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Stress Responses of Rats with Septal Lesions

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BRICK, J., R. G. BURRIGHT AND P. J. DONOVICK. *Stress responses of rats with septal lesions.* PHARMAC. BIOCHEM. BEHAV. 11(6) 695–700, 1979.—Rats which had received septal lesions or underwent control surgery were exposed to stress in four experiments. We examined plasma corticosterone (Experiment 1), gastric ulceration (Experiment 2), motor activity (Experiment 3) and whole brain norepinephrine (Experiment 4) following 3 hours of prone-restraint-stress conditions at 21°C or 5°C. Rats with septal lesions had higher steroids, less severe ulceration, greater motor activity and higher brain norepinephrine than controls following stress. Changes in hyperemotionality and differences in coping strategies are discussed.

Septal Stress Temperature Restraint Activity Norepinephrine Corticosterone Ulcers

IN RATS, one of the most dramatic alterations following septal lesions is the rage syndrome [5,6]. Many aspects of the septal rage syndrome could be interpreted as an increase in stress reactions. The adrenal-pituitary system is an important part of an organism's mechanism for coping with environmental stress [23]. The release of glucocorticoid hormones [1] and gastrointestinal lesions [31] are often used as an index of the degree of emotionality.

In a previous study [28], no differences in basal levels of corticosterone were found between undisturbed animals with septal lesions and controls, but septal lesions prevented a rise of corticosterone in response to an air blast. However, these results are inconsistent with the report [22] that following 5 seconds of handling or three minutes exposure to a novel open field environment, rats with septal lesions release corticosterone sooner and in greater quantities than did controls. The importance of environmental influences on this differential corticosterone measure is further supported by the fact that, in both studies, undisturbed rats with septal lesions and controls showed no differences in their level of this hormone. However, the hormonal response to stressful conditions is unresolved.

A variety of stressors, including immobilization and low temperatures, are known to cause a decrease in the steady state levels and an increase in turnover of central nervous system norepinephrine (NE) [7, 12, 25, 32, 34]. It has been suggested that septal-lesion induced emotional reactivity may be due to altered monoamine levels, and that lesions of the septal area decrease whole brain NE, but not turnover [4]. However, no change in NE levels following septal lesions also has been reported [13]. Because of these discrepancies in the literature we decided a more comprehensive examination of the effects of septal lesions on stress reaction was warranted. Therefore, we examined changes in corticosterone, gastric lesions ("stress ulcers"), and whole brain NE in rats with septal lesions or controls following three hours restraint at 5° and 21°C. We also attempted to measure activity during restraint since septal lesions alter motor activity, and brain NE may be involved in such behavior [32].

GENERAL METHOD

Subjects

Subjects were adult male albino rats of the Sprague-Dawley strain (Taconic Farms, Inc.) which weighed approximately 250–300 g at the beginning of each experiment when they were 75 days old. Rats were experimentally naive unless stated otherwise. All rats were housed in individual cages with Purina Rat Chow and tap water available ad lib except on the day of the experiment when they were food deprived for 20 hours. Although a pilot study indicated that 20 hours food deprivation had no significant effect on basal levels of corticosterone (or norepinephrine), rats were food deprived to facilitate the development of gastric lesions; since food deprivation itself may have stressful effects, this procedure was used in all experiments. The vivarium in which the animals were housed was maintained on a 12:12 hr light/dark cycle (lights on 0700 hr) and temperature was maintained at approximately 21°C. Subjects were handled three days prior to surgery and one day following surgery and were allowed 10–14 days to recover before testing.

Surgery

Half of the subjects in each experiment were randomly assigned to the septal group, the remainder were assigned to the non-lesioned, operated control group. For surgery, animals were anesthetized with sodium pentobarbital (50 mg/kg IP) supplemented with local injections of lidocaine hydrochloride. A Kopf stereotaxic instrument with a modified head holder was used to position the electrode. The modification consisted of a nose clamp to immobilize the head and maintain the surface of the skull in a horizontal plane. The tip of the electrode was lowered 5.5 mm from the surface of the dura at a 25 degree angle toward the midline. Control animals underwent the same procedure except that the electrode was not lowered. Septal lesions were produced electrolytically by passing a 1.5 mA anodal DC current for 20 seconds through the uninsulated tip (approximately 0.25 mm) of a stainless steel insect pin (00 ga) to a rectal cathode. Rats were tested 10–14 days after surgery unless otherwise stated.

