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Conditioned and Unconditioned Influences on Body Temperature and Ethanol Hypothermia in Laboratory Rats

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YORK, J. L. AND S. G. REGAN. *Conditioned and unconditioned influences on body temperature and ethanol hypothermia in laboratory rats.* PHARMAC. BIOCHEM. BEHAV. 17(1) 119–124, 1982.—Both a naive group and a group of chronically handled rats were observed to develop hyperthermia when their cages (rats in situ) were removed from their usual positions on cage rack shelving and placed upon the laboratory bench for a period of 60 minutes. That procedure apparently functioned as a stressful unconditioned stimulus for the naive group. The extent of hyperthermia was more pronounced in the chronic group, presumably owing to the classical conditioning of environmental cues to the stressful events that had repeatedly been associated in the past with placement of the cage onto the benchtop. Doses of naloxone (10 mg/kg) and of ethanol (1 g/kg) that normally produced negligible effects on body temperature were found to significantly reduce the hyperthermia that developed when cages were placed onto the benchtop. The hypothermic response to 2 g/kg of ethanol was lessened in both groups by placement of the cages onto the benchtop.

Body temperature Stress Ethanol Naloxone

THE manner in which laboratory rats are handled has long been suspected of having an effect upon their body temperature. Stressful events, in particular, are known to produce hyperthermia ("emotional hyperthermia" [7]), which probably involves the mediation of catecholaminergic systems [11,15]. The routine handling of laboratory rats has been reported to be stressful when the animals are first exposed to human handling [7,13]. Repeated daily manipulations, such as picking up and weighing, are believed to diminish the extent of stress hyperthermia through the process of habituation [7,20].

The above factors must be considered and monitored in order to obtain accurate measurements regarding the influence of drugs, such as ethanol, on body temperature. In particular, the response of the temperature-regulating systems to the routine maneuvers imposed upon the animal should be allowed to reach a steady-state level before the influence of experimental conditions is tested. We have observed that simply changing the location of our rats' home-holding cages within a small room may have a reliable and significant influence upon the body temperature of the animals. Moreover, the extent of hyperthermia was actually greater in chronically handled than in a matched group of naive animals. We report here those observations and additional data regarding some possible underlying factors that may influence body temperature and the response to ethanol.

METHOD

Subjects and Environment

Sixteen female CD strain rats (Charles River, Wilmington, MA), 5–11 months of age, were housed one per cage in clear polycarbonate cages (21 h × 45 l × 25 w, cm) with bedding of wood shavings. The cages were permanently placed (5 per shelf) on mobile, five-shelf animal housing racks 1.72 m high (0.5×1.5 meter shelves, with spacings of 34 cm between shelves; Wahman Manufacturing Co., Timonium, MD). The racks were housed against the wall inside a Warren-Shearer controlled environment room (temperature 20.5–21.5°C; relative humidity 45–64%; sound level 68 dB; mean light 35.5±14.6 ft candles/cm^2) in which a 12-hour light-dark cycle prevailed. Fluorescent fixtures with diffusion gratings were used for lighting. Eight of the animals (chronic handling group) had been part of an experiment conducted in the homeroom in which they had been weighed, had rectal temperatures taken, were injected with ethanol (1.0–3.0 g/kg, twice weekly for 8 weeks) or saline and returned to their home spaces on the rack shelf, and 60 minutes later were subjected to rotarod (motor performance) measurements on a daily basis for 8 weeks. Weighings, drug injections, and temperature and rotarod measurements had been obtained by moving the home cage from the cage rack (R) to a benchtop surface (B) located along the opposite side of the environment room (approximately 6 ft distant). The

