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A model to measure anticipatory anxiety in mice?*

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Abstract. Among animals from the same cage, mice removed last had a higher temperature compared to those removed first. This phenomenon a) persisted 2 and 24 h later; b) was present regardless of the number of the animals (5, 10, 15 and 20) in each cage, c) was independent of whether the number of animals was reduced or maintained constant in the cage and d) could even be observed by reversing the order of removal of the animals from the cage. In addition, the fewer the animals allocated to a cage the greater the percentage of those which became hyperthermic. This rise in rectal temperature of mice removed last was prevented by diazepam (2.5 and 5 mg/kg PO, 30 min), nitrazepam (2 and 4 mg/kg PO, 30 min) but not by imipramine (15 and 30 mg/kg PO, 60 min) or haloperidol (0.5 and 1 mg/kg PO, 60 min) and was observed in a greater percentage of mice following subcutaneous yohimbine treatment (2 mg/kg, 60 min). This phenomenon does not seem to depend on physical exercise due to an attempt to escape, since no correlation appears to exist between motor activity (open-field) and rise in rectal temperature. These data would seem to indicate that hyperthermia in the last animals may represent a new tool for studying the neurobiology of anticipatory(?) anxiety.

Key words: Animal model of anxiety – Hyperthermia – Mouse – Anxiolytics

In the course of experiments aimed at determining the effect of drugs on rectal temperature in mice, we observed that, among animals of the same cage, those removed last had a higher temperature as compared to those removed first. This phenomenon was observed even when the order of removal of the animals from the cage was reversed and regardless of the number of mice (5, 10, 15 or 20) allocated to a cage.

Body temperature of rats increases when recorded on subsequent measurements (Poole and Stephenson 1977; Eikelboom and Stewart 1981) and this has been interpreted as a conditioned response in anticipation to handling or insertion of the rectal probe (Eikelboom 1986).

In view of the above, the phenomenon we observed was interpreted as indicative of a state of anxiety due to expectation of an (unknown or known?) event. The present work was aimed at substantiating this hypothesis.

Materials and methods

General

Male Swiss mice (Nossan, Italy) 25–30 g body wt, were housed at constant room temperature (22 ± 1° C) and relative humidity (55 ± 5%), with food and water ad lib., and 12 h light-dark cycle (light on: 6.00 a.m.). Mice were allocated to groups of 5, 10, 15 or 20 to a cage. Experiments were carried out between 9.00 a.m. and 1 p.m., at least 1 week after the arrival of the animals: during this period mice were handled (removed and touched) every other day.

Rectal temperature

Temperature measurements were carried out in the same room where mice were housed. Rectal temperature was measured by inserting a thermistor probe for a length of 2 cm into the rectum of the mouse which was restrained manually. Digital recording of the temperature was determined to the nearest 0.1° C by means of a U. Sachs instrument. The probe, dipped into silicon oil before insertion, was held in the rectum for about 20 s. When diazepam, nitrazepam, imipramine or haloperidol were used, the rectal temperature was recorded in the first and last 3 out of 15 mice allocated to each cage. The intermediate nine animals were removed without any recording. Temperature recordings were carried out by the same person who handled animals prior to the experiment.

Measurement of motor activity

Motor activity was evaluated in an open-field (36 × 60 cm) divided in 12 × 12 cm squares, one mouse was placed in the corner of the box and the number of squares crossed with all four paws within a 3-min period was recorded.

