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HYPOTHALAMIC THERMO-RESPONSIVE NEURONES IN THE NEW-BORN RAT

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SUMMARY

1. Single unit activities were recorded from the neurones in the preoptic area and anterior hypothalamus of developing new-born rats (aged 1–24 days old) during thermal stimulation of the brain. During the first 2 weeks of life, about 80 % of these neurones had low spontaneous firing rates between 0·1 and 5 impulses/sec at 38 °C hypothalamic temperature (T_{hyp}).

2. Out of 640 units studied, 118 units increased the firing rate upon elevation of T_{hyp} (warm-units) and fourteen showed the opposite type of response to temperature changes (cold-units). Warm-units were found in the rats of all the age span studied and cold-units were recorded in the rats more than 8 days old.

3. Thermal coefficients of warm-units and cold-units varied between +0·11 and +2·47 and between −0·10 and −0·49 impulses/sec. °C, respectively. Number of warm-units with higher rates of firing and greater thermal coefficients, comparable to those of warm-units in the adult, gradually increased with growth. The thermal responsiveness of warm-units, when expressed by Q_{10}, are already high even in the immediate neonatal period. Their Q_{10} values were in the range between 2 and 38·5 (mean 6·4).

4. Units responding to extrahypothalamic temperatures were only found in the rats more than 14 days old.

5. All the six warm-units tested increased the firing rates following subcutaneous injections of capsaicin, while the majority of thermo-unresponsive units were not affected by this drug.

6. It is suggested that thermo-responsive neurones in the preoptic area and anterior hypothalamus in the new-born rat have attained some degree of electrophysiological maturity, despite their slowly firing characteristics.

INTRODUCTION

Studies on thermoregulatory responses in the new-born rat have shown that some functions such as heat production responses appear at early stages of ontogeny (Taylor, 1960; Thompson & Moore, 1968), while the other functions, such as behavioural and cutaneous vasomotor responses, begin to operate later (Conklin & Heggeness, 1971; Fowler & Kellog, 1975). Since different functions may involve the

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neuronal systems that develop at different rates, the ontogenic study of the neuronal activities may yield some understanding of the central mechanisms of thermoregulation. In the adult animal, the preoptic area and anterior hypothalamus contains thermo-responsive neurones which may be involved in the central control of thermoregulation (see review, Eisenman, 1972). To our knowledge, such thermo-responsive units have not been demonstrated in the new-born animal. Henderson, Luckwill & Nayernouri (1971) failed to find such neurones in new-born rabbits 5–12 days old although some neurones in the thalamus, reticular formation and hippocampus responded to changes in brain or peripheral temperatures.

In the rat, the maturation within the hypothalamus is an entirely postnatal event (Coggeshall, 1964; Torda, 1976). In particular, the hypothalamus is one of the last structures to attain adult levels of monoamines (Loizou, 1972) and the hypothalamic aminergic system is related in some way to thermoregulation. For the study of the ontogeny of hypothalamic functions, the rat would thus seem to be a good choice.

In order to see the postnatal development of the hypothalamic neuronal systems of thermoregulation, the responses of neurones in the preoptic area and anterior hypothalamus to hypothalamic and extrahypothalamic temperatures were studied in the new-born rat. It has been known that warm-responsive neurones in this area in the adult rat are specifically excited by capsaicin (Nakayama, Suzuki, Ishikawa & Nishio, 1978), a pungent substance known to stimulate warm-detectors responsible for thermoregulation (Jancsó-Gábor, Szolcsányi & Janscó, 1970*a*, *b*; Hori & Harada, 1977). The effect of capsaicin on the neuronal activities was used as an index of the maturity of equivalent neonatal neurones. Some of the preliminary results have been reported elsewhere (Hori & Shinohara, 1977).

METHODS

Preparations. Seventy new-born rats, aged 1–24 days and weighing 5–48 g, were used. After being anaesthetized with urethane (10–20 mg/10 g, I.P.), the animal was mounted on a stereotaxic instrument and the head was held as rigidly as possible. To avoid distortion of the brain at a young age when the skull was still flexible, the ear bars were carefully pushed together until the first slight resistance was just felt. Putting small pieces of clay at both sides of the skull helped to hold the head sufficiently rigid to permit electrophysiological recording.

Before this study, a pilot study was done to establish the stereotaxic position of the preoptic area and anterior hypothalamus in 1–24 day old rats. It was found that dorsoventral growth of the brain reached almost its maximum at 10 days of age and that the relative position of this region and bregma did not change significantly during 1–24 days of age, although the anterior shift of neural structures is known to be still in progress at 5 weeks (Sherwood & Timiras, 1970). With the skull of the rat horizontal between bregma and lambda, the stereotaxic co-ordinates for the preoptic area and anterior hypothalamus were found to be 0·5 mm rostral to bregma, 0–1 mm from the mid-line and 5–6 mm (1–4 day old), 6·5–8 mm (5–9 day old) and 8–9 mm (10 day old and elder) below the surface of the skull. Conductive heating and cooling were done by means of a water-perfused thermode. The thermode tube (0·8 mm, o.d.) was implanted, 0·5 mm rostral to bregma, 2 mm from the mid-line and to a depth of 5–8 mm below the surface of the skull. Three millimetres from the thermode, a re-entrant tube containing a thermistor was implanted for the measurement of hypothalamic temperature (T_{hyp}).

Single unit recording. At the same distance from the thermode but on the contralateral side of the brain, a glass micro-electrode containing pontamine sky-blue acetate was inserted into the preoptic and anterior hypothalamic areas for recording single unit activities. The positions of

electrode tips were verified histologically by a dye-marking technique (Hellon, 1971). Recordings in which the electrode tips were observed outside the appropriate area were excluded from the data in this paper. The micro-electrode was connected to a field-effect transistor preamplifier leading to an oscilloscope and an audiomonitor. The output of the oscilloscope amplifier was fed into a band-pass filter (100–2000 Hz) and a level discriminator. The discriminator output was displayed on another beam of the oscilloscope, and was in turn led into a linear counting device set to recycle every second or every 10 sec. The counter output and hypothalamic temperature were recorded on an ink-writing oscillograph. During the experiment, the animal was placed on a water-circulating chamber made of thin metal plates (surface area of the chamber was 150 × 70 mm) so that the rectal temperature (T_{re}) and skin temperatures could be controlled. During thermal stimulation of the preoptic and anterior hypothalamic areas, the animal's rectal temperature was maintained at 38 °C.

In order to determine whether neurones in the preoptic and anterior hypothalamic areas receive the input from extrahypothalamic thermosensors, thermal stimulation was applied to the chest and abdominal surface area. While holding hypothalamic temperature at neutral levels (37–38 °C), the water temperature of the above-mentioned metal chamber was controlled so that the chest and abdominal skin temperatures were varied between 27 and 40 °C. Due to the small mass of new-born rats, thermal stimulation of skin readily caused the changes in rectal temperature and we had difficulties in determining whether the neurones responded to skin temperature or extrahypothalamic deep body temperature or both. If a unit changed its firing rate to thermal stimulation of chest and abdominal surface area, the unit was considered to respond to extrahypothalamic temperatures (T_{exhyp}) including skin and extrahypothalamic deep body temperatures.