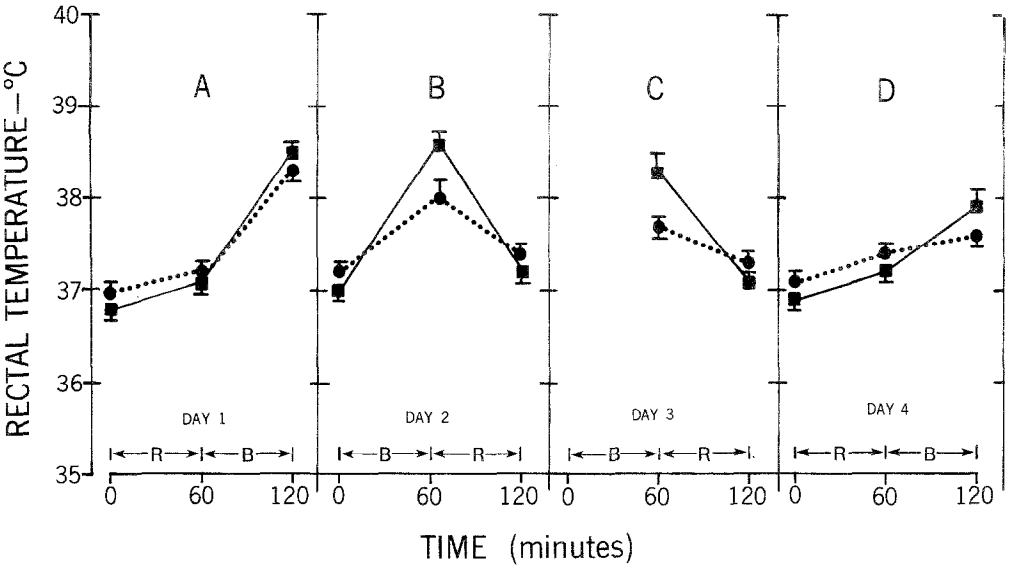

FIG. 1. Rectal temperature as influenced by cage location in chronically handled (■---■) and naive (●---●) rats, N=8 each. In A and B, at time zero, the home cage containing the rat was moved from the shelving rack (R) to the laboratory benchtop (B), and the rectal temperature was immediately determined. The designation B or R indicates where the cage remained for the ensuing 60 minutes when rectal temperatures were redetermined and the resting position of the cage (B or R) was reversed. In C, rats in their home cages were placed onto the benchtop at time 0 and allowed to remain there for 60 minutes. Rectal temperatures were then determined for the first time at 60 minutes (abscissa), and the cages were returned to their home positions on the cage rack for an additional 60 minutes. In D, the entire cage rack with cages in position was moved out of the controlled environment room into a much larger laboratory maintained at approximately the same temperature and humidity. After a 60-minute placement in the middle of that area, rectal temperatures were again taken (60 minutes, abscissa), and the rat cages were placed upon the benchtops within the large laboratory area for an additional 60 minutes.

other 8 animals (naive group) were similar in their age distrubutions but had been handled only briefly once per week for the purpose of cage cleaning. All animals were allowed continuous access to Teklad rat and mouse diet and to drinking water. The studies described in this report spanned a period of 7 weeks. The mean body weight at the beginning of the study was 397.5±14.2 (g±SEM) for the chronic group and 414.2±8.0 for the naive group.

Procedure

Rectal temperatures were measured by means of a Yellow Springs Instrument Co. Model 45TA Digital Thermometer fitted with a No. 423 small animal probe. In order to determine temperatures, the rat cage was first placed upon the benchtop, and the stainless steel grid cover was removed. The rat was grasped at the base of the tail with thumb and forefinger, and the hindquarters were elevated such that only the forepaws remained in contact with the cage bottom. The probe was then gently inserted 6 cm into the rectum and was allowed approximately 40 seconds to stabilize before readings were recorded. The rats were then weighed and injected with drug, if required. After initial temperature measurements were made, the cage (with rat in situ) was allowed to remain on the benchtop (B) for an additional 60-minute period or was returned to its normal position on the cage rack shelf (R) for the 60-minute test period. The effect on body temperature of reversing the placement of the cages (B vs R) was also determined for the period 60–120 minutes after the initial measurement.

