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COMPLEMENTARY LOWERING OF THE BEHAVIOURAL AND PHYSIOLOGICAL THERMOREGULATORY SET-POINTS BY TETRODOTOXIN AND SAXITOXIN IN THE CAT

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SUMMARY

1. Injections of tetrodotoxin or saxitoxin (25 ng in 0·10 ml.) into a lateral cerebral ventricle caused deep body temperature of cats to fall approximately the same amount (2° C) whether the animals were resting in the cold (4° C) or were responding to escape heat.

2. Continuous exposure to heat either prevented the hypothermic response or enhanced the level of tachypnoea required to lower body temperature. Tetrodotoxin also caused hypothermia when an animal was lever pressing to obtain heat in the cold environment.

3. These results provide evidence that agents which alter the set-point for physiological thermoregulatory activity produce a complementary shift in the behavioural set-point as well.

INTRODUCTION

Elevation of the set-point for physiological thermoregulatory responses by pyrogens is accompanied by behavioural responses which facilitate the development of fever (Weiss, Laties & Weiss, 1967; Gale, Mathews & Young, 1970; Cabanac, Duclaux & Gillet, 1970). However, no studies have been reported in which thermoregulatory behaviour has been examined after administration of an agent which lowers the physiological set-point. Tetrodotoxin has been shown to produce hypothermic responses characterized by (1) activation of heat-loss mechanisms at environmental temperatures above as well as below the thermoneutral temperature, (2) the absence of compensatory shivering during development of hypothermia and (3) the persistence of the ability to regulate against superimposed thermal stresses (Borison, McCarthy, Clark & Radhakrishnan, 1963; Clark & Coldwell, 1973). These characteristics suggest that tetrodo-

toxin lowers the set-point for physiological thermoregulation. Saxitoxin, the agent responsible for paralytic shellfish poisoning (Kao, 1966), is thought to act by the same cellular mechanism as tetrodotoxin (Kao, 1972; Narahashi, 1972). The results of the experiments described below indicate that both tetrodotoxin and saxitoxin cause complementary shifts in behavioural and physiological thermoregulatory set-points.

METHODS

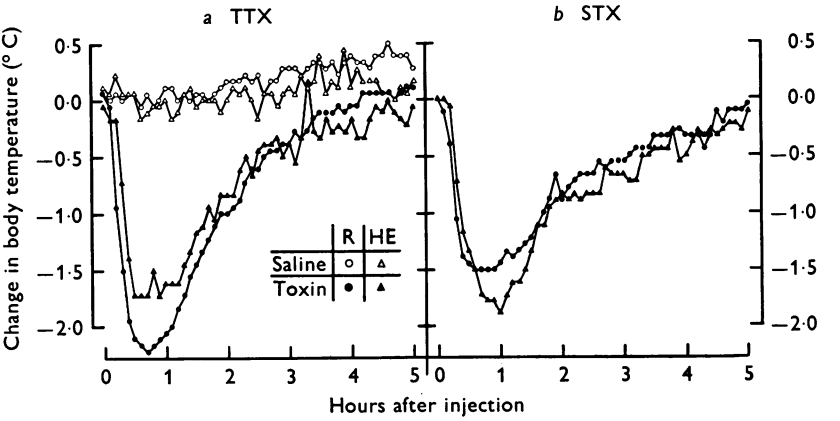
Procedures for care and feeding of adult mongrel cats, for making injections into a lateral cerebral ventricle, for automatically recording body temperature from the retroperitoneal space (T_{rp}) and for avoiding contamination by pyrogens have been described previously (Clark & Moyer, 1972; McCarthy & Borison, 1966).

The behavioural apparatus (Plate 1) consisted of a stainless-steel cage 61 cm long, 41 cm wide and 33 cm high over which was suspended, at a height adjusted between 10 and 22 cm, a battery of six 250 W infra-red heat lamps and a small fan (450 cfm). This arrangement differed from that of Weiss *et al.* (1967) primarily in that the cats were free to move anywhere within the cage. Depression of a lever turned off the heat lamps and activated the fan. The lamps remained off and the fan remained on for as long as the lever was held down. The total length of time the lamps were on during successive 30 min periods was noted from cumulative time meters. The pattern of lever pressing was recorded simultaneously with the animal's temperature on the same chart.