In a limited number of neurones, the effect of capsaicin (8-methyl-6-nonenoyl-vanillylamide, Merck) was studied. A 10% solution of capsaicin was prepared as described by Jancsó-Gábor & Szolcsányi (1967) and was injected subcutaneously, in doses of 1·5–41·3 μg/10 g of body weight for the first injections and 5·7 μg–1·5 mg/10 g for the subsequent injections. A unit was considered to be responsive to capsaicin if its mean firing rate during any 5 min period within 30 min after injection was 50% greater than the mean firing rates during 5 min period before injection.

Analysis of data. The units with unstable and irregular firing and a signal-to-noise ratio of less than four were rejected for studies of thermal responses. To confirm the results, thermal responsiveness of units was examined at least twice. Two methods for characterizing the thermal responsiveness of the units were used: the thermal coefficient (the slope of thermal-response curve) (Boulant & Hardy, 1974) and the Q_{10} (Eisenman, 1972). The thermal-response curve was determined for each unit by plotting the firing rate as a function of hypothalamic temperature. Each datum point represents a mean firing rate during a 10 sec interval at which there were no abrupt changes in firing rate and temperature. The slope of linear regression (impulses/sec. °C) was taken as the thermal coefficient. The Q_{10} was calculated by the method reported previously (Eisenman & Jackson, 1967). A unit having absolute value of more than 0·1 impulses/sec. °C for thermal coefficient and 2·0 or more for Q_{10} was considered thermo-responsive. There were no units showing conflict between these two criteria. Units were considered responsive to extrahypothalamic temperature if the absolute value of their thermal coefficient exceeded 0·1 impulses/sec. °C.

RESULTS

Spontaneous firing rates of neurones in the preoptic area and anterior hypothalamus. Spontaneously firing neurones were found in the preoptic and anterior hypothalamic areas of new-born rats even on the first day of life. The firing patterns of units varied from regular to irregular. The units showing irregular and sporadic firing patterns were discarded in the present study. A total of 640 units giving regular firing were recorded for long enough to allow thermal studies. The spontaneous firing rate at a hypothalamic temperature of 38 °C was measured for each unit from a record over at least a 5 min interval. The means (± S.E.) of these firing rates in new-born rats are presented in Fig. 1 and Table 1. During the first week of life, the firing rate

at a hypothalamic temperature of 38 °C remained low, between 0·1 and 7·1 impulses/sec, and the mean value of each age group was less than 3. With advance of ages, the number of units having greater firing rates gradually increased. While the neurones having firing rates in excess of 5 impulses/sec corresponded to less than 10% of the

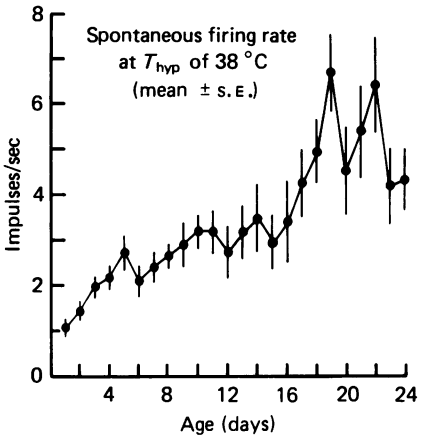


Fig. 1. Mean (± S.E.) spontaneous firing rates of preoptic and anterior hypothalamic neurones at hypothalamic temperature (T_{hyp}) of 38 °C in new-born rats at 1–24 days of age.

TABLE 1. Spontaneous firing rates of neurones in the preoptic area and anterior hypothalamus at 38 °C hypothalamic temperature (T_{hyp}) in the new-born rat

Days of age	Number of units	Mean firing rate (± S.E.) at 38 °C of T_{hyp} (impulses/sec)		Number of units having firing rates of: 5 or less (impulses/sec)	Over 5 (impulses/sec)
1–2	37	1·34 ± 0·17	$P < 0·005$	37 (100%)	0 (0%)
3–4	67	2·09 ± 0·19	n.s.	64 (95·5%)	3 (4·5%)
5–8	121	2·50 ± 0·18	n.s.	110 (90·9%)	11 (9·1%)
9–12	87	3·04 ± 0·26	n.s.	72 (82·8%)	15 (17·2%)
13–16	82	3·37 ± 0·33	$P < 0·005$	64 (78·1%)	18 (21·9%)
17–20	122	5·08 ± 0·41	n.s.	83 (68·0%)	39 (32·0%)
21–24	124	5·27 ± 0·49		81 (65·3%)	43 (34·7%)

total neurones recorded in 1–7 day old rats, the percentage increased to 34·7% in 21–24 day old rats. From Fig. 1 and Table 1, it appears that there are two critical periods for increase of mean spontaneous firing rate at 38 °C between 1–2 days and 3–4 days of age and between 13–16 and 17–20 days of age.

Thermo-responsive neurones. Out of 640 units in the preoptic and anterior hypothalamic areas, 118 units (18·5%) responded to an elevation of hypothalamic temperature with an increase in firing rate and were designated warm-units, by analogy with those described in adult mammals. Fourteen (2·2%) units showed the opposite type of response to temperature changes (cold-units). The remaining 508 units did not respond to changes of hypothalamic temperature between 32 and 42 °C.

The percentage of distribution of warm-units was only 8·8% (6 of 68) in 1–3 day old rats, but increased at the age of 4–5 days and remained almost constant (between

17·2 and 24·4 %) after 5–24 days. Combining all the data obtained from 5 to 24 day old rats, 20 % (107 of 536) of neurones in the preoptic and anterior hypothalamic areas were warm-responsive. Fig. 2 shows typical examples of the firing rate records of warm-units in the rats aged 1, 2, 4 and 6 days old. These units fired at low