Histology

Rats used in biochemical experiments could not be used for histology. Therefore, approximately 10% of lesioned rats in such experiments were randomly removed from assay experiments and used for histology. For those rats decapitated for biochemical data, brain perfusion was not possible. Instead, brains were placed in a rapid hardening solution of one part 40% Formalin to 1 part 95% ethanol [19]. Otherwise rats were perfused intracardially with normal saline, followed by 10% buffered Formalin. Brains were removed and fixed in 10% buffered Formalin. Sections were stained with a metachromic stain technique [8] which showed cell bodies and fiber tracts.

Immobilization

The apparatus consisted of a 6 cm diameter tube made of 7.5 mm mesh which was closed off at one end with a wooden block. Rats were placed in the tube with two 0.5 cm diameter dowels placed at the base of their tails to prevent escape. The dowels were adjusted to provide a snug fit, without pinching the rat or forcing it into a contorted position. All behavioral testing was done in a sound attenuated testing room or environmental temperature chamber.

Histological Results

Microscopic examination revealed large bilateral damage restricted to the precommissural septum [10]. Typically there was complete destruction of the medial and lateral nuclei; in some cases there was partial sparing of the lateral septum. Lesions frequently extended into the wall of the lateral ventricles, but were restricted to the area ventral to the corpus callosum and usually dorsal to the anterior commissure. In some cases the diagonal band of Broca was also damaged. There was no apparent relationship among differences in these large lesions and the behavioral or biochemical data reported.

EXPERIMENT 1: PLASMA CORTICOSTERONE LEVELS FOLLOWING RESTRAINT

Since rats with septal lesions are often described as hyperemotional, and adrenal activity is often taken as an index of emotionality, we measured plasma corticosterone following restraint-cold stress.

All testing was done at the same time during the light phase of the 12:12 light/dark cycle each day, beginning at 1300 hr and ending at 1600 hr. Rats (n=36) were assigned randomly to septal or control surgical groups and then to one of three treatment groups: no stress (21°C), restraint stress (21° or 5°C). Six rats per day (3 from each surgical group) were deprived of food for 20 hr and then secured in the restraining apparatus and placed in the cold room (or a quiet testing room). After three hours, animals were removed from the cold room (or testing room) and the restraining apparatus and decapitated. Non-stressed rats were simply removed from their home cages to a separate room a few feet away and decapitated. The total time from removal to sacrifice was less than 20 sec. A microfluorimetric assay for plasma corticosterone was used [11], and brains were removed and placed in the rapid hardening solution.

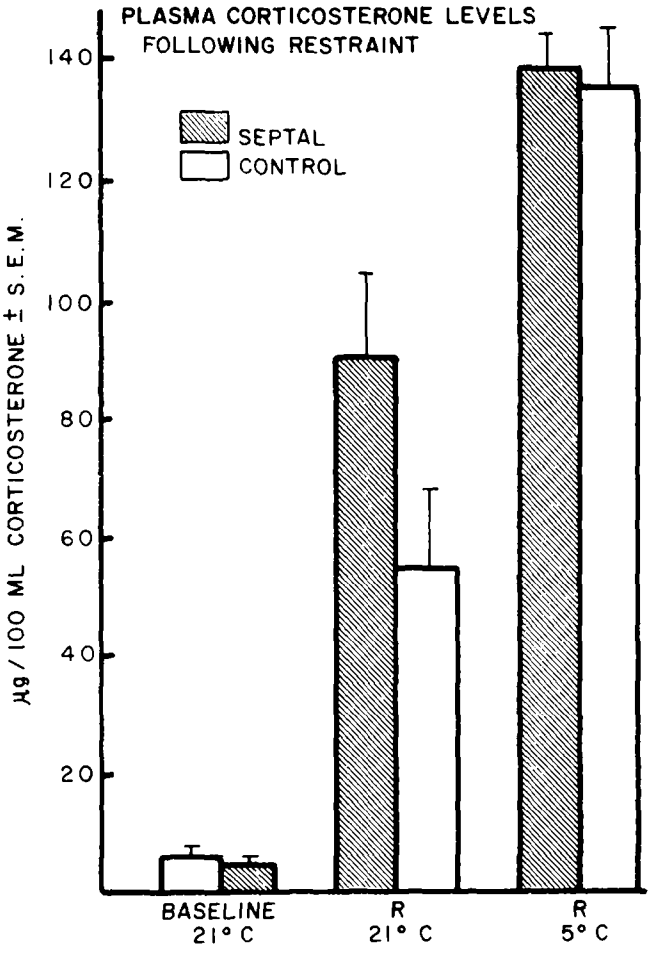

FIG. 1. Plasma corticosterone levels following restraint.

