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Effects of hypothermia on Pavlovian conditioning in the rabbit: I. Nictitating membrane response

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The nictitating membrane of New Zealand White rabbits was classically conditioned during normothermia and severe hypothermia. After 100 daily acquisition trials for 5 days, the severe hypothermic rabbits only had a mean of 2% conditioned responses in comparison to the 94% conditioned response performance reached by the normothermic rabbits on the 3rd day. The topography of the unconditioned response for the severe hypothermic rabbits consisted of a reduction in amplitude, longer latencies, and no habituation.

It was the purpose of this experiment to determine the effect of severe hypothermia on the Pavlovian conditioning of the rabbit nictitating membrane response in comparison to normothermic rabbits. On the basis of previous research in hypothermia with other species and instrumental paradigms, e.g., Essman and Sudak, 1963 and Sudak and Essman, 1963, 1965, little or no learning was expected in the rabbits which were experiencing severe hypothermia during the conditioning sessions.

Severe hypothermia was defined as a decrease in rectal temperature of 10°C from a normal temperature of 37°C. Rectal temperature is an easily accessible and relatively accurate index of brain temperature. Sarajas and Putkonen (1968) cooled the body and brain temperatures to 20°C in rabbits. Rectal and brain temperatures dropped simultaneously. The absolute difference between rectal and brain temperatures never was greater than 2.5°C.

The following experiment was preceded by a long series of pilot studies. In all cases, we obtained similar results. Therefore, the decision to use only four rabbits in this experiment was based on the apparent potency of manipulations in rectal temperature as a variable in Pavlovian conditioning.

Method

Subjects. The subjects were four male New Zealand White rabbits 40-50 days old at the beginning of the experiment. The rabbits were housed individually in wire mesh cages at room temperature (22°C) and had access to ad-lib food and water.

Apparatus. The rabbits were restrained in Plexiglas boxes, similar to those used by Gormezano (1966), during acquisition training and refrigeration. A stainless steel suturing clip was attached to the skin approximately 3 mm below, and another one 3 mm posterior to, the right eye of each rabbit to deliver the unconditioned stimulus (US). A nylon loop suture was placed through the nictitating membrane of the right eye.

During acquisition training, a potentiometer was positioned on the head of the rabbit. The armature of the potentiometer was connected to the nylon loop suture. Lateral movements of the nictitating membrane deflected the armature and were recorded on a Beckman dynograph located in an adjacent room. Paper speed was 100 mm/sec. Upward deflection of the dynograph recording pens of at least 1 mm during the conditioned stimulus (CS) constituted a conditioned response (CR).

The freezing compartment of a refrigerator was used in conjunction with ice packs to induce hypothermia. The ice packs consisted of commercial plastic sandwich bags filled with water and then frozen. A commercial heating pad was used in cases in which the body temperature continued to decrease after the induction of hypothermia.

Rectal temperature was recorded with a mercury thermometer (Philadelphia Thermometer Co.) inserted into the rectum approximately to a depth of 30-35 mm.

The rabbits, in the restraining boxes, were placed in styrofoam chests during acquisition training. The walls of the chests were 3 mm thick. The inner dimensions of the chests were 50 x 29 x 31 cm. The wall at one end of each chest was removed, and each chest was positioned in front of a speaker with continuous white noise and a speaker for transmitting the CS tone.

The CS was a 1,000-Hz tone of 250 msec duration (70 dB, SPL). The US was a 2-mA ac shock, 50 msec in duration. The offset of the CS was simultaneous with the onset of the US. The mean intertrial interval was 60 sec. A Lab K computer and Tally tape reader controlled the presentation of stimulus events.

Procedure. Four rabbits were randomly assigned to a hypothermic group and a control normothermic group. On the day preceding the start of acquisition training, the rabbits were completely shaven of all fur while under nembutal anesthesia (.25 cc/kg IV in the ear vein). The nylon loop was sutured into the nictitating membrane while the rabbits were anesthetized. The stainless steel clips for delivering the US were attached to the skin near the right eye a few minutes prior to acquisition training.

Acquisition training consisted of 100 CS-US conditioning trials per day for 5 consecutive days. Two animals were run simultaneously.

On each day, preceding acquisition training, the rabbits in the hypothermic group were placed in the freezing compartment of a refrigerator for approximately 1-1/2 h, while in the restraining box, until the rectal temperature had fallen 10°C from a normal of 37°C to 27°C. The door of the freezing compartment remained open 1.5 cm to provide ventilation. Ice packs were maintained on the back of rabbits while they were in the freezer and during acquisition training. The amount of ice packs was adjusted individually for each rabbit to maintain rectal temperature at 27°C during acquisition.

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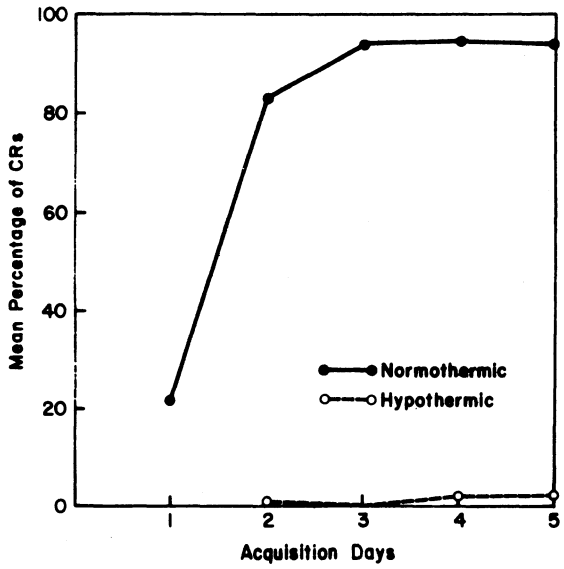

Figure 1. Mean percentage of CRs for 5 days of CS-US pairings for rabbits in a state of normothermic or severe hypothermic rectal temperature.