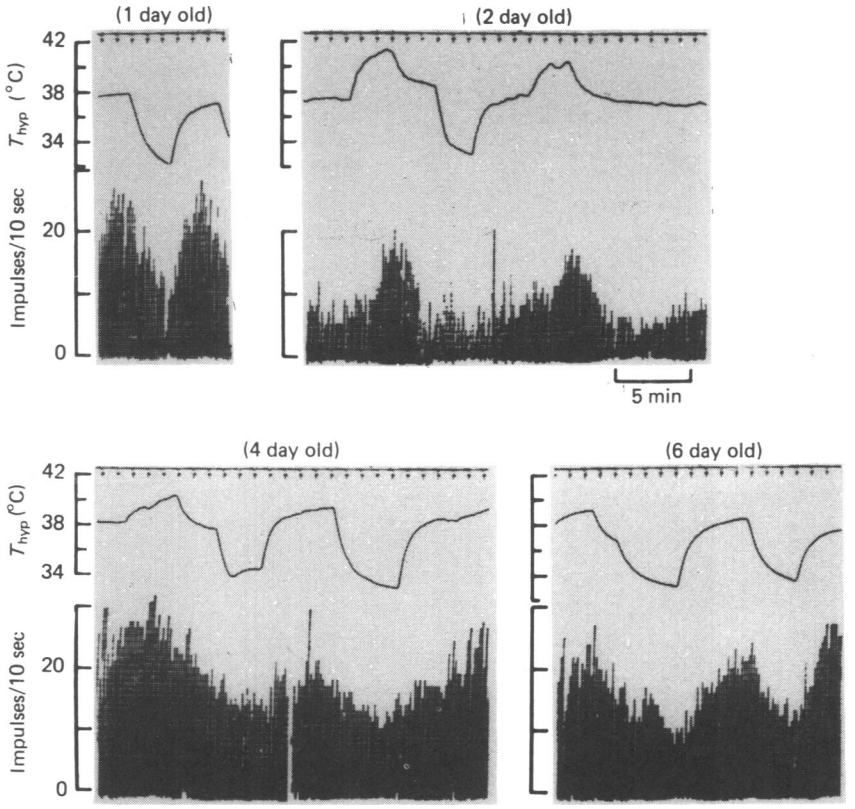
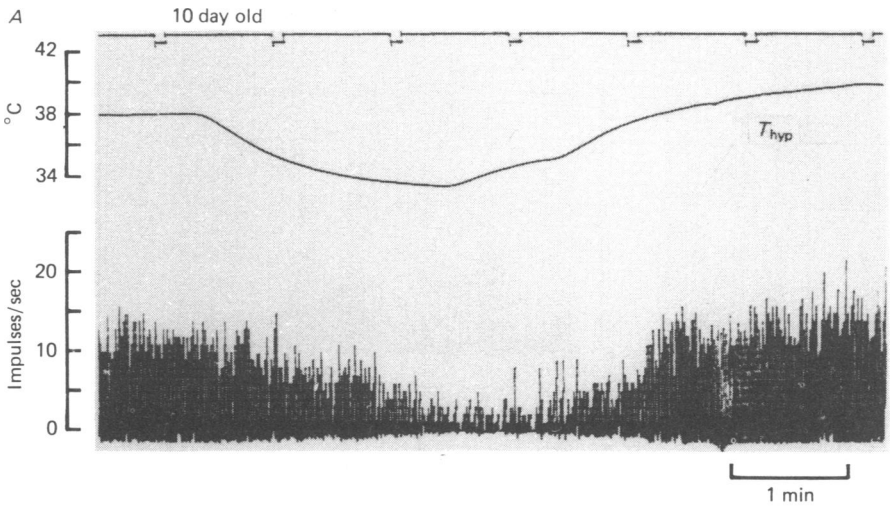


Fig. 2. Firing rate responses of warm-units to changing hypothalamic temperature (T_{hyp}) in 1, 2, 4 and 6 day old rats.

frequencies of less than 2·5 impulses/sec, but they responded consistently to changes in hypothalamic temperature. The temperature coefficients of these units were 0·33, 0·15, 0·19 and 0·21 impulses/sec. °C for 1, 2, 4 and 6 day old rats, respectively. Their Q_{10} was greater than 2, ranging between 3·2 and 10·2. In the older pups, we more frequently found warm-units having higher firing rates and temperature coefficients greater than +1 impulses/sec. °C, values which have been commonly observed in the adult mammals. Example records of such 'adult-like' units found in 10 and 18 day old rats are shown in Figs. 3 and 4, respectively. A warm-unit in a 10 day old rat (Fig. 3) had a temperature coefficient of +2·3 impulses/sec. °C and a Q_{10} of 24·3. Two warm-units shown in Fig. 4*C*, *D* were recorded in the same animal. One unit (Fig. 4*C*) had low spontaneous firing rate at 38 °C of less than 5 impulses/sec, while the unit shown in Fig. 4*D* had a high rate of 23·2 impulses/sec. Even in the 24 day old rats, slowly firing warm-units were frequently found among adult-like rapidly firing warm-units. In the rats of 21–24

days of age, 55·6% (15 of 27) of warm-units had firing rates at 38 °C of less than 5 impulses/sec, while in the adult rat and guinea-pig the percentage of such slowly firing units was only 10·3% (4 of 39) (Boulant & Bignall, 1973).

It has previously been pointed out that warm-units in the preoptic and anterior



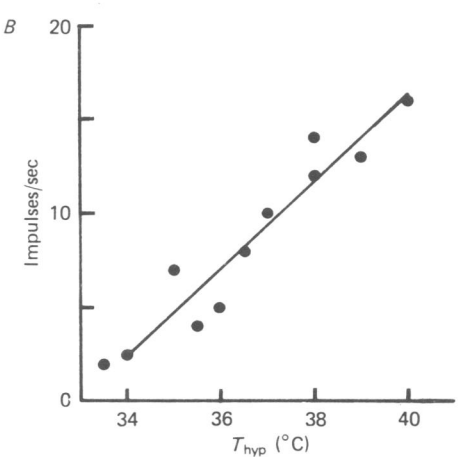


Fig. 3. Firing rate responses (*A*) and thermal response curve (*B*) of an 'adult-like' warm-unit in a 10 day old rat. Some data points in *B* were obtained from the records not shown in *A*.

hypothalamic areas are more readily found among those units having higher spontaneous firing rates (Boulant & Bignall, 1973). In the new-born rats, too, 30·2% (39 of 129) of units having firing rates at 38 °C greater than 5 impulses/sec were warm-responsive, while the percentage of warm-units in the units having firing rates at 38 °C of 5 impulses/sec or less was only 15·5% (79 of 511). This relationship was observed in all age groups studied.

No cold-units were found in the rats younger than 1 week of age. The numbers of cold-units did not increase with age and even in 21–24 day old rats the percentage of cold-units recorded was only 4 % (5 of 124). Example records of cold-units are shown in Fig. 5. The firing rates at 38 °C of cold-units were low (between 0·48 and 4·58 impulses/sec; mean 1·91). Since cold-units in the adult have generally low firing

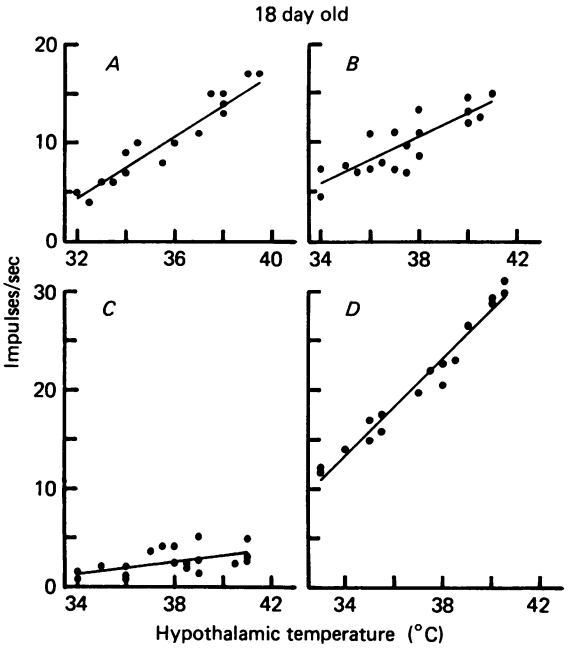


Fig. 4. Thermal response curves of warm-units in 18 day old rats. Units *A* and *B* were recorded from different animals and Units *C* and *D* from the same animal.

rates, very small increases in firing rates of cold-units during ontogeny might be undetected.