Procedure

Experiment 1 (*Fig. 1*). Animals were allocated 5, 10, 15 or 20 to a cage. The time spent to record rectal temperature in all animals in a cage depended on the number of mice

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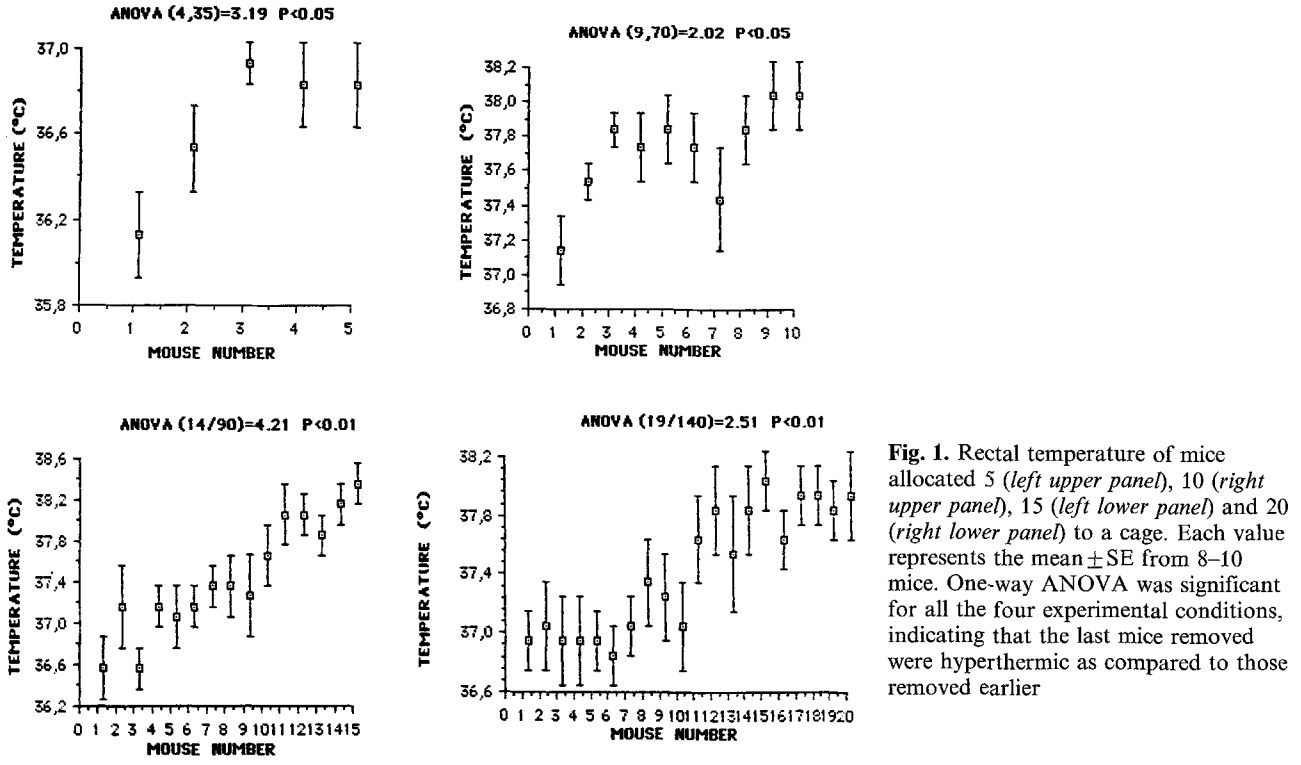

Fig. 1. Rectal temperature of mice allocated 5 (*left upper panel*), 10 (*right upper panel*), 15 (*left lower panel*) and 20 (*right lower panel*) to a cage. Each value represents the mean ± SE from 8–10 mice. One-way ANOVA was significant for all the four experimental conditions, indicating that the last mice removed were hyperthermic as compared to those removed earlier

allocated to the cage (generally 1 min for each mouse). After each measurement animals were removed to another cage.

Experiment 2 (*Fig. 2*). The rectal temperature of mice (15 to a cage) was measured at various times (15 min, 2 and 24 h) after the first measurement. Each animal was used for two temperature recordings: for the first recording and for one of the other times chosen (15 min or 2 or 24 h). After each measurement animals were removed to another cage.

Experiment 3 (*Fig. 3*). After rectal temperature measurements, mice (15 to a cage at the beginning) were removed to another cage or again allocated to the same cage.