The influence of naloxone, ethanol, and saline on body temperature with different cage placements was determined. All drugs were injected intraperitoneally. Ethanol (95%, U.S. Industrial Chemicals, Tuscola, IL) was dissolved in saline to form a solution of 10 g/100 ml (10% w/v). Naloxone (Endo Laboratories, Garden City, NY) was dissolved in saline to form a solution of 0.1 g/100 ml. The different experiments exploring the influence of cage location on body temperature were generally performed on successive days beginning at 1:30 p.m. (indicated as time "zero" in the figures). Two days of inactivity were allowed to intervene between the tests with naloxone and also between tests with different doses of ethanol.

RESULTS

Figure 1A and B illustrates the basic observations that served as a stimulus for this study. When rectal temperatures were taken at time zero and animals (in their cages) were returned to their usual position on the cage rack, very little change in rectal temperature was observed 60 minutes later (mean change in temperature +0.20±0.12°C naive group vs +0.25±0.13°C for the chronic handling group). However, if the cages (rats in situ) were allowed to remain on the countertop for the period 60–120 minutes, a significant elevation in body temperature was observed in both groups at the

120-minute mark (mean change in temperature from time 0: 1.26±0.10°C for the naive group vs 1.71±0.10°C for the chronic handling group). Thus, the elevation in rectal temperature was significantly greater in the chronic handling group than in the naive group ($p<0.01$, Student's t-test). However, inasmuch as the pharmacological histories of the naive and chronic groups were also different, caution must be exercised in attributing differences entirely to the different handling experiences of those two groups.

The data in Figure 1B illustrate the same effects as those depicted in Fig. 1A, but in reverse sequence. When rectal temperature was determined at time zero and the cages were allowed to remain on the benchtop for the period 0–60 minutes, a significant elevation in body temperature was observed in both groups of animals, the elevation being greater again in the chronic handling group (1.54 ± 0.14°C) than in the naive group (0.85±0.13°C). The body temperatures regressed to the point of not being significantly different when the animal cages were returned to their usual position on the cage rack for the period 60–120 minutes.

It can be seen that in the complete absence of handling at time zero (Fig. 1C), when the rat cages were moved from the cage rack to the benchtop and allowed to remain there for 60 minutes, the rectal temperatures of the chronic treatment group became elevated to a greater extent than those observed in the naive group (1.39±0.17 vs 0.60±0.10°C, $p<0.01$, Student's t-test). Movement of the entire 25-unit cage rack, with the cages remaining in their usual shelf positions, from the controlled environment room (sound 68 dB; light 35.5±14.6 ft candles/cm^2) down the hall to a larger laboratory (sound 55.5 dB; light 94.5±28.8 ft candles/cm^2), maintained at the same temperature and humidity, produced a small elevation in temperature 60 minutes later in both groups (Fig. 1D). After the 60-minute reading, cages were allowed to remain on the benchtop for another 60 minutes. That exposure again resulted in a significantly greater elevation in body temperature in the chronic group (1.00±0.24°C) than in the naive group (0.53±0.12°C). However, the elevations in temperatures were not nearly so great as those observed after placement of animals on the benchtop for 60 minutes in their homeroom (cf. Fig. 1A, B).

The data in Fig. 2 illustrate that the effects of ethanol on body temperature were differentially affected by the placement of the rat cage onto the benchtop or back into its usual position on the cage rack shelf. Sixty minutes after the injection of saline (0.0 dose of ethanol, abscissa, equivolume to 2 g/kg dose of ethanol), rectal temperatures were observed to increase only slightly if the rats were placed back into their home shelf position for the 60-minute waiting period. However, as previously observed (cf. Fig. 1), if the rats were allowed to remain on the benchtop for the 60-minute waiting period, body temperatures were found to be markedly elevated in both the naive and the chronic groups, but more so in the chronic group. Reversing the cage position (B vs R) after the 60-minute measurement produced an effect similar to that previously described (cf. Fig. 1).