Thermo-responsiveness of neurones in preoptic and anterior hypothalamic areas. Thermal response curves for all the warm- and cold-units are shown in Fig. 6. Almost all of them responded linearly to hypothalamic temperature over a range between 34 and 42 °C. Two warm-units found in 19 and 23 day old rats responded to temperatures over 37 °C but not to temperatures in the hypothermic range. In the remaining units, no clear inflexion point was found in the thermal response curves. Summarized data of temperature coefficients and Q_{10} are presented in Table 2. The number of units having greater temperature coefficients appeared to increase with growth. However, the temperature coefficients of warm-units even in 24 day old rats were still low in the range between +0·22 and +2·2 impulses/sec. °C with the average of $+0{\cdot}71 \pm 0{\cdot}17$ (S.E.), which is significantly lower than those of warm-units recorded in the adult mammals. On the other hand, thermo-responsiveness of preoptic and anterior hypothalamic neurones in the new-born rat, when expressed in Q_{10}, are comparable to those of warm-units in the adult. The Q_{10} of a warm-unit found in a 1 day old rat was already high (2·5) and those of warm-units in 1–4 day old rats were in the range between 2·03 and 12·75.

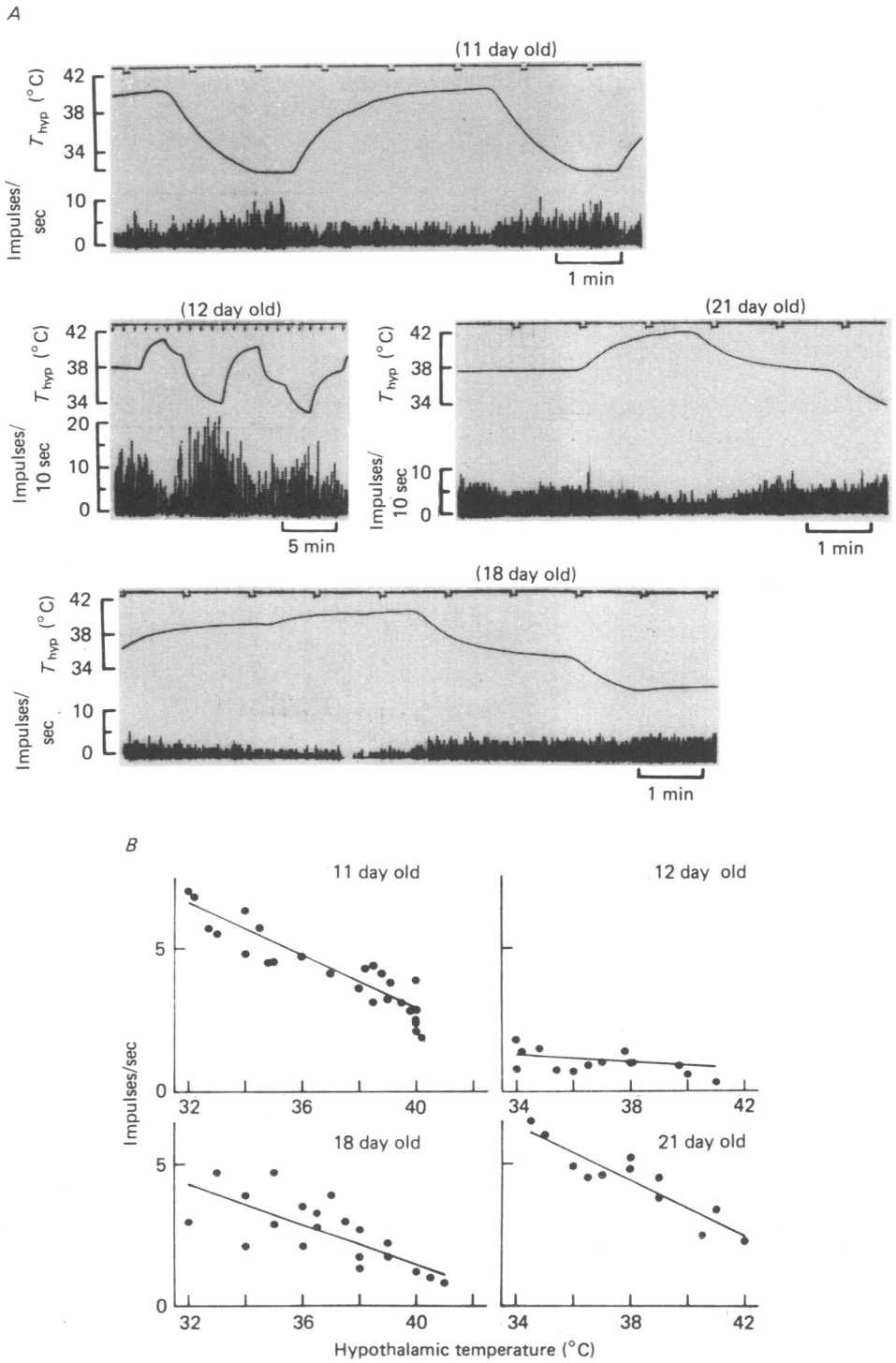


Fig. 5. Firing rate responses (*A*) and thermal response curves (*B*) of cold-units in 11, 12, 18 and 21 day old rats. Some data points in *B* were obtained from the records not shown in *A*.

TABLE 2. Thermal responsiveness of thermo-responsive neurones

		Warm-units					Cold-units	
		Number of units	Thermal coefficients (impulses/sec. °C)		Q_{10}		No. of units	
Days of age	Total no. of units	(%)	Mean ± S.D.	Range	Mean ± S.D.	Range	(%)	Thermal coefficients (impulses/sec. °C)
1–4	104	11 (10·6)	0·26 ± 0·08	0·15–0·42	5·57 ± 3·85	2·03–12·75	0 (0)	
5–8	121	21 (17·4)	0·32 ± 0·05	0·12–1·04	4·33 ± 3·75	2·08–18·74	2 (1·7)	−0·18, −0·26
9–12	87	18 (20·7)	0·73 ± 0·50	0·16–2·31	9·38 ± 7·97	3·00–24·30	4 (4·6)	−0·14, −0·10, −0·45, −0·10
13–16	82	20 (24·4)	0·39 ± 0·16	0·10–0·69	4·74 ± 4·26	2·03–15·95	1 (1·2)	−0·31
17–20	122	21 (17·2)	0·78 ± 0·58	0·15–2·47	7·12 ± 8·31	2·06–36·50	2 (1·6)	−0·34, −0·27
21–24	124	27 (21·8)	0·65 ± 0·47	0·11–2·17	7·07 ± 8·77	2·01–38·52	5 (4·0)	−0·12, −0·49, −0·22, −0·35, −0·43
Total	640	118 (18·5)	0·55 ± 0·45		6·38 ± 6·84		14 (2·2)	Mean ± S.D. −0·27 ± 0·13

The temperature coefficients of cold-units also tended to increase with growth, although there was no statistical difference ($P > 0{\cdot}1$) in the mean temperature coefficient of cold-units between 8–13 day old rats ($-0{\cdot}22 \pm 0{\cdot}05$ (S.E.) impulses/sec. °C) and 18–24 day old rats ($-0{\cdot}32 \pm 0{\cdot}05$).