Experiment 4 (*Fig. 4*). The rectal temperature was again recorded in mice (15 to a cage) 24 h after the first measurement by reversing the order of removal, i.e. the rectal temperature of the mouse recorded as first on the first day was recorded as last on the second day and vice versa (the rectal temperature of the mouse recorded as last in the first day was recorded as first on the second day).

Experiment 5 (*Table 1 and Fig. 5*). Since the rise in body temperature of the last animals removed compared to the first ones is a very reproducible phenomenon, the experiments with diazepam, nitrazepam, imipramine and haloperidol were carried out by utilizing only the first 3 and the last 3 mice of a cage containing 15 animals. When yohimbine was administered, all animals ($n=20$) of a cage were used, since we thought that an anxiogenic drug would be more likely to anticipate the rise, rather then simply increase the rise of body temperature.

Experiment 6 (*Fig. 6*). This experiment was carried out by first measuring the rectal temperature of animals, then putting them in an open field in order to record motor activity and finally recording again their body temperature. In these experiments, the animals were allocated to the same cage after temperature recording.

Drug treatment

Nitrazepam (2 and 4 mg/kg IP), diazepam (2.5 and 5 mg/kg PO), imipramine (15 and 30 mg/kg PO) and haloperidol (0.5 and 1 mg/kg PO) were administered 30, 30, 60 and 60 min before the rectal temperature recording, respectively, to the first and last three animals in a cage containing 15 mice. Yohimbine (2 mg/kg) was administered subcutaneously 60 min before rectal temperature measurement to all the animals housed in groups of 20 to a cage.

Drugs and sources

Nitrazepam and diazepam (Roche, Milan, Italy), imipramine (Ciba-Geigy, Saronno, Italy) and haloperidol (Lusofarmaco, Milan, Italy) were solu-suspended in 0.5% (w/v) carboxymethylcellulose, 0.4% (w/v) Tween 80 and 0.9 (w/v) NaCl. Yohimbine (Aldrich, Beerse, Belgium) was dissolved in saline.

Statistics

Values represent mean ± SE from eight to ten mice. Homogeneity of variance was checked before data processing. Results were evaluated by one-way or factorial analysis of variance (ANOVA) according to the experimental design. Drugs were administered to animals chosen by means of a completely randomized schedule (Borsini 1985).

Results

Regardless of the number of mice (5, 10, 15 or 20) allocated to each cage, it was observed that body temperature of

