drop may have been due to a disruption of breathing as this pup was transected in the rostral medulla. Finally,

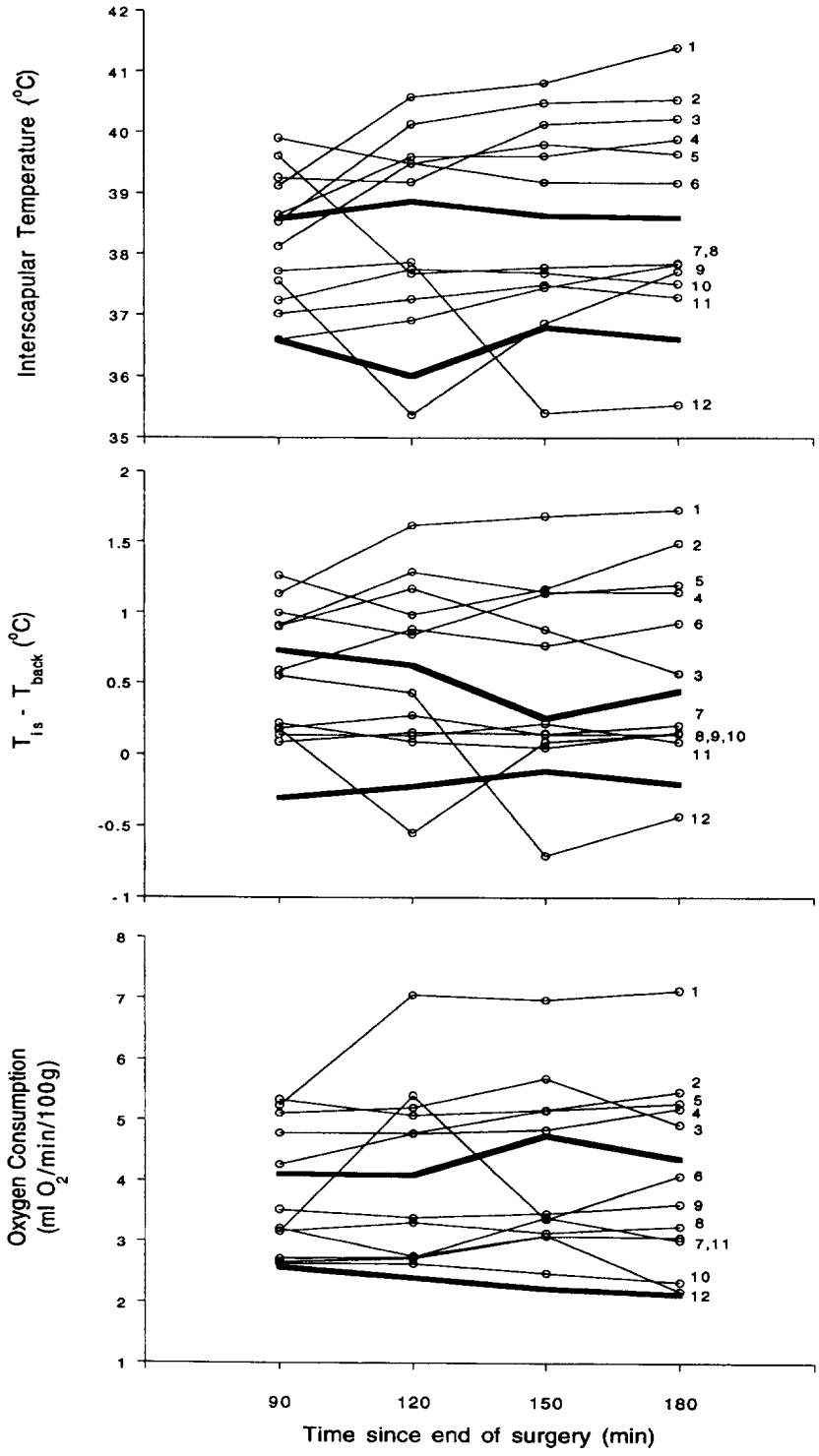

Fig. 1. Interscapular temperature (top), $T_{is} - T_{back}$ (middle), and oxygen consumption (bottom) at the 4 post-surgical time points for each of the 1- to 3-day-old pontine transected pups. The dark lines in each plot are confidence intervals that were derived from the mean values for the 6 control pups ± 2 standard deviations. The identifying numbers to the right of each plot correspond to the numbers in Fig. 2.