RESULTS AND DISCUSSION

All rats struggled against the restraining apparatus. More than half the time there was vocalization within a minute of being placed in the restrainer. Rats sometimes twisted onto their backs and tried biting the mesh. About half the rats urinated and defecation occurred occasionally. As indicated by the rise in plasma corticosterone (see Fig. 1), however, restraint plus cold was the most stressful condition, $F(2,30)=126.7, p<0.001$. At 5°C there was no effect of surgical treatment, perhaps due to a ceiling effect. However, for rats restrained at 21°C, there were significantly higher levels of plasma corticosterone in animals with septal lesions (Dunnett test, $p<0.01$).

These results are consistent with other findings of: increases in plasma corticosterone following various forms of stress [1,23], no changes in basal corticosterone following septal lesions [22,28], but higher levels of corticosterone in rats with septal lesions than in controls following stress ([21] but see [28]). Such data may be due to changes in adrenal activation threshold [27].

EXPERIMENT 2: GASTRIC LESIONS FOLLOWING RESTRAINT

The development of gastric lesions (so-called "stress ul-

cers") is often associated with highly stressful environmental situations. We therefore examined gastric ulceration in rats from septal lesion and operated control groups after restraint in the cold.

METHOD AND PROCEDURES

Fifteen rats with septal lesions and 15 control rats were food deprived for 20 hours prior to being weighed and restrained in a cold room (5°C) for three hours. Six rats from each group had been used one month earlier in a spatial alternation experiment after receiving septal lesions 6 weeks prior to the present study. The remaining rats were experimentally naive. The data from these two differentially experienced groups did not differ statistically and therefore were combined appropriately. Following the three hour period of restraint, rats were sacrificed with a 50 mg dose of sodium nembutal, reweighed, stomachs removed and opened by incision along the lesser curvature. Stomachs were pinned to a styrofoam board and washed with tap water. The number, length, and width of each lesion was measured using a Zeiss dissecting microscope. For neurohistology, animals were perfused intracardially with 10% Formalin after removal of the stomach.

RESULTS AND DISCUSSION

In keeping with previous investigations [14, 29, 30], the total length of gastric lesions for each rat was used as the principal measure of extent of ulceration. Results were analyzed using non-parametric statistics (Mann-Whitney).

As expected (cf. [28]) all gastric lesions were found in the lower glandular portion of the stomach, none in the upper rumenal area. No ulcers were found in the duodenum. The total length of ulceration in control rats was longer than that found in rats with septal lesions ($U=33$, $n=15$, $p<0.001$, see Fig. 2). However, there was no significant difference between surgical groups in: mean frequency of ulcers (mean=2.1 for septals, 3.7 for controls); percentage of rats that developed ulcers (66%, 78% respectively); or in percent weight lost (0.6%, 1.2%) during testing. These results suggest that rats with septal lesions were not as susceptible as controls to severe gastric ulceration induced by restraint in the cold.