The 1.0 g/kg dose of ethanol was not expected to produce hypothermia to an appreciable extent [1], and indeed, only a small decrease in body temperature (as compared to the saline value) was observed 60 minutes later when the animals were placed back onto the cage rack shelf immediately after drug injection (solid symbols, Fig. 2). However, the 1.0 g/kg dose of ethanol attenuated the rise in body temperature typically seen when rats were allowed to remain on the benchtop for the 60-minute waiting period (open symbols, Fig. 2).

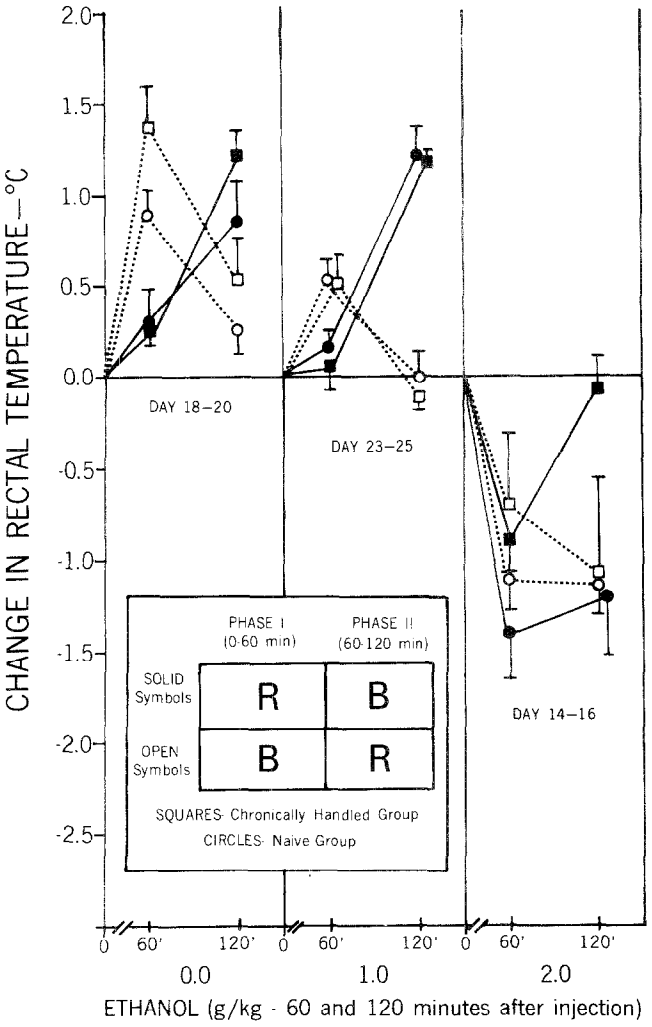

FIG. 2. Location-dependent effects of ethanol in chronically handled (squares, N=8) and naive (circles, N=8) rats. At time zero subjects were weighed, rectal temperatures taken, and injections of saline (0.0 dose of ethanol) or ethanol (1.0–2.0 g/kg) were administered. Subjects were then immediately returned to their cages. During Phase I (0–60 minutes), the home cage was either returned to its usual location on shelving racks (R) (solid symbols) for 60 minutes, or the cage was allowed to remain on the laboratory bench (B) (open symbols) for 60 minutes. After the 60-minute period of undisturbed placement, rectal temperatures were again measured, and the positions (R vs B) of the home cages were reversed from those prevailing during Phase I (see figure inset for summary of procedures). After another 60 minutes of undisturbed placement in the designated location (Phase II, 60–120 minutes), rectal temperatures were again determined at time 120 minutes (abscissa). Ordinate: Change in rectal temperature from that existing at time zero, immediately prior to the administration of drug. Brackets indicate positive or negative aspects of the standard error of the mean.