Rectal temperature was measured at the beginning, middle, and end of acquisition training.

The rabbits in the normothermic control group were placed in an inoperative freezer compartment for 1-1/2 h each day before acquisition training was initiated.

Results

Figure 1 shows the mean percentage of CRs for 5 days of acquisition training for the normothermic control group and the hypothermic group. No CRs were given by the hypothermic rabbits on Days 1 and 3. The mean percentage of CRs on Days 2, 4, and 5 were 1, 2, and 2, respectively. In contrast, the normothermic rabbits reached a mean 94% CR rate on the 3rd day of acquisition training. The difference in CR performance between the two groups is reflected in a statistically significant main effect for temperature groups [$F(1,2) = 359.31$, $p < .005$], and Groups by Days interaction [$F(4,8) = 93.96$, $p < .001$].

The daily mean amplitudes of the unconditioned responses (UCRs) to the ac eyeshock US for the normothermic and hypothermic groups are represented in Figure 2. UCR amplitude was measured at the point of highest deflection of the dynograph recording pens after US onset from a pre-CS onset baseline.

The progressive decrease in the amplitude of the UCRs over the 5 days of acquisition training in the normothermic rabbits indicates UCR habituation. The hypothermic rabbits had an overall lower UCR amplitude than the normothermic rabbits and did not demonstrate UCR habituation. The mean UCR amplitude averaged over the 5 days of acquisition for the normothermic and hypothermic groups was 17.53 mm and 4.85 mm, respectively. This difference in mean amplitude did not yield a statistically significant main effect for temperature groups [$F(1,2) = 15.06$] because of the small df. However, a significant Groups by Days interaction [$F(4,8) = 7.32$, $p < .01$] provides statistical verification for the difference in UCR amplitude so visually apparent in Figure 2.

UCR latency in the normothermic group was ascertainable up to Trial 40, the trial on which the first CR was given in that group on Day 1 of acquisition training. UCR latency was defined as the time elapsed in milliseconds between US onset and UCR onset. The mean UCR latency was significantly shorter ($t = 4.6$, $df = 2$, $p < .05$) in the normothermic rabbits (3.63 msec) in comparison to the hypothermic rabbits (4.92 msec) during the first 39 trials of Day 1.

DISCUSSION

As expected, Pavlovian conditioning of the rabbit nictitating membrane was extensively impaired by severe hypothermia. During 5 days of acquisition training, the highest level of conditioned responses attained by the hypothermic rabbits was an average of 2% CRs. In contrast, the normothermic rabbits reached an asymptotic level of conditioned responses at 94% as early as the 3rd day of training. Extending the acquisition training sessions beyond 5 days is not feasible because of the ravaging effect of severe hypothermia on the rabbit. Hypothermia also affected the topography of the UCR. The hypothermic rabbits had reduced UCR amplitudes and longer UCR latencies than the normothermic rabbits. Habituation of the UCR was observed in the normothermic rabbits but not in the hypothermic rabbits.

Most researchers attribute the learning deficit associated with hypothermia to disruption of the learning process in the central nervous system (e.g., Gehres, Randall, Riccio, & Vardaris, 1973;

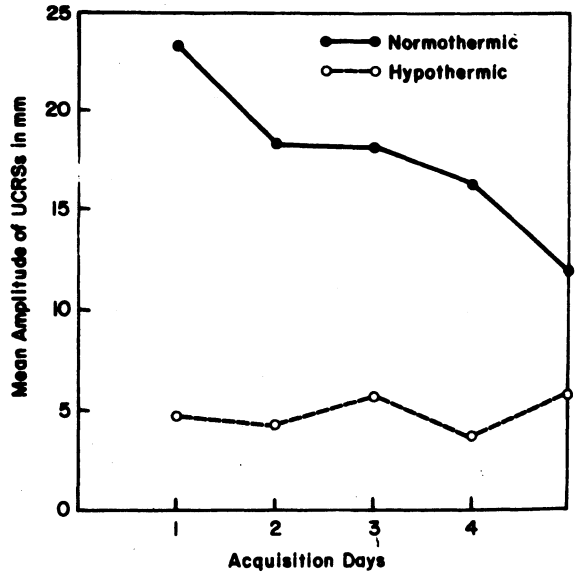

Figure 2. Daily mean amplitudes in millimeters of the unconditioned responses to the eyeshock US for rabbits in a state of normothermic or severe hypothermic rectal temperature during the conditioning process.

Gerard, 1955; Riccio, Hodges, & Randall, 1968; Sarajas & Putkonen, 1968). Most speculations in the literature are minor variations of the notion that severe hypothermia depresses neural activity. The topography of the UCR for the hypothermic rabbits, however, did not exclude the possibility that the hypothermia interfered with the contraction of the nictitating membrane. The extensive shivering of the animal may have disrupted the performance, rather than the learning, of the membrane response.

A second experiment was conducted with heart rate, a response which is continually emitted. Failure to condition this ongoing response is strong indication that hypothermia primarily affects learning, rather than interfere with the voluntary or nonvoluntary performance of a response. This experiment is reported by Stava and Hupka, 1976.

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