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Brain Norepinephrine and 5-Hydroxytryptamine as a Function of Time after Avoidance Training and Footshock[1]

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BAUER, R. H. *Brain norepinephrine and 5-hydroxytryptamine as a function of time after avoidance training and footshock.* PHARMAC. BIOCHEM. BEHAV. 1(6) 615–618, 1973.–Male Sprague-Dawley rats acquired a one-way avoidance task more rapidly 10 min and 24 hr after partial avoidance training or inescapable shock in the start box than 3.5 hr after these treatments. Endogenous levels of brain 5-hydroxytryptamine (5-HT) were not altered 10 min, 3.5 hr, or 24 hr after avoidance training or inescapable shock in the start box. Norepinephrine (NE) was lower 10 min and 3.5 hr after training and inescapable shock but not 24 hr later. Since neither 5-HT nor NE exhibited a U or inverted U function following avoidance training or footshock, these results indicate that endogenous levels of 5-HT and NE are not related to poorer avoidance at intermediate retest intervals.

Avoidance Norepinephrine 5-Hydroxytryptamine

AT THE present time there is considerable controversy regarding the theoretical interpretation of poorer avoidance at intermediate retest intervals (1–8 hr) as compared to those either earlier or later. A number of papers suggested that poor avoidance at intermediate intervals is due to an initial increase and subsequent decrease of fear [6]. decreased locomotion and an increase in incompatible responses, which interfere with avoidance, are presumed to be related to fear. More recently, Spear, Klein, and Riley [14] have suggested that physiological changes which follow a stressful event may be responsible for a memory retrieval deficit 1–8 hr after aversive conditioning. Internal conditions present during learning are assumed to be altered at intermediate retention intervals and consequently memory of originial learning is more difficult to retrieve. Thus, poorer avoidance 1–8 hr after training may be mediated by a mechanism similar to state dependent learning found with various drugs [12].

At the behavioral level, a number of workers have examined this U-shaped retention function but relatively little research has been devoted to investigating physiological changes which might account for this phenomenon. Earlier papers suggested that a decrease in corticosterone, which parallels the initial performance decrement but not the subsequent improvement in avoidance, was responsible for the animal's inability to cope behaviorally with stress [10]. However, more recent evidence has not supported the hypothesis that alteration of the pituitary-adrenal system is related to avoidance deficits 1–8 hr after training [7, 13, 16]. Therefore, evidence relating the pituitary-adrenal system to this phenomenon is at best controversial.

Although the pituitary-adrenal system may not account for poorer avoidance 1–8 hr after training, evidence suggests that alteration of brain norepinephrine (NE) may be related to this phenomenon. A number of studies have reported reduced levels of endogenous brain NE shortly after a variety of stressors [4,11] and a further decrease 2–4 hr following stress [2,15]. Weiss, Stone and Harrell [19] found that rats which could escape and avoid shock have increased NE when sacrificed 20–40 min after training but animals that could neither escape nor avoid showed no change. Drugs which release NE also increase activity and avoidance [8, 15, 17]. These results suggest that poststress reduction of brain NE can perhaps account for avoidance deficits 1–8 hr after training.

The major purpose of the present experiment is to determine if rats sacrificed 3.5 hr after avoidance training have lower levels of endogenous brain NE and/or 5-hydroxytryptamine (5-HT) than those sacrificed 10 min or 24 hr after training. In addition, rats given inescapable and unavoidable shock in the start box of a one-way avoidance apparatus 5 min and 24 hr prior to avoidance training

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acquired the one-way avoidance task more rapidly than rats trained 4 hr after this treatment [1]. Inescapable and unavoidable footshock was, therefore, administered in the start box of a one-way avoidance apparatus in an attempt to replicate these results and determine if NE or 5-HT of trained and stressed animals is differently affected. Thus, the present experiment examined the possibility that altered brain NE and/or 5-HT are related to poorer avoidance at intermediate retest intervals and a test of the generality of the Weiss, Stone, and Harrell [19] results.

METHOD

Animals and Experimental Design

The animals were 156 experimentally naive 120–180 day old male albino Sprague-Dawley rats weighing 300–450 g. These animals were maintained on ad lib food (Purina rat chow) and water, and housed in the laboratory in individual cages for at least two weeks before experimental treatment. Light onset and offset in the colony room were at 6:00 a.m. and 6:00 p.m. respectively. All experimental treatments were given between 11:00 a.m. and 4:00 p.m. These rats were run by two experimenters who were not acquainted with previous research on this phenomenon.