Body temperature changed in the expected directions when the positions of the cages were reversed for the period 60–120 minutes. The ability of the 2.0 g/kg dose of ethanol to lower body temperature was also found to be more pronounced when the rat cages were placed back onto the cage rack shelf for the 0–60-minute waiting period. The result-

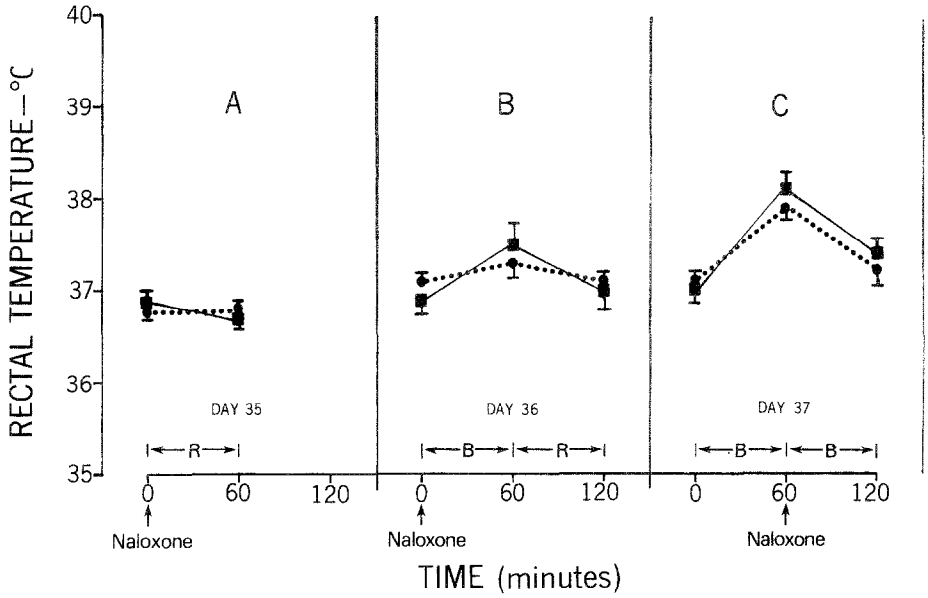

FIG. 3. Influence of naloxone on body temperature in chronically handled (■---■) and naive (●---●) rats, N=8 per group. In A and B, after rectal temperatures were taken at time zero, naloxone (10 mg/kg) was injected, and the animals were returned either to their designated positions on the cage rack shelf (R, Experiment A) or to the benchtop (B, Experiment B) for a 60-minute period. In C, after initial temperature measurements, rats were placed onto the benchtop (B) for the period 0–60 minutes. At 60 minutes, naloxone (10 mg/kg) was injected, and the animals were allowed to spend an additional 60 minutes on the benchtop before the final temperature measurement was made at 120 minutes. Brackets indicate positive or negative aspect of the standard error of the mean.

ant hypothermia in the chronically handled group was almost completely eradicated by simply placing the cage onto the benchtop for the 60–120-minute waiting period. The hypothermic effects of ethanol (2 g/kg) are expected to be nearly maximal after 120 minutes [1]. The hypothermia observed at 60 minutes in the group placed onto the benchtop (0–60 minutes) was further extended by placement of those animals back into the cage rack for the period of 60–120 minutes.

Naloxone (10 mg/kg, IP, equivolume to 2 g/kg dose of ethanol), injected at time zero, immediately after temperatures were measured, was observed to have very little influence on body temperature when the cages were returned to their home shelves (Fig. 3). That drug also prevented the elevation in temperature expected from the placement of cages onto the benchtop for the period 0–60 minutes (Fig. 3). Moreover, naloxone caused temperatures to return nearly to normal when the drug was injected into animals that had already experienced an elevation of temperature owing to placement onto the benchtop (Fig. 3), even though the cages were allowed to remain on the benchtop after the injection of naloxone. Note that the basic observation reported in Figs. 1 and 2, i.e., hyperthermia induced by placement of the cages onto the benchtop, is still present on day 37 (Fig. 3) of the experiment.