Responses of preoptic and anterior hypothalamic neurones to extrahypothalamic temperatures. Forty-nine units recorded in 2–24 day old rats were studied for their

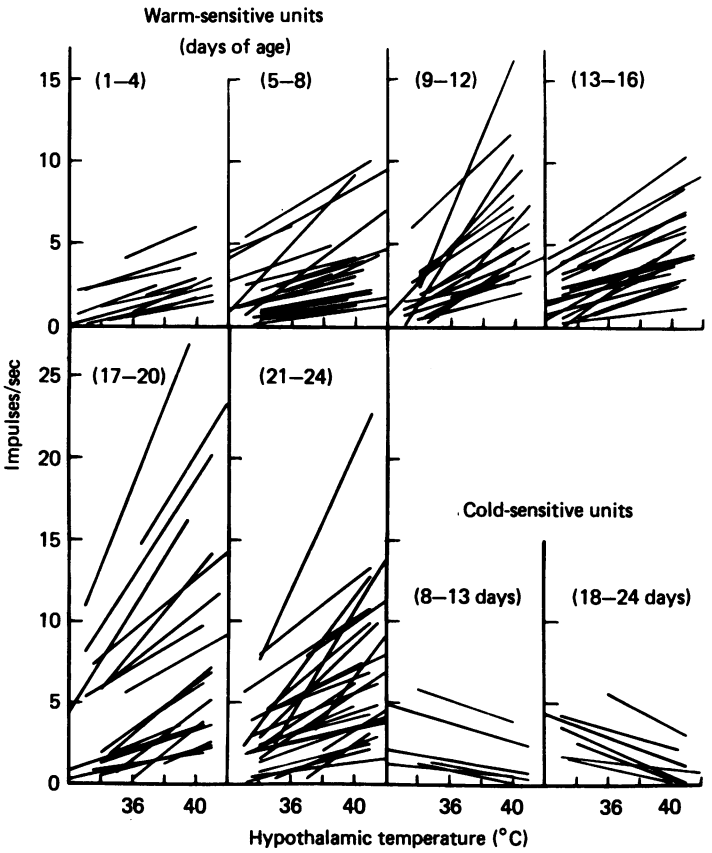


Fig. 6. Thermal response curves of thermo-responsive units in preoptic and anterior hypothalamic areas.

thermal responsiveness to changes in extrahypothalamic temperatures (T_{exhyp}) while holding hypothalamic temperature at neutral levels (37–39 °C). To do this, it was necessary to cool and warm the chest and abdominal surface area for long enough to demonstrate reliable changes in firing rates. As described in Methods, thermal stimulation of skin for more than 5 min usually resulted in changes in extrahypothalamic deep body temperature, as evidenced by changes in rectal temperature (T_{re}). Rectal temperature seldom fell below 34 °C in the present study, while core temperatures as low as 32 °C are not considered unphysiological in the new-born rat. According to Conklin & Heggeness (1971), high rates of oxygen consumption were maintained in the new-born rat with a core temperature as low as 30 °C at which metabolic activity is inhibited in the adult. Despite these temperature extremes, a

majority of preoptic and anterior hypothalamic units (39 of 49) did not change their firing rates during these temperature changes.

Fig. 7 demonstrates the examples of neurones which were responsive to changes in extrahypothalamic temperature. The units A and B responded to hypothalamic temperature changes as warm-units while the unit C was unresponsive to such

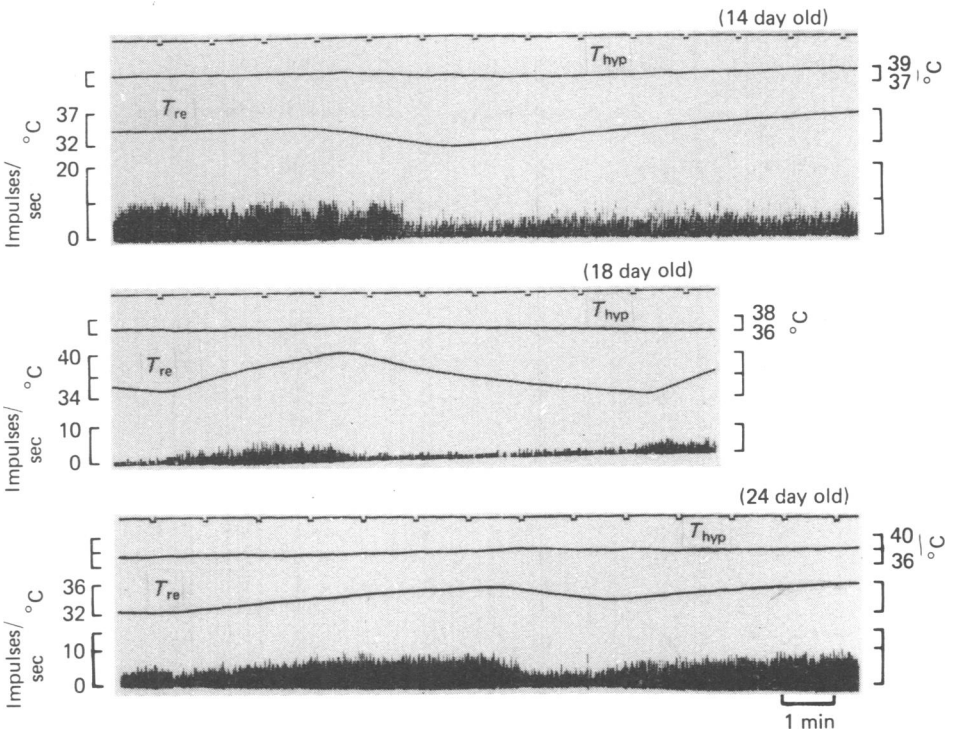


Fig. 7. Firing rate responses of units in preoptic and anterior hypothalamic areas responding to extrahypothalamic temperatures. T_{re}, rectal temperature. *A* and *B* respond to hypothalamic temperature change as warm-units. *C*, unit unresponsive to hypothalamic temperature change.

changes. Unit A was considered responsive to extrahypothalamic deep body temperature but not to skin temperature. On the other hand, units B and C altered their firing rates as soon as the skin temperature changed, but when the skin temperature reached a new steady level the firing rates of the units still continued to change. Therefore, these two units were taken to respond to both skin and extrahypothalamic deep body temperatures.