Seventy-two animals received one-way avoidance training (AT) with a tone CS. A tone and inescapable and unavoidable footshock were presented in a semirandom order in the start box of the one-way avoidance apparatus for 72 additional animals (RTS, i.e., random tone and shock). One-half of these two groups was given one-way avoidance training 10 min, 3.5 hr, or 24 hr later (hereafter referred to as the retest) while the remaining animals were sacrificed at one of these time intervals and their brains later analyzed for NE and 5-HT. There were 12 animals in each of these 12 independent groups. Twelve untreated animals were also sacrificed and their brains analyzed for NE and 5-HT.

Apparatus

The walls of the one-way avoidance apparatus were Plexiglas and the floor was constructed of 2 mm in dia. stainless steel bars placed 14 mm apart (center–center). The start box and goal box were each 30 cm long, 12 cm wide, and 16 cm high. A 4.0 cm high hurdle and hand operated door separated the two compartments. The side of the door facing the goal box was black. Raising and lowering the door between the two compartments activated and terminated the CS, US, and a timer. The CS was a 75-*db* 4,5000 CPS tone generated by a Mallory Sonalert (Model SC628H) placed 30 cm above the apparatus. The US was a 1.5-ma footshock generated by a Foringer shock generator and scrambler.

Procedure

During avoidance training and the retest the start box walls were covered on the outside with medium gray paper and the goal box walls were covered with black paper. The animals were adapted to the start box for 30–60 sec and a trial was begun only when the animal was facing the door. Avoidance training required the rat to cross from the start box to the goal box before the 5 sec CS-US interval elapsed. When the rat crossed into the goal box the door was lowered and the animal was confined there for 5 sec. The animals spent the 30 sec intertrial interval in a 40 cm high holding box. All animals were transported in their home cage and handled by the base of the tail.

The AT animals were trained to one avoidance following at least one escape and returned to the colony room until sacrificed or receiving a retest consisting of 15 additional avoidance training trials 10 min, 3.5 hr or 24 hr later. A total of 36 AT animals (n = 6 per group) received avoidance training and the retest before any of the RTS animals were run. This procedure was followed because the CS and US were presented in the start box of the avoidance apparatus for the RTS animals and it was, therefore, impossible to use a yoked control design. Thus, the number of CSs and USs and duration of the USs received by the RTS animals was determined on the basis of the avoidance training results of the first 36 AT animals. These AT animals received a mean of 5.2 shocks with a mean duration of 1.1, 2.7, 1.0, 0.7 and 0.5 sec respectively before an avoidance. The RTS animals received five inescapable and unavoidable shocks of these durations. Duration of the five tones was always 5 sec and tone and shock were separated by 5–15 sec. The gray paper was removed from the start box walls and a white card was placed over the start box door when animals received the CS and US at random in the start box. The 30 sec intertrial interval was spent in the same holding box used during training and the retest. The RTS animals were returned to the colony room until sacrificed or receiving 15 retest trials, as described above, 10 min, 3.5 hr, or 24 hr later.

Sacrificed animals were transported from the colony room to a room adjoining the experimental room and decapitated with large shears. Their brains were removed and immediately frozen at −17°C before determination of endogenous NE and 5-HT by the method of Laverty and Taylor [9]. The brains were coded and NE and 5-HT analysis was done blind.

RESULTS

Behavioral

The median number of trials to the first avoidance during one-way avoidance training was not significantly different for the first (6.1) and last (5.0) 36 AT animals. These results indicate that the number and duration of shocks received by RTS animals constituted an adquate control. Median number of trials to the first avoidance during training for animals given the retest (5.2) or sacrificed (4.7) also did not differ significantly. Therefore, retested and sacrificed animals received a similar number of footshocks.

Since number of avoidances during the retest and trials to the first avoidance yielded a similar pattern of results only trials to the first avoidance are reported. Figure 1 presents the median number of trials to the first avoidance during the retest for the two treatment groups at each of the three retest intervals. The heavy black dot represents the median number of trials to the first avoidance during avoidance training for sacrificed and retested animals. Inspection of Fig. 1 suggests that animals retested 10 min and 24 hr after avoidance training or footshock required fewer trials to the first avoidance than animals retested 3.5 hr later. Mann Whitney U tests revealed that animals retested 10 min and 24 hr after avoidance training made the first avoidance in fewer trials than those retested 3.5 hr later ($p<0.01$). Rats retested 10 min and 24 hr after footshock also avoided in significantly fewer trials than animals retested 3.5 hr after footshock ($p<0.01$). Comparisons