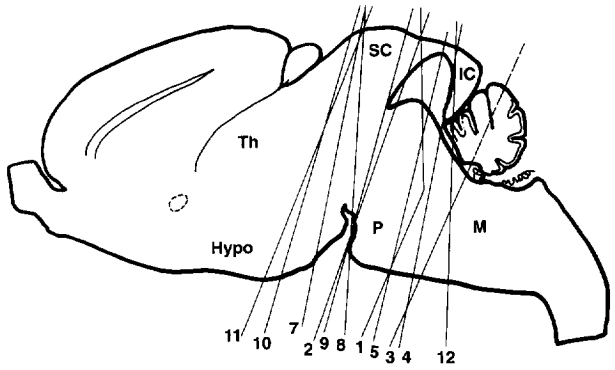

Fig. 2. A sagittal view of the brain of a 2-day-old pup with the location of the pontine transections superimposed upon it. The identifying numbers correspond to the numbers in Fig. 1. The transection for pup #6 is not shown because it traversed the brainstem at an angle, ranging from the anterior medulla to the anterior pons. Hypo, hypothalamus; Th, thalamus; P, pons; SC, superior colliculus; IC, inferior colliculus; M, medulla.

there was no apparent effect of age on response to transection in this group as 3 of the 6 pups that increased thermogenesis (pups 3, 5, and 6 in Figs. 1 and 2) were one day of age.

3.2. Effect of basal forebrain transection on non-shivering thermogenesis

Again, because there were no qualitative differences between the data for the 1- to 2- and 8- to 9-day-old pups, only the data for the younger pups are presented here.

Air temperature within the chamber was maintained within a 0.4°C range at the 30, 60, and 90 min time points. Mean air temperature ranged from 35.1 to 35.5°C across all three time points. At no time were there any significant differences in mean air temperature (30 min: $t = 1.062$, $df = 9$, NS; 60 min: $t = 0.171$, $df = 9$, NS; 90 min: $t = 0.009$, $df = 10$, NS). These temperatures are within the 1- to 2-day-old pup's thermoneutral zone, as determined by our observations and those of others [16].

Behaviorally, transected pups tended to remain quiet throughout the test. Thus, it is not necessary to consider motor activity as an explanation for the results that follow.

None of the sham pups became hyperthermic during the post-surgical period. For these pups, mean interscapular temperature was 34.1 ± 0.4°C at 30 min, 37.6 ± 0.2°C at 60 min, and 37.7 ± 0.2°C at 90 min. The respective values for the transected pups were 33.7 ± 0.4°C, 38.6 ± 0.2°C, and 38.8 ± 0.4°C. The differences at 60 and 90 min are significant (30 min: $t = -0.753$, $df = 9$, NS; 60 min: $t = 2.925$, $df = 9$, $P < 0.05$; 90 min: $t = 2.214$, $df = 10$, $P = 0.05$; see Fig. 3, top panel). On an individual basis, the maximum interscapular temperature recorded during the 90 min post-surgical period never exceeded 38.5°C in the sham pups. In contrast, in the transected pups, maximum interscapular temperature exceeded 40°C in 1 of 6 cases, 39°C in 3 of 6 cases, and 38.5°C in 5 of 6 cases.