Presented in Table 3 are the summarized results on the responses of forty-nine units to T_{exhyp}. Six units including two warm-units and one cold-unit in 2, 4, 8, 9 and 10 day old rats did not respond to extrahypothalamic temperature change. Units responsive to extrahypothalamic temperature change were only found in the rats older than 14 day old. Out of forty-nine units, nine units responded with positive temperature coefficients and one with a negative coefficient. Of six units showing thermal responsiveness to both hypothalamic and extrahypothalamic temperatures, four units responded to both with the same type (positive) of temperature coefficient

and two units had different types of coefficients. Although the number of units studied was small, this is consistent with previous findings in adult mammals that the majority of central neurones having dual thermal responsiveness respond to both temperatures with same type of temperature coefficient (Boulant & Hardy, 1974; Hori & Harada, 1976).

TABLE 3. Responses of the preoptic and anterior hypothalamic neurones to hypothalamic (T_{hyp}) and extrahypothalamic temperatures (T_{exhyp})

Days of age	No. of units	Responses to T_{hyp}			Responses to T_{exhyp}		
		None	+	−	None	+	−
2	2	2	0	0	2	0	0
4	1	0	1	0	1	0	0
8	1	0	0	1	1	0	0
9	1	0	1	0	1	0	0
10	1	1	0	0	1	0	0
14	9	5	4	0	8	1	0
15	3	2	1	0	3	0	0
16	4	4	0	0	3	1	0
17	2	1	1	0	1	0	1
18	7	6	1	0	5	2	0
19	3	2	1	0	2	1	0
21	4	3	1	0	3	1	0
22	5	3	2	0	4	1	0
23	1	0	0	1	1	0	0
24	5	2	2	1	3	2	0
Total	49	31	15	3	39	9	1

Responses of preoptic and anterior hypothalamic neurones to capsaicin. Six warm-units and ten thermally unresponsive units found in 2–24 day old rats were studied for their responsiveness to subcutaneous injection of capsaicin (1·5–41·3 μg/10 g body wt.). The results are summarized in Table 4. All the six warm-units found in rats aged 2, 6, 8, 14 and 22 days increased their firing rates in response to capsaicin injection. A typical example of the firing rate responses of a warm-unit (Table 4, unit 5) to capsaicin is shown in Fig. 8. The unit increased its firing rate to a maximum of more than 4 times basal during the period 4–36 min after injection. When the same dose of capsaicin was subsequently given 65 min after the first injection, the unit again showed an excitatory response but less in magnitude and for a shorter period. The two warm-units (units 2 and 6, Table 4) decreased their firing rates initially for periods of 2–12 min and 4–15 min respectively, and then increased them to a maximum of 250% of the original level.

On the other hand, only two (units 10 and 15, Table 4) of the ten thermally unresponsive units responded to capsaicin, one with a decrease and the other with variable changes in firing rate. The firing rate of unit 10 decreased 11 min after capsaicin injection and did not return to the preinjection level for the observation period of 60 min. Unit 15 was studied for the responses to repeated injection of capsaicin with increasing doses. In response to the first injection (1·5 μg/10 g), the unit initially decreased and then increased its firing rate. However, the unit did not respond to the

TABLE 4. The effect of capsaicin on the firing rate of warm-units (W) and thermally unresponsive (no) units. ↑, increase in firing rate; ↓, decrease in firing rate; and no, no response. Number in parentheses indicates the period at which the response occurred. Units 5, 13, 15 and 16 were studied more than twice.

Unit no.	Days of age	Type of unit	Dose of capsaicin (μg/10 g body wt.)	Responses of units to capsaicin	Periods of observation (min)
1	2	W	14·5	↑ (6 min–)	11
2	6	W	8·2	↓ (2–12 min)–↑ (13 min–)	15
3	6	W	7·1	↑ (5 min–)	12
4	8	W	6·7	↑ (3 min–)	7
5	14	W	4·3; 4·3	↑ (4–36 min); ↑ (4–27 min)	65, 62
6	22	W	2·7	↓ (4–15 min)–↑ (16 min–)	22
7	2	no	13·9	no	40
8	5	no	41·3	no	75
9	5	no	41·3	no	43
10	8	no	5·7	↓ (11 min–)	60
11	10	no	4·9	no	40
12	14	no	4·3	no	12
13	17	no	2·9; 2·9; 5·8	no; no; no	43, 66, 28
14	20	no	2·2	no	12
15	24	no	1·5; 3·0; 150	↓ (5–17 min)–↑ (19–80 min); no; ↓ (1–5 min)	87, 28, 30
16	24	no	8·3; 8·3	no; no	26, 29

second injection (3 μg/10 g). This seems to indicate a desensitization of the cell to the first injection of capsaicin (Jancsó-Gábor *et al.* 1970*b*). In fact, even 100 times the initial dose of capsaicin on the third injection elicited only a slight inhibition of the unit activity for a short period (1–5 min). The remaining eight units were not affected

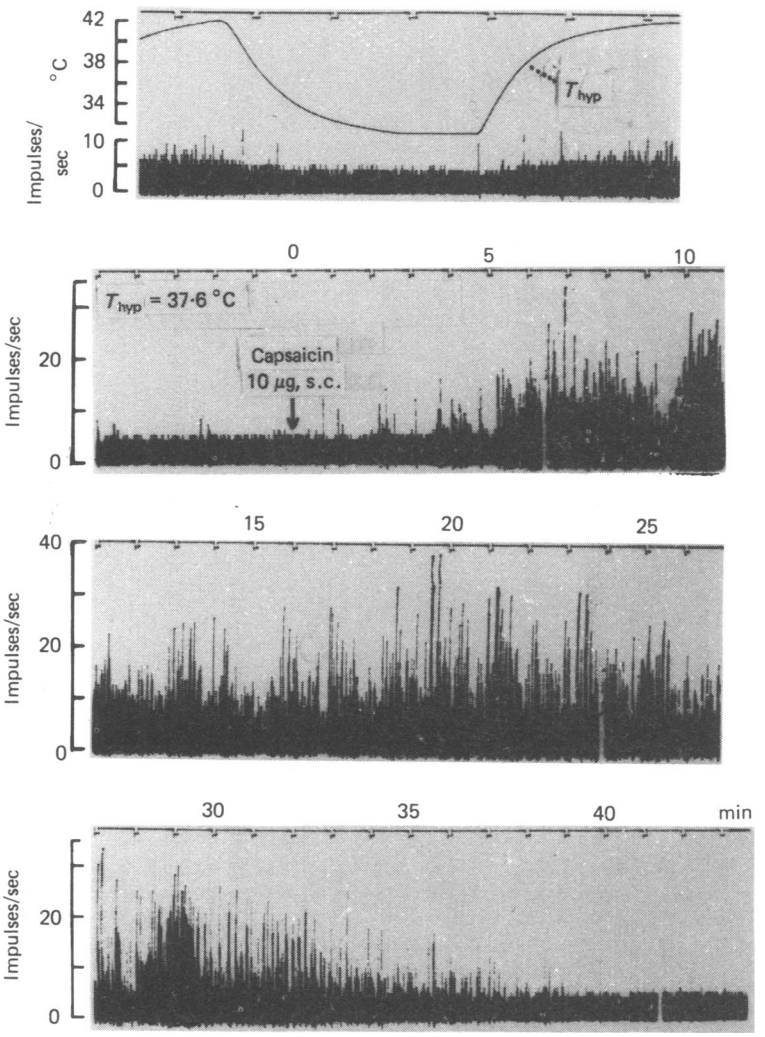


Fig. 8. Effect of capsaicin on the firing rate of a warm-unit in a 10 day old rat. Upper panel, responses to changing hypothalamic temperature (T_{hyp}). Capsaicin was injected at an arrow, while holding hypothalamic temperature at 37·6 °C.