Initially the cats were trained to escape heat at a room temperature of about 22° C. With the lamps set at an intermediate height, between eight and fifteen sessions of 15–120 min were required before the animals could safely be allowed to respond without assistance. After several more unassisted sessions, the animals depressed the lever approximately 90 % of the time. Subsequent training sessions, during which the height of the lamp-fan assembly was adjusted until a position was found for each cat at which it pressed the lever between 40 and 70 % of the time, and all further experiments were carried out at an environmental temperature of $4 \pm 1°$ C. Once training was completed, each cat was given a series of four trials in a randomly determined order. In two of these trials ('resting' trials) the behavioural apparatus was not turned on. The cat was simply placed in the cage, and its T_{rp} was recorded. In the other two trials, termed 'heat-escape' trials, the behavioural apparatus was operative. A given cat was placed in the cage at the same time of day on each trial with the lamp-fan assembly in the position determined during the training sessions. One hour later, the animal was given an intraventricular injection (0·10 ml.) of either tetrodotoxin or saline solution. Thus the four trials consisted of all combinations of tetrodotoxin or saline injection with resting or heat-escape behaviour. The average of T_{rp}'s recorded at 6 min intervals during the half-hour period immediately before injection was taken as the base line from which changes in T_{rp} were measured. The total time spent lever pressing during this same period was used as the base line for changes in behaviour. The experiment was terminated 5 hr after the injection, and the ventricular cannula was flushed with saline solution if tetrodotoxin had been given. After tabulating the changes in T_{rp} at 6 min intervals from the time of injection until the end of the experiment, a thermal response index (TRI) was determined with a calculator (Clark & Coldwell, 1973) such that one unit of TRI is equivalent to a 1° C change lasting for 1 hr. Trials were spaced at least 48 hr apart, and the interval between tetrodotoxin injections was at least 7 days to avoid possible development of tolerance. The dose of tetrodotoxin used (25 ng) was chosen to cause

a moderate degree of hypothermia with a minimum of side effects which might impair behavioural activity. Following the trials with tetrodotoxin, some of the same animals were tested with saxitoxin in both resting and heat-escape trials.

Tetrodotoxin (Sankyo, Tokyo) was purchased from Calbiochem, Los Angeles. Stock solutions of tetrodotoxin and saxitoxin in saline solution were stored at 4° C.



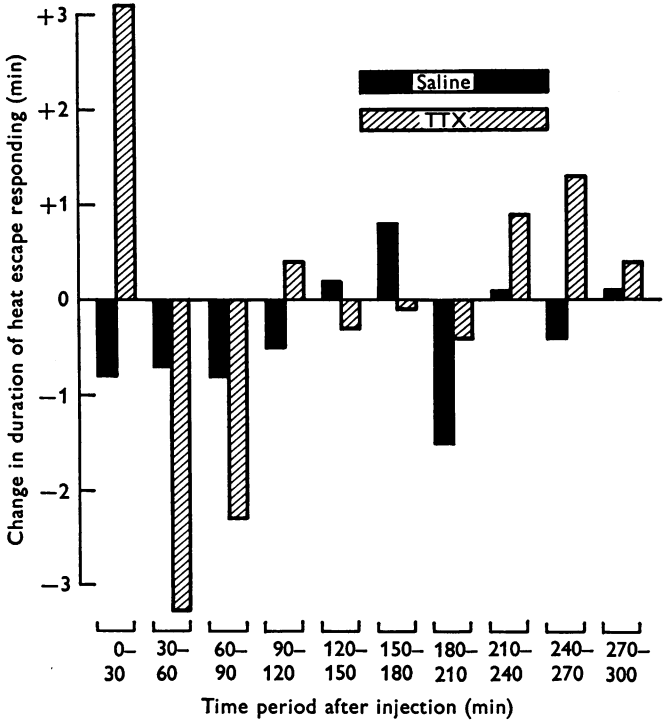
Text-fig. 1. Changes in retroperitoneal temperature after intraventricular injections of toxin or saline solution to cats which were resting (R) or lever pressing to escape heat (HE). *a*, tetrodotoxin (TTX), mean values in five cats. *b*, saxitoxin (STX), mean values in four cats.