A comparison of interscapular and back-skin temperatures shows that the hyperthermia induced by transection was the result of heat production by BAT. The bottom panel of Fig. 3 presents the $T_{is} - T_{back}$ data for the 1- to 2-day-old pups at the three post-surgical time points. Interscapular temperature significantly exceeded back-skin temperature by the 60 min time point (30 min: $t = 0.472$, $df = 8$, NS; 60 min: $t = 2.561$, $df = 9$, $P < 0.05$; 90 min: $t = 3.344$, $df = 10$, $P < 0.01$). The data for $T_{is} - T_{back}$ makes it clear that all pups were producing heat by BAT early in the test: This was no doubt a result of the use of

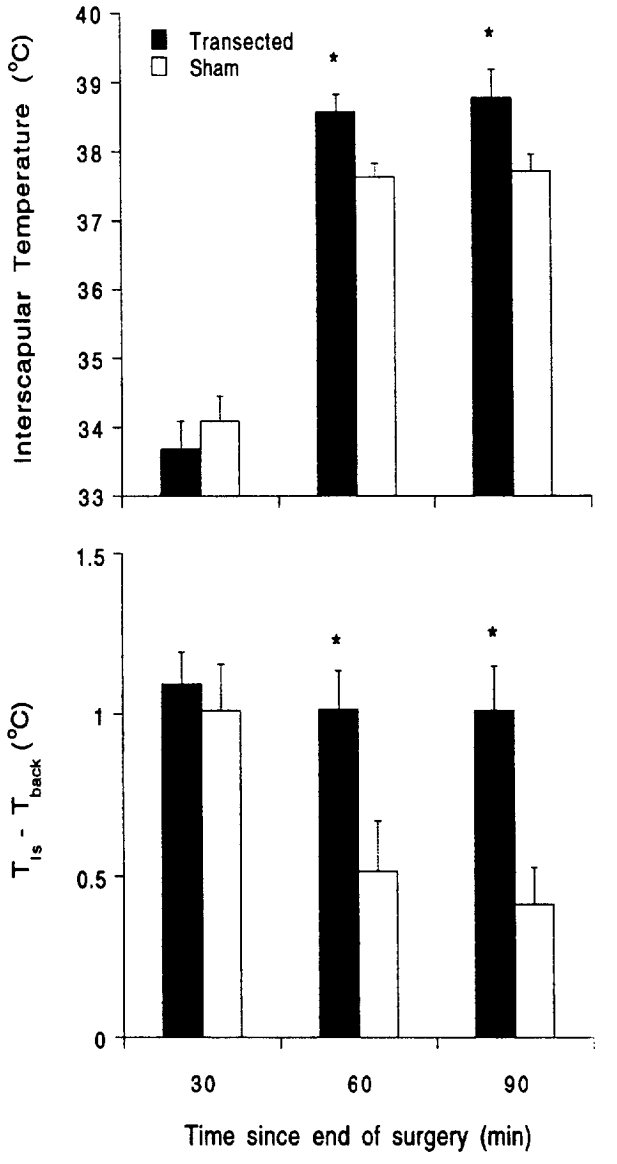

Fig. 3. Mean interscapular temperature (top) and mean $T_{is} - T_{back}$ (bottom) for the 3 post-surgical time points for the 1- to 2-day-old basal forebrain transected and sham pups. $5 \leq n \geq 6$. Mean ± S.E.M. * $P < 0.05$ in relation to Sham, unpaired t-test.

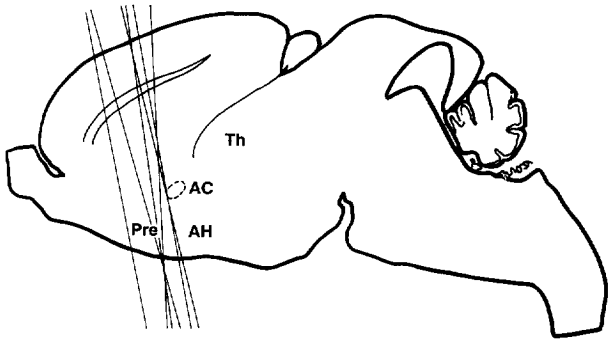

Fig. 4. A sagittal view of the brain of a 2-day-old pup with the location of the basal forebrain transections superimposed upon it. Pre, preoptic area; AH, anterior hypothalamus; Th, thalamus; AC, anterior commissure.

hypothermia anesthesia. Over the course of the recording session, however, the differential between interscapular and back-skin temperature decreased in the sham pups but remained elevated in the transected pups.