by capsaicin. Thus, capsaicin excited warm-units in the preoptic and anterior hypothalamic areas in the new-born rat, but the majority of thermally unresponsive units were not affected. This finding is in good agreement with that of Nakayama *et al.* (1978) in the adult rat.

DISCUSSION

Spontaneous firing rates of preoptic and anterior hypothalamic neurones. The present study demonstrates a gradual increase in spontaneous firing rates of preoptic and anterior hypothalamic neurones in the rat during postnatal period. It has also been shown in other species and in other neural areas that ontogeny is characterized by an increase in firing rates (Hyvarinen, 1966; Deza & Eidelberg, 1967; Huttenlocher, 1967; Henderson *et al.* 1971), although the spontaneous firing rates of lateral hypothalamic neurones in 8–21 day old rats have already reached characteristically low adult levels (Almli, McMullen & Golden, 1976). According to Boulant & Bignall (1973), 62·8% (79 of 113) of preoptic and anterior hypothalamic neurones in the adult rat and guinea-pig have spontaneous firing rates in excess of 5 impulses/sec. Although the proportion of neurones having spontaneous firing rates at 38 °C over 5 impulses/sec increases in number with age in new-born rats, their percentage in the total population of neurones is still only 34·7% at 21–24 days of age. This indicates that the activities of some preoptic and anterior hypothalamic neurones in new-born rats have not reached the adult level even at 3 weeks of age.

The events which determine the increase in firing rates of preoptic and anterior hypothalamic neurones during the postnatal period are not known. The evidence presently available suggests at least two factors. One is the development of the metabolic processes in the neurones necessary for the rapid restoration of membrane potentials (Huttenlocher, 1967). Although the final cell divisions in the medial preoptic area in the rat occur prenatally (Ifft, 1972), cytological development is an entirely postnatal event. In particular, most cells do not exhibit any cytological features suggestive of active neuronal metabolism during the first 5 days after birth (Reier, Cullen, Froelich & Rothchild, 1977). A correlation between electrical activity and biochemical development appears to exist in the cerebral cortex of neonatal cat (Huttenlocher, 1967) and rat (Himwich, 1962). Another factor is the increase in the synaptic connexions of neurones in the preoptic and anterior hypothalamic areas, including connexions among these neurones and between these neurones and the periphery. Since developmental changes in dendritic arborization and in electrical activity coincide in time (Huttenlocher, 1967), the sparsity of excitatory synaptic input may explain, at least in part, the slow firing rates of preoptic and anterior hypothalamic neurones in the young rat. Dendritic growth in the rat brain begins after 6 days of age and becomes rapid after 12 days (Himwich, 1962). The number of synapses per unit area is relatively small in the medial preoptic area of 1–5 day old rats, but it increases rapidly during 10–15 days and by 25 days adult levels are reached (Reier *et al.* 1977). Myelination of the fibre tracts in the central nervous system is almost non-existent in the 10 day old rat and develops between 21 and 39 days of age, but adult levels are not reached until 39 days of age (Sherwood & Timiras, 1970). Proliferation of monoamine-containing nerve terminals in the hypothalamus is still in progress in the rat at 3 weeks of age (Loizou, 1972). Poor connexions between hypothalamic neurones and the periphery have also been demonstrated electrophysiologically in the lateral hypothalamus (Almli *et al.* 1976) and in the preoptic area and anterior hypothalamus in the present study. These facts are compatible with the present finding that more than half of the preoptic and anterior hypothalamic

neurones even at 3 weeks of age still have spontaneous firing rates at 38 °C of less than 5 impulses/sec.

The two critical periods in the increase of mean firing rate observed in the present study correlated approximately with the morphological, biochemical and electrophysiological developments of the brain. The first critical period (2 and 3 days of age) coincides with the time at which biochemical development and cortical differentiation have just started and strychnine spikes can appear in the cortex. As stated above, the number of warm-units started to increase at 4–5 days. The second period (16 and 17 days) corresponds to the time immediately after the so-called 'critical period' (4–10 days) (Himwich, 1962; Reier *et al.* 1977). This period is characterized by the appearance of cytological features suggesting elevated metabolism, rapid growth of dendrites and high rate of synaptogenesis in the medial preoptic area (Reier *et al.* 1977). Biochemically, there is a rapid accumulation of protein, amino acids and development of enzymatic activities in the brain. Electrophysiologically, this is the time when the mature type electroencephalogram appears and auditory evoked potential can be recorded (Himwich, 1962).

Thermal responsiveness of preoptic and anterior hypothalamic neurones. In the present study, about 20 % of neurones responded to change in hypothalamic temperature with positive temperature coefficients in 5–24 day old rats. Similar results have been obtained in the urethane-anaesthetized adult rat: 23 % by Jahns & Werner (1974) and 22·5 % by Boulant & Bignall (1973). Studies in other adult mammals also indicate more or less similar proportions of warm-units in the preoptic and anterior hypothalamic areas (Nakayama, Hammel, Hardy & Eisenman, 1963; Hardy, Hellon & Sutherland, 1964; Eisenman & Jackson, 1967; Guieu & Hardy, 1970; Hori & Nakayama, 1973; Boulant & Hardy, 1974). On the other hand, the percentage of cold-units in the new-born rat was very small, at most only 4 % in 21–24 day old rats, whereas those of adult rat have been described as being 13 % (Jahns & Werner, 1974) and 20 % (Boulant & Bignall, 1973). It had been previously proposed that the cold-units were interneurones and their thermal responsiveness was brought about by the inhibitory inputs from nearby warm-units (Boulant & Hardy, 1974). If this suggestion is right, poor development of synapses in the preoptic and anterior hypothalamic areas of new-born rat may explain the sparsity and the late appearance of cold-units during ontogeny.