RESULTS

Body temperature before injection. When the animals were simply resting during the initial half-hour in the cold environment, there was no consistent change in T_{rp}. However, in the heat-escape experiments, T_{rp} rose an average of 0·6° C (range 0·1–1·5° C). During the second half-hour the mean base line temperature (ten trials) while resting was 38·5° C and during heat escape was 39·1° C. T_{rp} was considerably more variable during heat escape than in the resting trials.

Administration of tetrodotoxin to resting animals. Text-fig. 1*a* shows that T_{rp} decreased approximately 2° C after tetrodotoxin was injected into the lateral cerebral ventricle of resting cats. The maximum decrease in T_{rp} and the TRI for each trial are listed in Table 1*a* along with mean values. Although T_{rp} declined more rapidly in the present study than after injection of the same dose at an environmental temperature of 22° C (Clark & Coldwell, 1973), there was considerable overlap in the maximum degree of hypothermia and in the TRI's between these two studies. Thus the hypothermic response was not greatly enhanced in the cold environment. In spite of the low dose of tetrodotoxin injected, ataxia was apparent during the phase of declining T_{rp} although it varied considerably in degree among

cats. Other common effects (Borison *et al.* 1963; Clark & Coldwell, 1973) included tachypnoea, which seldom exceeded rates of 150/min; defaecation and emesis, which were usually preceded by increased vocalization and mydriasis; and nystagmus.



Text-fig. 2. Mean differences (five cats) between the time spent depressing the lever in the indicated period and the time spent responding in the 30 min pre-injection control period.

Administration of tetrodotoxin to animals during heat-escape behaviour. During the hypothermic response to tetrodotoxin the cats continued to press the lever to escape heat. Other than being delayed (6–9 min), the mean hypothermic responses were essentially the same as when the cats were resting (Text-fig. 1*a*, Table 1*a*). Mean changes in heat-escape behaviour in successive 30 min periods after injection of either tetrodotoxin or saline solution are shown in Text-fig. 2, and individual responses for the first 2 hr are tabulated in Table 1*a*. No remarkable changes in mean durations of thermoregulatory behaviour were seen after injection of saline solution, although there were often sizeable changes in individual responses. However, during the initial 30 min after tetrodotoxin injection, when T_{rp} was declining rapidly, four of the five animals increased lever

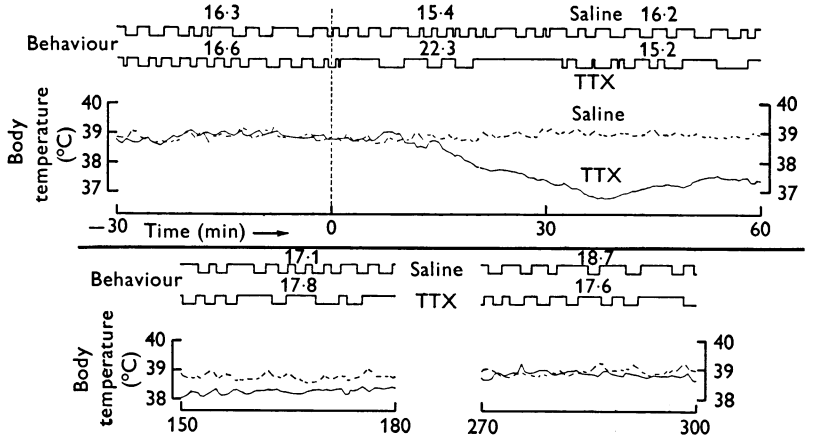
TABLE 1. Effects of tetrodotoxin and saxitoxin (25 ng) on retroperitoneal temperature and heat-escape behaviour. Environmental temperature = 4° C