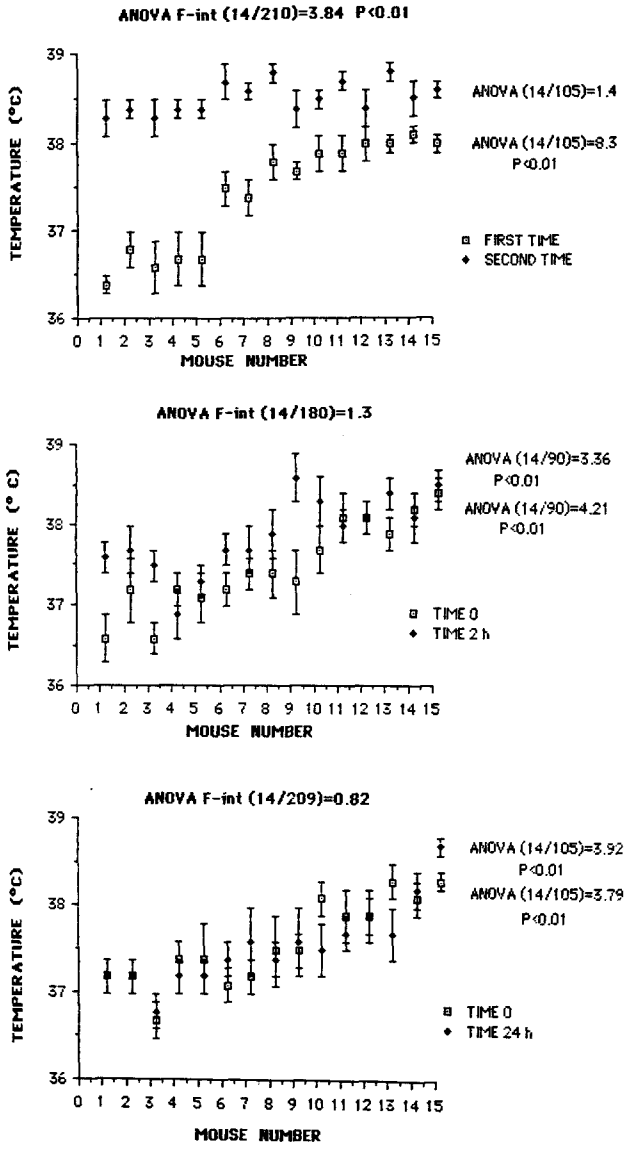

Fig. 2. Rectal temperature of mice 15 min (*upper panel*), 2 (*middle panel*) and 24 (*lower panel*) h after the first temperature recording. Mice were housed 15 to a cage. Each value represents the mean ± SE from 8–10 mice. Factorial ANOVA revealed a significant effect only when the rectal temperature was again recorded 15 min after the first measurement

those animals removed as last was higher when compared to that of mice removed as first (Fig. 1). This rise in rectal temperature was no longer observable 15 min after the first recording, since in this case all animals had a high rectal temperature (Fig. 2); however, it was again observed 2 and 24 h later (Fig. 2) and every day for 3 weeks (data not shown). The hyperthermia of the last animals removed was present regardless of whether the number of animals was reduced at each measurement or maintained constant in the cage (Fig. 3) and even after reversing, on the subsequent day, the order of removal of mice from the cage (Fig. 4).

The rise in rectal temperature was prevented by diazepam and nitrazepam, but not by imipramine or haloperidol (Table 1). Yohimbine increased the number of mice which developed hyperthermia (Fig. 5).

No correlation was found between motor activity (open-field) and rise in rectal temperature (Fig. 6).

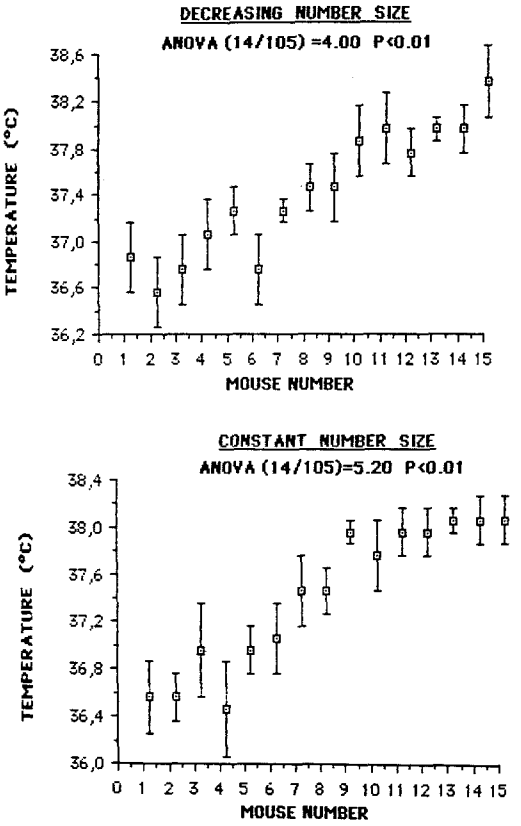

Fig. 3. Rectal temperature of mice whose number in the cage was reduced at each measurement (mice were moved to another cage; *upper panel*) or maintained constant (mice were again allocated to the same cage; *lower panel*). Factorial ANOVA [*F*int(14/210) = 0.55] did not reveal statistical interaction between the two experimental conditions. Mice were housed 15 to a cage. Each value represents the mean ± SE from 8–10 mice