The mean value of temperature coefficients of warm-units and the proportion of units having higher temperature coefficients tended to increase with age in the new-born rat. However, the mean temperature coefficients of warm-units even in 21–24 day old rats ($+0{\cdot}65 \pm 0{\cdot}47$ (S.D.) impulses/sec. °C) did not reach adult levels, which are +4·2 impulses/sec. °C in the cat (Nayakama *et al.* 1963), +7 in the dog (Hardy *et al.* 1964), +4·4 in the rabbit (Hori & Nayakama, 1973), +1·68 in the rabbit (Boulant & Hardy, 1974) and +3·2 in the rat (T. Hori & K. Shinohara, unpublished observation). Thus, the temperature coefficients of warm-units in the new-born rat are about one tenth of those of warm-units in adult mammals. Rather, they are comparable to those of warm-units found in the preoptic and anterior hypothalamic areas of the Australian blue-tongued skink (Cabanac, Hammel & Hardy, 1967), which were between +0·09 and +1·0 impulses/sec. °C. These reptilian units also

had characteristically low spontaneous firing rates. On the other hand, the Q_{10} of warm-units in the new-born rat even at the immediate neonatal period are comparable to those of the units in the adult mammals. There is disagreement about how best to describe the thermal responsiveness of neurones (Guieu & Hardy, 1970; Eisenman, 1972). As pointed out by Guieu & Hardy (1970), the Q_{10} designation tends to favour the slowly firing units. On the other hand, the temperature coefficient expression is apparently not suitable for comparison of thermal responsiveness of units having quite different basal rates of firing. The spontaneous firing rate at 38 °C of warm-units in the new-born rat varied from 0·7 to 23·2 impulses/sec with a mean of 4·7 ± 3·7 (S.D.), while about 90 % of warm-units in the adult rat have spontaneous firing rates over 5 impulses/sec (Boulant & Bignall, 1973). Thus, it is suggested that the Q_{10} expression is more suitable than the temperature coefficient for comparative study of the thermal responsiveness of units in the new-born and adult rats. Thermal responsiveness of warm-units in the new-born rat are thus similar to those of units in the adult mammals, when expressed as Q_{10}.

Temperature coefficients of cold-responsive neurones in the new-born rat were in the range between −0·1 and −0·49 impulses/sec. °C, with the average of −0·27 ± 0·13 (S.D.). In adult mammals, slightly greater values for temperature coefficients of cold-units have been reported: −1 impulses/sec. °C in the dog (Hardy *et al.* 1964), −2·0 in the rabbit (Hori & Nakayama, 1973) and −1·68 (Boulant & Hardy, 1974) in the rabbit. Again, temperature coefficients of cold-units in the new-born rat are similar to those of units in the skink, which ranged between −0·26 and −0·7 impulses/sec. °C (Cabanac *et al.* 1967).

Henderson *et al.* (1971) have reported the absence of thermo-responsive neurones in preoptic and anterior hypothalamic areas of 5–12 day old rabbits. This may indicate the slower maturation of hypothalamic neurones in the young rabbit. Indeed, it has been pointed out that the morphological, biochemical and neurophysiological developments of whole brain of the rabbit take up a longer period of time than those of the rat (Himwich, 1962).

Responses of preoptic and anterior hypothalamic neurones to extrahypothalamic temperatures. In the present study, the percentages of units responding to changes in extrahypothalamic temperature were 20·4 % (10 of 49) in 2–24 day old rats and 23·3 % (10 of 43) in 14–24 day old rats. These figures are comparable to those of units in the adult rabbit (29·4 %, 40 of 136) (Boulant & Hardy, 1974), but they are far smaller than those of hypothalamic units responding to scrotal skin temperature in the adult rat (74·6 %, 94 of 126) (Ishikawa & Nakayama, 1977). This may reflect the immature properties of hypothalamic neurones in the new-born rat with respect to connexions between the hypothalamus and peripheral thermoreceptors.

Thus, it appears that neurones in the preoptic and anterior hypothalamic areas of the rat are already endowed with the ability to respond to local temperature at birth, while responsiveness to peripheral temperature develops later, probably beginning at about 10–14 days of age. The late appearance of neuronal responsiveness to peripheral inputs in ontogeny has been observed by others. No units in the lateral hypothalamus responded to pain stimulation applied to the skin in 8–21 day old rats, despite their adult-like characteristics of local osmosensitivity (Almli *et al.* 1976).

In the new-born rabbit, thalamic neurones responding to peripheral temperature were not recorded until 9 days of age (Henderson *et al.* 1971).

Responses of preoptic and anterior hypothalamic neurones to capsaicin. The present results indicate that all the neonatal warm-units examined responded to capsaicin, while thermally unresponsive units did not. Even a warm-unit found in a rat as young as 2 day old responded to capsaicin. The finding suggests another similarity in properties between new-born and adult neurones. The responses to capsaicin, i.e., increased activities of warm-units and decreased activities of cold-units in the adult rat (Nakayama *et al.* 1978), are appropriate to cause the hypothermia observed in the unanaesthetized rat when capsaicin is given systemically or into the preoptic and anterior hypothalamic areas (Jancsó-Gábor *et al.* 1970*a*, *b*; Hori & Harada, 1977). Since the warm-units in the new-born rat are facilitated by capsaicin, direct injection of capsaicin into the hypothalamus may be expected to produce hypothermia in this animal. In fact, we observed hypothermic responses in the new-born rat, when capsaicin was given either intrahypothalamically or subcutaneously (T. Hori & S. Tsuzuki, unpublished observation).

It has been reported that serial injections of increasing doses of capsaicin resulted in decreased responsiveness, the animals finally becoming completely unresponsive (desensitization). These capsaicin-desensitized rats had permanent impairment of both autonomic (Jancsó-Gábor *et al.* 1970*a*) and behavioural (Hori & Harada, 1977) responses to heat. Based on the results obtained from physiological and morphological studies, previous workers (Jancsó-Gábor *et al.* 1970*a*, *b*) attributed the thermoregulatory deficits of capsaicin-desensitized rats to the dysfunction of warm-receptors located in the hypothalamus and peripheral tissues. Quite recently, we have observed that rats which had been desensitized by capsaicin in the early postnatal period showed the same deficiency in heat-defense responses when they became adults (T. Hori, S. Tsuzuki & K. Shinohara, unpublished observation).

The maturity of preoptic and anterior hypothalamic thermo-responsive neurones in the new-born rat. The striking similarities between the thermo-responsive neurones in new-born and adult rats, in terms of neurone population, Q_{10}, responsiveness to peripheral temperatures and responsiveness to capsaicin, may suggest some degree of electrophysiological maturity of neurones in preoptic and anterior hypothalamic areas in the new-born rat, despite their slowly firing characteristics. It is suggested that these slowly firing thermo-responsive neurones may develop into the thermo-responsive neurones with higher firing rates in the adult. At present, there is little data on the ontogeny of hypothalamic thermoregulatory function in the new-born rat, which can be correlated with the present electrophysiological findings. All that is available is our own unpublished finding on the hypothermic responses to intrahypothalamic capsaicin. This suggests that the preoptic area and anterior hypothalamus in the new-born rat contain the neural structures which lower the body temperature in response to capsaicin. One can imagine that at least some of these capsaicin-responsive structures are the slowly firing warm-units which are assumed to develop into the warm-units of the adult.

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