DISCUSSION

The observation that both chronically handled and naive animals developed hyperthermia when their cages were placed upon the benchtop in the room in which they were housed (controlled environment room) suggests that there was something intrinsically stressful about placement of the individual cage into an open space. Relevant to that notion is the observation that movement of the entire cage rack (with individual cages remaining in their usual positions) into an unfamiliar laboratory possessing different visual and sound stimuli resulted in only a very slight hyperthermia. Furthermore, the finding that movement of the individual cages onto the benchtop in the unfamiliar laboratory produced only a slight additional increase in body temperature suggests that stimuli specific to benchtop placement in the controlled environment room were particularly important. Those stimuli were more effective in producing hyperthermia in the chronically handled group than in the naive group, an observation suggesting that owing to the stressful maneuvers repeatedly performed in the past on those animals (weighing, temperature measurements, drug injections, and rotarod trials), surrounding stimuli acquired the ability, through classical conditioning, to elicit stress hyperthermia [6].

Other investigators [8, 10, 16, 17] have reported that a compensatory response (i.e., hyperthermia) may be elicited by stimuli that had repeatedly accompanied chronic injections of ethanol sufficient to produce hypothermia in the past. According to that view, reflex-like compensatory physiological events necessary to restore homeostasis (normothermia) are brought into play each time an animal is exposed to ethanol hypothermia. Environmental stimuli present during those events may become conditioned via Pavlovian processes to elicit those reflex-like events which if allowed to operate in the absence of ethanol, result in hyperthermia [18,19]. We do not feel that the development of a

compensatory hyperthermia to the hypothermia effects of ethanol was operating in our animals, owing to the fact that they had received only a few spaced exposures to ethanol (see Method section) and, moreover, had exhibited the hyperthermia only when placed onto the benchtop and not when returned to their home cage rack for the waiting period.

Conversely, it appears unlikely that a stress hyperthermia was operating in the studies by Le *et al.* [16] or Mansfield and Cunningham [17], as saline injections were not observed to weaken the conditioned compensatory hyperthermia in animals made tolerant to ethanol. Animals used by those experimenters were also not exposed to the stress of rotarod performance measurement employed in the present study, a procedure which may have contributed importantly to the response observed in the present study. Stress hyperthermia was probably not a factor also in the study by Crowell *et al.* [10], as a comparison group of rats chronically injected with saline in a distinctive room did not evidence hyperthermia during placebo testing.

The existence in the brain of endogenous opiate-like substances (endorphins) that may function as neuromediators of many kinds of physiological responses has now been established [9, 14, 22]. Endorphins have been suggested to mediate some aspects of the response to stressful stimuli which, through a complex series of events, may result in the accumulation of body heat [2, 5, 6]. Naloxone is suspected of blocking the access of endorphins to critical target tissue. Interestingly, we observed naloxone to have no influence on body temperature when animals were placed back into their home shelving units immediately after injection of the drug. Naloxone has been reported by others also to have a negligible effect on normal body temperature in unstressed animals [23]. However, that drug attenuated the elevation in body temperature normally expected when animals were allowed to remain on the countertop and also effectively lowered body temperature in animals whose body temperature was elevated owing to placement onto the benchtop for 60 minutes. These findings are in harmony with others [20,21] which suggest that the endorphins may mediate some aspect of the expression of the hyperthermia we have observed in our experiments. Of course, other as yet poorly understood actions of naloxone may also be involved.

Much like naloxone, the 1.0 g/kg dose of ethanol also attenuated the rise in body temperature expected when animals were allowed to remain on the benchtop for a 60-minute period, particularly in the chronically handled group. Yet, the same dose of ethanol had little effect upon body temperature when the rats were returned to their home shelf positions. We do not, however, wish to strongly suggest, on the basis of that observation, that ethanol also brings about the blockade of opiate receptors involved in stress hyperthermia. It is more likely, for instance, that the drug has a more generalized disrupting action upon the neural tissue subserving temperature regulation [12].

The present observations call attention to a phenomenon which may operate in studies which involve maneuvers that may be stressful and to which the animals may not habituate. Thus, a hyperthermic response may become conditioned to stimuli repeatedly associated with the stress of handling and drug injection. Our finding that such an "anticipatory response" ([7]; cf. also [3,4]) may develop and interact with the effects of drugs emphasizes the need for caution in experiments which employ body temperature as a dependent variable, as well as experiments in which changes in body temperature may influence a dependent variable.

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