Discussion

A rise in body temperature was observed only in the last few animals removed from the cage. This rise in temperature was no longer observable when temperature was again measured 15 min later. This is not surprising, because the insertion of the rectal probe is a form of stress which is known to increase body temperature (Poole and Stephenson 1977) and therefore the animals removed first also developed hyperthermia. However, the rise in temperature in the animals removed last as compared to the first was again observable 2 and more distinctly 24 h later. It is worth noting that this phenomenon could be demonstrated even after reversing the order of removal, which means that the hyperthermia of the last animals depends only on the order of removal and is not a characteristic of any particular animal.

The rise in rectal temperature in the animals removed last as compared to first does not appear to be attributable to a) compensatory thermogenic effects due to the decrease in number of mice in the cage, since it was observed even when their number was maintained constant or b) physical exercise due to repetitive attempts to escape, since no correlation seems to exist between motor activity and rise in rectal temperature.

Although rise in body temperature was observed regardless of the number (5, 10, 15 or 20) of animals allocated to a cage, it is worth noting that the fewer the animals

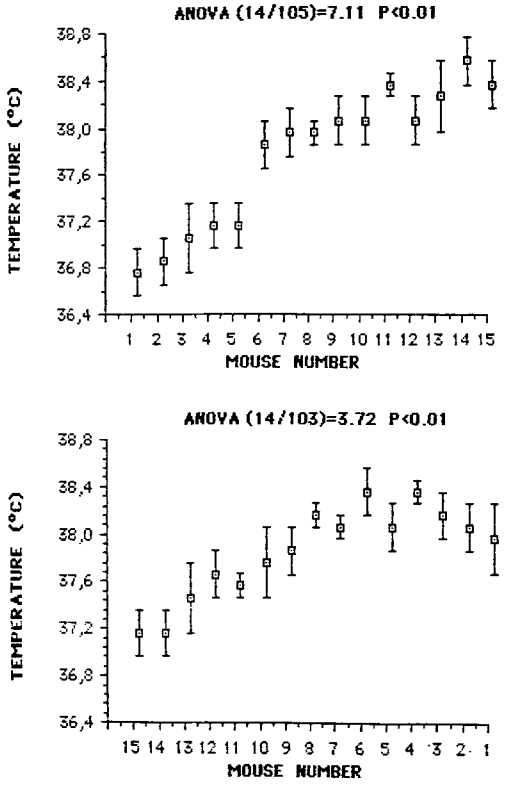

Fig. 4. Rectal temperature of mice before (*upper panel*) and after (*lower panel*) reversing the order of the removal, 24 h from the first measurement. Factorial ANOVA [Fint(14/210) = 1.52] did not reveal statistical interaction between the two experimental conditions. Mice were housed 15 to a cage. Each vaue represents the mean ± SE from 8–10 mice

the greater the percentage of those which became hyperthermic, which might indicate that the fewer the animals, the more quickly the remaining ones realize that something is going to happen to them. In other words, the third mouse removed was hyperthermic as compared to the first one when mice were allocated 5 or 10 to a cage but not when allocated 15 or 20 to a cage. This seems to exclude that disturbance (i.e. introduction of the hand in the cage, removal of the cage from the housing shelf to working table) may completely account for the phenomenon observed. The time elapsing between the removal of the first and last animals was the only difference between the first and last animals; therefore it is possible that the expectation of an (unknown or known?) event could be responsible for the effect we observed. The fact that the rise in body temperature was prevented by diazepam and nitrazepam (anxiolytics), but not by haloperidol (neuroleptic) or imipramine (antidepressant) would suggest that mechanisms related to anxiety are involved. This is also substantiated by the fact that yohimbine, an anxiogenic compound, increased the number of mice which developed hyperthermia by anticipating the onset of this phenomenon.