		Temperature								Behaviour. Time period after injection (min)							
		Maximum decrease (°C)				TRI (Δ° C × hr)				0–30		30–60		60–90		90–120	
		Resting		Heat escape		Resting		Heat escape		Change in time pressing lever (min)*							
Toxin	Cat no.	Saline	Toxin	Saline	Toxin	Saline	Toxin	Saline	Toxin	Saline	Toxin	Saline	Toxin	Saline	Toxin	Saline	Toxin
a	1	0·2	2·2	0·3	2·1	0·67	−4·07	−0·22	−3·16	−0·9	+5·7	−0·1	−1·4	+0·3	−3·6	+1·8	+1·4
	2	0·0	1·1	0·7	2·3	1·71	−0·29	0·74	−2·94	−2·6	+3·8	−3·9	+4·2	−3·2	−0·3	−4·1	+4·7
	3	0·2	1·5	0·5	1·9	0·98	−2·72	−0·20	−2·78	+2·5	−0·5	+2·7	−5·0	+2·3	+1·1	+3·0	+0·9
Tetrodo-	4	0·2	2·8	0·9	2·0	0·31	−4·84	0·36	−4·51	−0·8	+5·3	−0·9	−3·9	−1·7	+0·2	+0·5	+1·8
toxin	5	0·2	4·2	0·2	3·1	1·49	−6·51	0·60	−3·75	−2·3	+1·0	−1·4	−10·3	−1·5	−8·7	−3·5	−6·7
	Mean	0·2	2·4	0·5	2·3	1·03	−3·69	0·26	−3·43	−0·8	+3·1	−0·7	−3·3	−0·8	−2·3	−0·5	+0·4
b†	4			0·4	2·6			0·77	−4·69	0·0	−7·3	−4·7	−0·6	−1·4	+2·4	+0·5	+1·1
c	1		2·8		2·9		−5·07		−5·09		+9·8		−1·9		−1·6		−2·3
	2		1·7		1·4		−3·56		−2·93		−7·1		+0·4		+2·8		+1·7
Saxi-	3		2·3		1·8		−2·52		−3·48		−4·6		+1·5		−0·3		−1·7
toxin	5		1·1		2·3		−2·51		−4·22		−1·4		+3·2		−0·1		+6·8
	Mean		2·0		2·1		−3·41		−3·93		−0·8		+0·8		+0·2		+1·1

* Time spent responding in the indicated period minus the time spent responding in the pre-injection control period.

† Heat-reinforcement behaviour.

pressing time. During the subsequent hour, in which T_{rp} levelled out and began to rise, response times usually fell below control levels. Thereafter, the amount of time spent responding differed very little from that during the control period. Text-fig. 3 shows the patterns of lever pressing and the changes in T_{rp} of one cat during its heat-escape trials. The development of hypothermia after tetrodotoxin was associated with increased lever pressing. As recovery began in the second half-hour post-injection, lever pressing declined to slightly below the control level. Recovery was gradual and was complete before the end of the 5 hr trial.

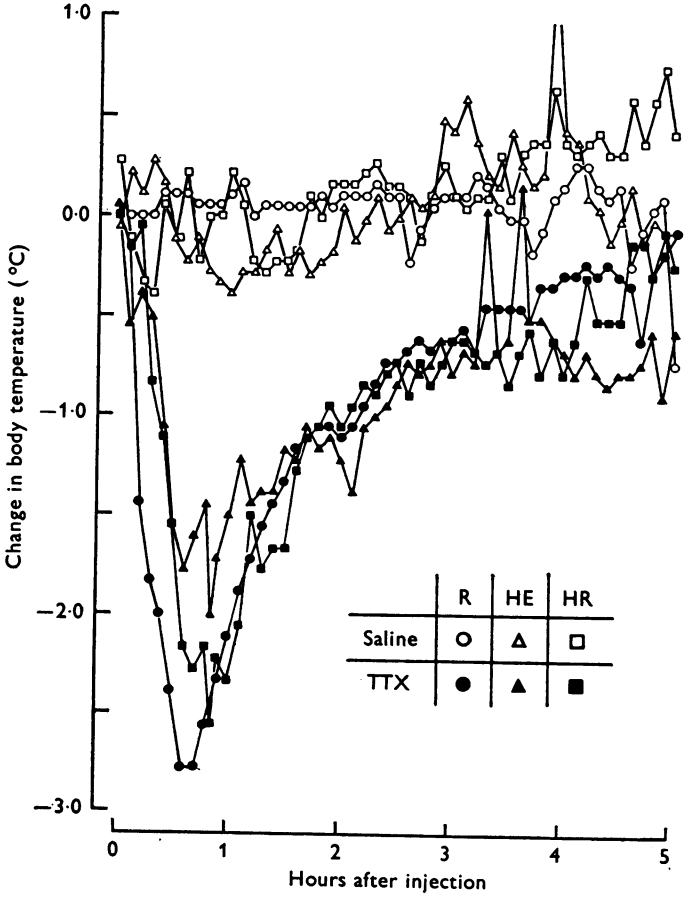


Text-fig. 3. Effects of tetrodotoxin injection on heat-escape behaviour and retroperitoneal temperature of cat no. 1. Portions of an actual record, which included the maximum effects and recovery, were traced to obtain this Figure. Tetrodotoxin or saline solution was injected at time zero. The upper portion of each panel shows the patterns of heat-escape behaviour. Upward deflexions indicate lever pressing. The numbers above these patterns are the total number of minutes spent responding during the corresponding 30 min period.