Although a combination of the time to put the post-surgical pup in the chamber and the time lag in the oxygen consumption system did not make it possible to gather reliable measurements at the 30 min time point, oxygen consumption at the other time points was elevated in the transected pups (60 min: 6.743 ± 0.661; 90 min: 6.451 ± 0.950) as compared with the sham controls (60 min: 4.503 ± 0.063; 90 min: 4.087 ± 0.206). These differences are significant (60 min: $t = 3.058$, $df = 9$, $P < 0.05$; 90 min: $t = 2.433$, $df = 10$, $P < 0.05$).

Fig. 4 presents a sagittal view of the brain of a 1-day-old pup on which the individual transections are superimposed. It can be seen that all of the transections were confined on their ventral aspect to the anterior hypothalamus and preoptic area and all of the transections were rostral to the anterior commissure. Interestingly, the most anterior of the six transections is that of the one pup that exhibited no signs of increased thermogenesis.

4. Discussion

These two experiments demonstrate that, even in newborn rats, components throughout the neuraxis contribute to the control of non-shivering thermogenesis. The first series of transections replicated and extended the results of Bignall et al. [3] by showing that there exists a system in the rostral pons or caudal midbrain of pups as young as 1 day of age that tonically inhibits heat production by BAT. These results are also consistent with transection experiments on adult rats [13,14].

That there exists an inhibitory system in the area of the midbrain/pons is not specific to thermoregulatory responses. For example, in a number of mammalian species, the response of fetuses and newborns to hypoxia is characterized by prolonged respiratory depression. Moreover, this respiratory depression can be prevented by transecting the brainstem at the junction of the midbrain and pons [8].

The newest and most surprising finding in this study is that basal forebrain transection also disinhibits BAT heat production in neonates. Although the data are not presented above, basal forebrain transection had an even more profound impact on the interscapular temperature of the 8- to 9-day-olds than on the newborns. Specifically, while interscapular temperature never exceeded 38.4°C during the 90-min test in the sham control pups, it exceeded 41°C in 3 of the 7 transected pups, 40°C in 5 of 7 pups, and 39.5°C in 6 of 7 pups.

The effect of basal forebrain transection reported here is different from the effect of hypothalamic ablation reported by Bignall et al. [3], who concluded from their experiment that the hypothalamus was not involved in thermoregulatory control in their 5-day-old subjects. As an explanation for this discrepancy between the two results, it is possible that their hypothalamic ablations destroyed an excitatory site in the hypothalamus while leaving the more caudal inhibitory site intact, as indicated by the description (and figure) of the ablation in their original report. In other words, hypothalamic ablation in the Bignall et al. [3] study produced results similar to the more rostral transections in Fig. 2 (pups 7–11).

Given the present results, it is reasonable to conclude that there exist at least two neural sites that excite BAT heat production and two neural sites that inhibit BAT heat production. The first excitatory site is likely located in the medulla or cervical spinal cord. The first inhibitory site, as described above, must be located at the junction of the midbrain and pons. The second excitatory site would then be situated between the most rostral transections in Fig. 2 and the most caudal transections in Fig. 4, that is, in the caudal to mid-hypothalamus; the presence of such a hypothalamic excitatory region would be consistent with the effects of hypothalamic stimulation on BAT thermogenesis [2,11]. The second inhibitory site is likely located in the basal forebrain, although we have little direct evidence to support this conclusion at this time other than to say that adult rats with medial preoptic lesions also exhibit increased thermogenesis [9].