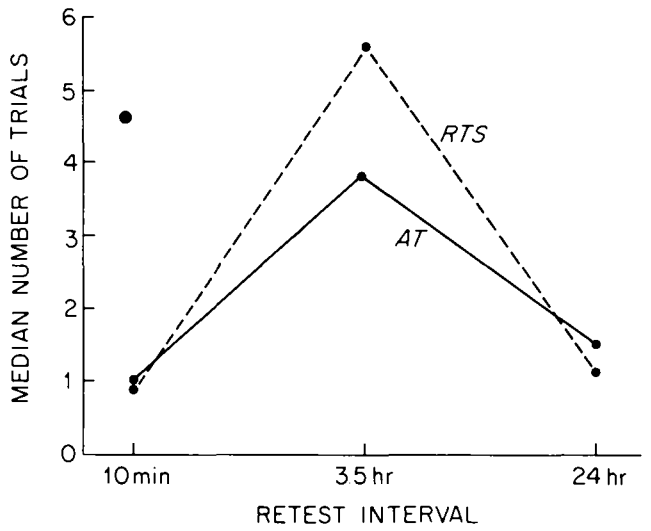

FIG. 1. Median number of trials to the first avoidance for AT and RTS animals tested at one of three retest intervals. The heavy black dot is the median number of trials to the first avoidance of AT animals during avoidance training.

between AT and RTS animals at each retest interval were nonsignificant. Thus a U shaped function of approximately the same magnitude was found for rats receiving avoidance training and the CS and US in the start box.

As would be expected, Fig. 1 also indicates that AT animals required fewer trials to the first avoidance when retested 10 min and 24 hr after training than during avoidance training. However, RTS animals also appear to require fewer trials to the first avoidance when retested 10 min and 24 hr after footshock than AT animals during avoidance training. Mann-Whitney U tests revealed that AT and RTS animals retested 10 min and 24 hr after treatment required fewer trials to the first avoidance than sacrificed animals during avoidance training ($p<0.05$; in these comparisons avoidance training data of 12 randomly selected sacrificed animals were included to avoid mixing correlated and uncorrelated data and to form samples of equal size). Trained and foot shocked animals retested 3.5 hr after treatment did not differ significantly from these sacrificed animals. These results indicate that when retested 10 min and 24 hr but not 3.5 hr later both AT and RTS animals required fewer trials to the first avoidance than naive animals during avoidance training.

Chemical

The upper panel of Fig. 2 presents mean 5-HT levels in μg/g of brain tissue for the control group and six experimental groups. As can be seen, 5-HT was not altered by avoidance training or footshock. A 2 x 3 factorial analysis of variance of the 5-HT data revealed no significant differences. *T*-tests between nonshocked controls and each of the six experimental groups were also nonsignificant.

The lower panel of Fig. 2 shows mean NE levels in μg/g of brain tissue for the control group and six experimental groups. Inspection of Fig. 2 suggests that trained and stressed animals have lower NE levels 10 min and 3.5 hr after treatment but not 24 hr later. Norepinephrine values for controls are similar to those reported previously with the present method of analysis [17]. A 2 x 3 factorial analysis of variance of the NE data revealed no significant effects of prior treatment or the treatment-sacrifice interval ($p<0.10$). Comparisons between nonshocked controls and each treatment group indicated that AT-10 min, AT-3.5 hr, and RTS-3.5 animals had signficantly lower NE levels ($p<0.01$; one-tailed). The RTS-10 min animals also had lower NE levels than controls but this difference was of lesser magnitude ($p<0.05$).