Administration of tetrodotoxin to an animal during heat-reinforcement behaviour. To control for the possibility that tetrodotoxin produced the behavioural effects by prolonged impairment of the ability to respond or by causing photic aversion, cat no. 4 was trained to press the lever to turn on the lamps and to turn off the fan (heat reinforcement). Similar changes in T_{rp} were produced (Text-fig. 4, Table 1*a*, *b*), regardless of whether the animal was resting or behaving to escape or to obtain heat. During the periods of developing hypothermia, the lever was depressed less on the heat-reinforcement schedule and more on the heat-escape schedule.

Saxitoxin. Four of the cats were also tested with saxitoxin (25 ng) while resting or responding to escape heat (Text-fig. 1*b*, Table 1*c*). In nearly all

respects, including side effects, the responses to saxitoxin were similar to those after tetrodotoxin. In the heat-escape trials, however, three of the animals given saxitoxin responded less during the first 30 min post-injection period than in the prior control period. This decrease seemed to be associated with more prolonged motor impairment and with defaecation or



Text-fig. 4. Changes in retroperitoneal temperature of cat no. 4 after injections of tetrodotoxin or saline solution when resting (R) or lever pressing for heat escape (HE) or heat reinforcement (HR).

emesis which was somewhat more delayed than after tetrodotoxin. Intraventricular administration of higher doses of saxitoxin to resting cats at environmental temperatures of 22 and 35° C produced responses comparable to those reported previously (Clark & Coldwell, 1973) after equal doses of tetrodotoxin.

Effects of an uninterrupted heat load on hypothermic responses to tetrodotoxin and saxitoxin. In two experiments tetrodotoxin or saxitoxin was injected after a control period of heat-escape behaviour, and the lever was deactivated so that depressing it no longer turned off the lamps. With the lamps set at a high position, one of the animals was still able to develop hypothermia but only by increasing its respiratory rate to twice the maximum reached in a previous heat-escape trial. This cat also depressed the lever from 80 to 90 % of the time even though this behaviour was ineffective. With the lamps set at the lowest position used, the other cat developed sustained respiratory rates greater than 250/min but, in spite of this increase, was unable to lower its T_{rp}. After 36–42 min, when the controls were reset to allow the cats to again regulate the lamps and fan, their T_{rp}'s and their respiratory rates rapidly declined.

DISCUSSION

The results of the present experiments indicate that tetrodotoxin produces a complementary lowering of the set-points for both behavioural and physiological thermoregulatory responses. Central administration of this toxin simultaneously caused T_{rp} to fall and initially increased the animals' motivation to escape heat. When the hypothermic response to intraventricular administration of tetrodotoxin was first studied (Borison *et al.* 1963), one of the most striking aspects of the response was that recovery from other effects of the toxin preceded the return of T_{rp} to normal. Cats which had recovered from motor disturbances and were able to locomote satisfactorily showed no apparent distress even though T_{rp} was several degrees below normal. Neither did they attempt to prevent heat loss by huddling. A question, therefore, arises concerning the animals' motivation to regulate body temperature. If provided with the opportunity to alter an external heat source, would an animal given tetrodotoxin act to minimize the hypothermic response by increasing the heat load, or would it regulate the heat source to maintain the same degree of hypothermia produced by physiological thermoregulatory processes alone? The former response would indicate that the low temperature was aversive and that the action of tetrodotoxin was solely to lower the set-point for physiological thermoregulation. The latter response would indicate that the hypothermia was not aversive and that tetrodotoxin lowers the set-point for behavioural as well as for physiological thermoregulation. Such a response was clearly the result in the present study because the cats developed similar hypothermic responses whether or not they had the opportunity to alter the heat source. Furthermore, soon after tetrodotoxin administration the animals usually increased the amount of time

spent responding to escape heat instead of acting to increase the heat load.