It has already been reported that body temperature can rise following stressful procedures (Delini-Stula and Morpurgo 1970; Pool and Stevenson 1977; Blasig et al. 1978; Hajos and Engerber 1986). We also observed this phenomenon, since the body temperature measured 15 min after the first insertion of the rectal probe is increased in all animals. When the effect of *physical stress* disappears, the temperature returns to normal and in this situation our experimen-

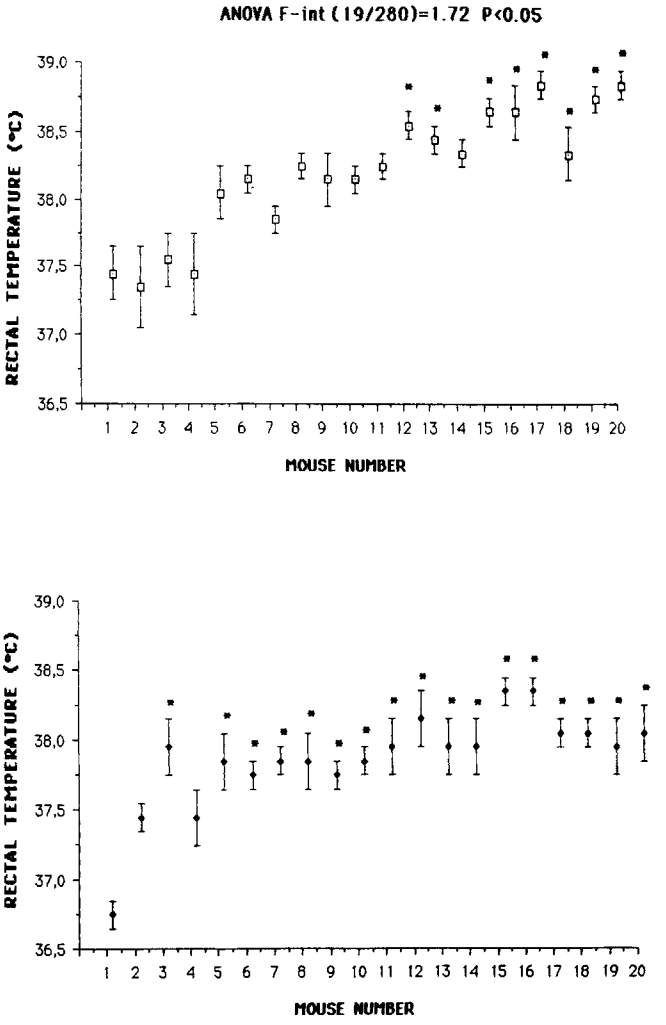

Fig. 5. Rectal temperature of mice (20 to a cage) administered with vehicle (*upper panel*) or yohimbine (*lower panel*). Factorial ANOVA revealed a significant effect which was due, as shown by post-hoc analysis (Tukey's test: * $P<0.01$), to the fact that a greater number of mice developed hyperthermia following yohimbine treatment. Each value represents the mean ± s.e. from 8 mice. *Open squares* represent vehicle; *closed diamonds* yohimbine

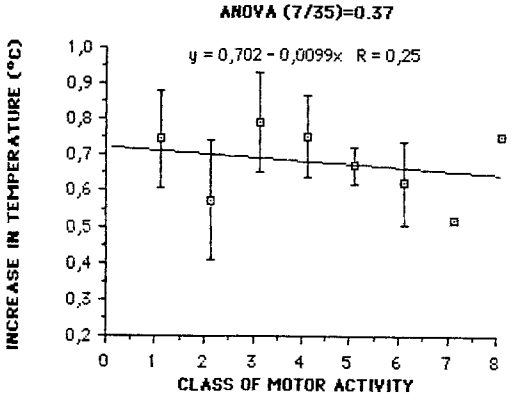

Fig. 6. Relationship between motor activity in the open-field and increase in rectal temperature after the open-field. Class of motor activity from 1 to 8 indicates respecitvely 0, 0–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79 squares crossed by each mouse. Each value represents the mean ± s.e. of increase in rectal temperature of 2–6 mice. Statistical analysis revealed that coefficient of regression was not significant