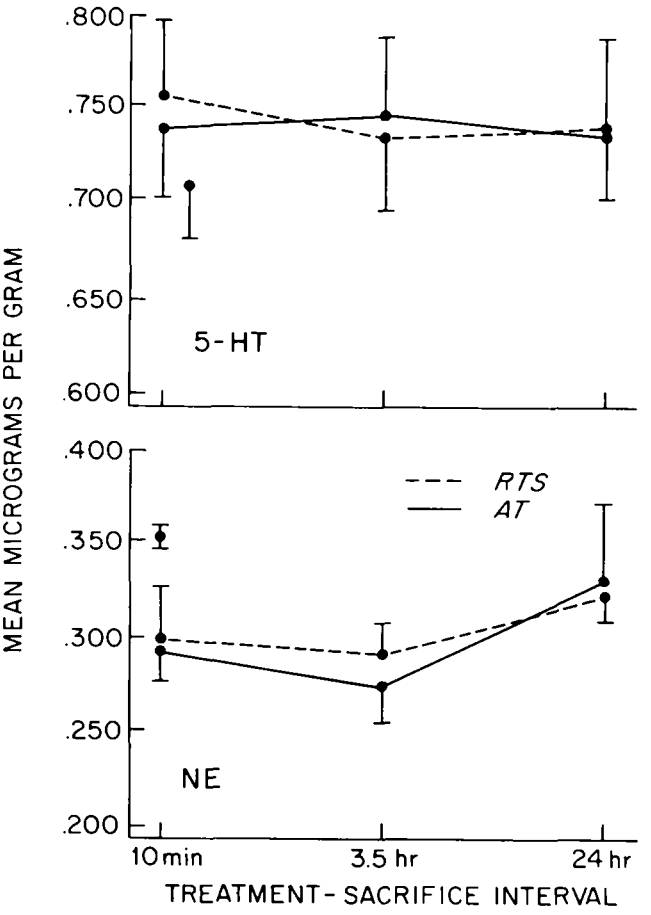

FIG. 2. Mean 5-HT and NE in μg/g of brain tissue for nonshocked controls (black dot), AT, and RTS animals sacrificed at one of the three intervals after treatment. Verticle lines ± SEM.

DISCUSSION

The present behavioral results indicate that a U-shaped avoidance function of approximately the same magnitude occurred following avoidance training and footshock in the start box of the avoidance apparatus. Furthermore, animals retested 10 min and 24 hr but not 3.5 hr after avoidance training or footshock were superior to naive animals. These results indicate that poor avoidance at intermediate retest intervals can occur in the absence of escape and avoidance training.

Poorer avoidance has previously been found with rats given unsignaled, unavoidable, and inescapable shock in the start box of a one-way avoidance apparatus 4 hr prior to avoidance training [1]. In contrast to these results, Brush [5] found no U shaped function when unsignaled shock

was given in a homogeneous shuttle box prior to avoidance training. Since results in the one-way avoidance task suggest that avoidance deficits at intermediate intervals are due to high levels of fear conditioned to specific apparatus cues failure to find this effect in a homogeneous shuttle box following unsignaled shock may be due to the animal's inability to discriminate the shocked from the nonshocked side.

The biochemical results indicate that rats receiving avoidance training and unavoidable and inescapable shock in the start box have lower whole brain NE levels than nonshocked controls 10 min and 3.5 hr after these treatments but not 24 hr later. These results are consistent with a number of other reports indicating that foot shock lowers NE [4,11]. Failure to find any change in 5-HT following avoidance training or inescapable shock is also consistent with previous reports [4, 11, 18]. Since neither NE nor 5-HT exhibited a U or inverted U function following avoidance training or footshock, alteration of these amines is apprently not related to inferior avoidance at intermediate retest intervals. These results suggest that changes in whole brain NE or 5-HT are not the mechanism responsible for either a deficient coping response or state depended memory retrieval. It also appears unlikely that regional changes in brain NE can account for poorer avoidance at intermediate intervals because footshock reduces NE uniformly throughout the brain [3,4]. However, these results do not completely eliminate NE or 5-HT from consideration since turnover rates of NE and 5-HT are increased after footshock [4,18]. Increased turnover of NE and/or 5-HT 1–8 hr following footshock may account for poorer avoidance at these intermediate retest intervals.

The present NE results are at variance with the reported NE increase following avoidance training but no change in yoked controls receiving unavoidable and inescapable shock [19]. A number of procedural differences could account for these discrepant findings. First, in the Weiss, Stone and Harrell study shock was administered to the tail and increased systematically across trials whereas in the present experiment footshock was used and intensity remained constant. A wide variety of stress situations are reported to reduce brain NE and it is, therefore, unlikely that these differences can account for the conflicting results. As in the present experiment, rats in Experiment 1 of the Weiss, Stone and Harrell study received approximately five shocks but were given a total of 70 trials requiring 2.5–3 hr. Therefore, time spent in the avoidance apparatus and level of avoidance training differed considerably in the two studies. As few as five footshocks a minute for 5 min [11] and six per minute for 15 min [4] are reported to decrease endogenous NE but six shocks per minute for 10 min alternated with 20 min of rest for 3 hr produce no change [18]. Therefore, there is a possibility that confinement in the avoidance apparatus for periods with no shock can account for these discrepant findings. However, level of avoidance training differed considerably and this factor remains a likely possibility.

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