The actual connectivity of these four excitatory and inhibitory sites cannot presently be discerned. Of course, it is not difficult to construct a number of diagrams involving interneurons, inhibitory and excitatory synapses, and direct and indirect connections that could explain the present results. We should note, however, that we cannot as yet rule out the possible contributions of non-neural, humoral factors in the elicitation of BAT heat production in transected pups [6,15]. We do suspect, however, that direct neural connections are involved in the phenomena reported here given that BAT is innervated at birth [5] and that newborn rats injected with tyramine, which causes the selective release of norepinephrine from nerve terminals,

exhibit increased BAT thermogenesis (Blumberg, unpublished data).

The present results suggest only some of the basic components that contribute to the modulation of BAT thermogenesis in neonates. More precise information regarding the location of these components, their connections, their interactions with other systems, and their recruitment during thermal challenge will be essential if we are to begin to understand how newborns integrate thermal information and respond to their thermal environment.

Acknowledgements

This research was supported by National Institute of Mental Health Grant MH50701 to M.S.B.

References

[1] Altman, J. and Bayer, S.A., *Atlas of Prenatal Rat Brain Development*, CRC Press, Boca Raton, FL, 1995.

[2] Amir, S., Stimulation of the paraventricular nucleus with glutamate activates interscapular brown adipose tissue thermogenesis in rats, *Brain Res.*, 508 (1990) 152–155.

[3] Bignall, K.E., Heggeness, F.W. and Palmer, J.E., Effect of neonatal decerebration on thermogenesis during starvation and cold exposure in the rat, *Exp. Neurol.*, 49 (1975) 174–188.

[4] Blumberg, M.S. and Alberts, J.R., Ultrasonic vocalizations by rat pups in the cold: An acoustic by-product of laryngeal braking? *Behav. Neurol.*, 104 (1990) 808–817.

[5] Derry, D.M. and Daniel, H., Sympathetic nerve development in the brown adipose tissue of the rat, *Can. J. Physiol. Pharmacol.*, 48 (1970) 160–168.

[6] Lempinen, M. Extra-adrenal chromaffin tissue of the rat and the effect of cortical hormones on it, *Acta Physiol. Scan.*, Vol. 62, (1964) Suppl. 231.

[7] Lipton, J.M. Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat, *Physiol. Behav.*, 3 (1968) 165–169.

[8] Martin-Body, R.L. and Johnston, B.M., Central origin of the hypoxic depression of breathing in the newborn, *Resp. Physiol.*, 71 (1988) 25–32.

[9] Nagel, J.A. and Satinoff, E., Mild cold exposure increases survival in rats with medial preoptic lesions, *Science*, 208 (1980) 301–303.

[10] Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, Orlando, FL, 1986.

[11] Perkins, M.N., Rothwell, N.J., Stock, M.J. and Stone, T.W., Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus, *Nature*, 289 (1981) 401–402.

[12] Phifer, C.B. and Terry, L.M., Use of hypothermia for general anesthesia in preweanling rodents, *Physiol. Behav.*, 38 (1986) 887–890.

[13] Rothwell, N.J., Stock, M.J. and Thexton, A.J., Decerebration activates thermogenesis in the rat, *J. Physiol.*, 342 (1983) 15–22.

[14] Shibata, M., Benzi, R.H., Seydoux, J. and Girardier, L., Hyperthermia induced by prepontine knife-cut: evidence for a tonic inhibition of non-shivering thermogenesis in anesthetized rat, *Br. Res.*, 436 (1987) 273–282.

[15] Slotkin, T.A. and Seidler, F.J., Adrenomedullary catecholamine release in the fetus and newborn: secretory mechanisms and their role in stress and survival, *J. Dev. Physiol.*, 10 (1988) 1–16.

[16] Spiers, D.E. and Adair, E.R. Ontogeny of homeothermy in the immature rat: metabolic and thermal responses, *J. Appl. Physiol.*, 60 (1986) 1190–1197.