The responses of resting cats to saxitoxin at environmental temperatures of 4, 22 and 35° C were similar to those after central administration of tetrodotoxin. This similarity suggests that saxitoxin also lowers the set-point for physiological thermoregulatory responses. Like tetrodotoxin, saxitoxin also lowered T_{rp} in the present study whether the animals were resting or lever pressing to escape heat. However, only one cat given saxitoxin initially increased its duration of lever pressing. Defaecation and vomiting interfered with thermoregulatory behaviour, at least transiently during the act, and, when these effects were relatively severe or prolonged, they probably accounted for the diminished duration of responding in these trials. The onset of the hypothermic response to both tetrodotoxin and saxitoxin was delayed in the heat-escape experiments, and hypothermia was preceded by a transient hyperthermia in some instances. This delay also seemed to be due in part to the severity of early motor impairment. Any impairment of behavioural capability associated with motor inco-ordination or other side effects would preferentially retard the development of hypothermia during the heat-escape trials, because the animals were exposed to heat during these trials. On the other hand, side effects would not prolong the latent period before hypothermia during the resting trials because physiological heat-loss mechanisms would not be required to dissipate additional heat from the lamps. Regardless of the cause of the delayed decline in T_{rp} and of the decrease in early post-injection lever pressing in some of the experiments, thermoregulatory behaviour clearly allowed hypothermia to be maintained for several hours once the side effects had diminished. If the animals had not depressed the lever at all, hypothermia either would not have occurred or would have developed only at the expense of still greater physiological heat-loss activity as evidenced by the experiments in which the heat load was uninterrupted for several minutes after either tetrodotoxin or saxitoxin injection. Hence any lever pressing to reduce the external heat load in the face of hypothermia can reasonably be considered as thermoregulatory behaviour. Further evidence that the behaviour was thermoregulatory was provided by cat no. 4 which was tested with tetrodotoxin on both heat-escape and heat-reinforcement schedules. On both schedules this cat developed hypothermic responses which were similar to the control response when resting and, in each case, it exhibited initial changes in responding which would facilitate hypothermia, namely, an increase in lever pressing on the heat-escape schedule and a decrease on the heat-reinforcement schedule. Therefore, the behavioural responses cannot be attributed to photic aversion or, except for transient effects already noted,

to the side effects of the toxins. It is clear that after administration of either toxin the animals regulated the external heat load to maintain a degree of hypothermia comparable to that produced by toxin-induced alterations of physiological thermoregulatory processes alone. This result is consistent with concurrent lowering of set-points for both behavioural and physiological thermoregulation.

Cabanac (1972), after considering the similarities between physiological and behavioural components of the thermoregulatory system, concluded that these two components are so closely related in the intact animal that behavioural responses can serve as a measure of the difference between actual inner temperature and a reference. This implies that a single set-point serves to govern both components. An alternative explanation is that separate set-points serve independently to govern the two major components of the system but are linked so that functionally they shift in complementary fashion. Agents such as endogenous pyrogen or bacterial endotoxins, which are considered to raise the thermoregulatory set-point, evoke complementary behavioural and physiological thermoregulatory responses. For instance, cats given I.V. injections of Piromen at an environmental temperature of 10° C developed only low grade fevers (Weiss *et al.* 1967). However, when the cats were able to press a lever to obtain heat from infra-red lamps the same dose of pyrogen elicited much larger febrile responses. In a similar setup at a thermoneutral environmental temperature, baboons increased their rate of lever pressing for heat reinforcement during the phase of rising temperature after Piromen injection. The rate of responding returned nearly to pre-injection levels once body temperature had reached a plateau (Gale *et al.* 1970). In another study when dogs, trained to control external sources of heat and cold, were given various pyrogens they responded more for heat than control animals at environmental temperatures below thermoneutrality and for less cold air at environmental temperatures above thermoneutrality (Cabanac *et al.* 1970). At present there is insufficient evidence to decide between the alternatives of a single set-point which subserves both behavioural and physiological thermoregulatory processes or separate set-points subserving each major component. If there are two set-points, tetrodotoxin and saxitoxin as well as pyrogens apparently shift both set-points in a complementary manner.

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EXPLANATION OF PLATE

Behavioural apparatus. The fan is placed within the circular shield which is just visible between the centre and right banks of lamps. The lamps and fan are in the lowest position used during this study. Cat no. 3 is shown depressing the lever. Wires lead from the cat's harness to a multipoint recorder.