Table 1. Effect of oral administration of various drugs on hyperthermia in mice removed at a later time

Treatment	Dose mg/kg	Time min	Rectal temperature (°C)		Δt	ANOVA interaction
			First	Last		
Vehicle	–	30	37.8±0.2	38.8±0.1[b]	+1.0	$F=3.78$
Diazepam	2.5	30	37.3±0.1	37.7±0.1[c]	+0.4	$df=2/48$
Diazepam	5	30	37.0±0.2[a]	36.9±0.2[c]	−0.1	$P<0.05$
Vehicle	–	30	37.3±0.1	38.4±0.2[b]	+1.1	$F=3.61$
Nitrazepam	2	30	36.4±0.2[a]	36.8±0.2[c]	+0.4	$df=2/49$
Nitrazepam	4	30	36.2±0.3[d]	36.1±0.4[c]	−0.1	$P<0.05$
Vehicle	–	60	36.7±0.3	38.5±0.2[b]	+1.8	$F=0.71$
Imipramine	15	60	36.2±0.2	38.1±0.1[b]	+1.9	$df=2/46$
Imipramine	30	60	36.5±0.2	37.9±0.2[b]	+1.4	n.s.
Vehicle	–	45	36.6±0.1	38.2±0.2[b]	+1.6	$F=1.20$
Haloperidol	0.5	45	36.3±0.2	38.4±0.1[b]	+2.1	$df=2/48$
Haloperidol	1	45	36.3±0.3	38.3±0.1[b]	+2.0	n.s.

Values represent mean±SE from 8–9 mice. The time refers to the time between drug administration and the temperature recording. First and last refer to the rectal temperature of the first and last three mice of three cages. n.s. = not significant. Tukey's test: [a] $P<0.05$, [d] $P<0.01$ vs vehicle-first; [b] $P<0.01$ vs respective first-group; [c] $P<0.01$ vs vehicle-last

tal procedure shows a rise in temperature only in mice removed last, suggesting that these animals may undergo *stress* no explanable on a physical basis (psychical?). It is worth noting that the rise in temperature in mice removed last was observable even after 3 weeks, suggesting that this phenomenon does not undergo tolerance, and this agrees with the fact that only hyperthermia due to physical but not psychical stress seems to decrease following chronic exposure to stressful stimuli (Eikelboom 1986). Even if further investigations are necessary, this model seems to represent a new tool for studying the psychobiological basis of anxiety, in particular the "alarm reaction" due to emotional stimuli as described by Selye (1950).

Finally, we would like to express a note of caution as regards specifically experiments aimed at evaluating the effect of drugs on rectal temperature, since, if the experimental design is not well planned (first mice versus last mice), the possibility exists that what is observed may depend on methodological rather than pharmacological differences.

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References

Blasig J, Hollt V, Bauerle U, Herz A (1978) Involvement of endorphins in emotional hyperthermia of rats. Life Sci 23:2525–2532

Borsini F (1985) Randomization program for AppleII computer. Brain Res Bull 15:279–281

Delini-Stula A, Morpurgo C (1970) The influence of fear evoking-situation on the rectal temperature of rats. Int J Psychobiol 1:71–75

Eikelboom R (1986) Learned anticipatory rise in body temperature due to handling. Physiol Behav 37:649–653

Eikelboom R, Stewart J (1981) Hypophysectomy increases the sensitivity of rats to naloxone-induced hypothermia. Life Sci 28:1047–1052

Hajos M, Engberg G (1986) Emotional hyperthermia in spontaneously hypertensive rats. Psychopharmacology 90:170–172

Poole S, Stephenson JD (1977) Core temperature: short comings of rectal temperature measurements. Physiol Behav 18:203–205

Selye H (1950) Stress and the general adaptation syndrome. Br Med J 17:1383–1392

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