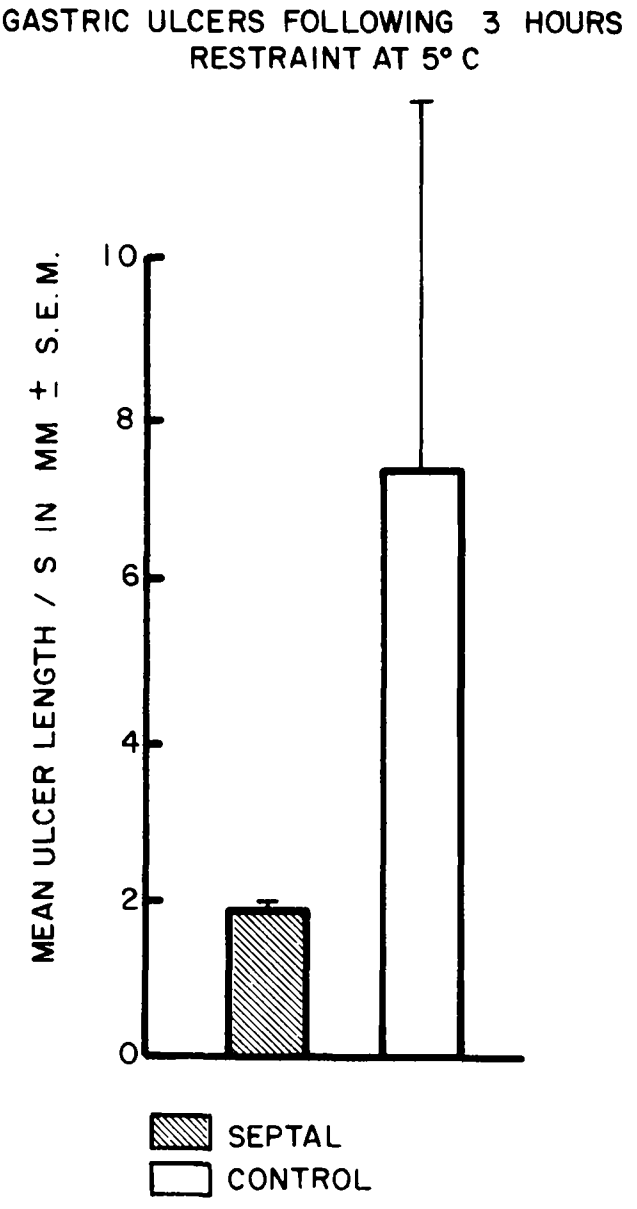

FIG. 2. Gastric ulcer length in septal and control rats.

EXPERIMENT 3: MOTOR ACTIVITY DURING RESTRAIN AND NON-RESTRAINT

Some investigators have reported that the probability of gastric erosions is inversely related to body temperature during restraint [2]. Since physical restraint is known to impair thermoregulation [3], the hypothesis was formed that an animal's resistance to physical restraint may alter its susceptibility to severe gastric ulcers. While we were unable to monitor body temperature, we measured motor activity of septal and control rats during restraint.

METHOD AND PROCEDURE

Rats (n=24; 12 septals, 12 controls) which had been food deprived for 20 hr were restrained in the testing room at 21°C for three hours as previously described, or left in their home cages (unrestrained condition). Stoelting activity monitors were calibrated using a pendulum which passed through the center of their electromagnetic fields. Activity level sensitivity was set at about "50" so that movements such as struggling in restrained rats, or ambulatory movement in unrestrained rats, were recorded. As will be seen below, restrained rats had higher activity scores than their unrestrained counterparts. It should be emphasized that these counts do not necessarily represent equivalent categories of behavior.

It was not feasible to record activity in the cold room due to mechanical and moisture related problems. However, the types of behavior we were measuring did not differ qualitatively between the two temperatures.

RESULTS AND DISCUSSION

Rats restrained at 21°C exhibited behavior similar to those restrained at 5°C, including gnawing the apparatus, vocalization, tail thrashing and attempts to escape and urination.

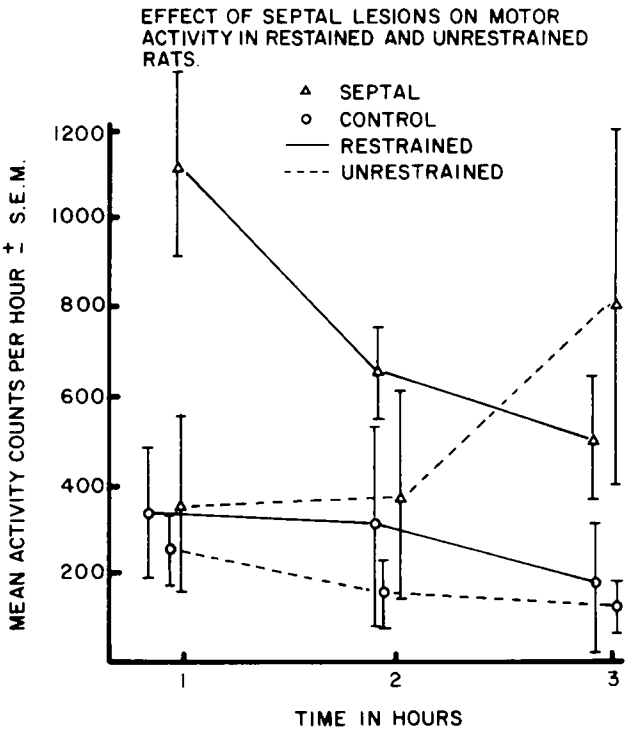

FIG. 3. Automated activity counts in restrained and unrestrained rats.

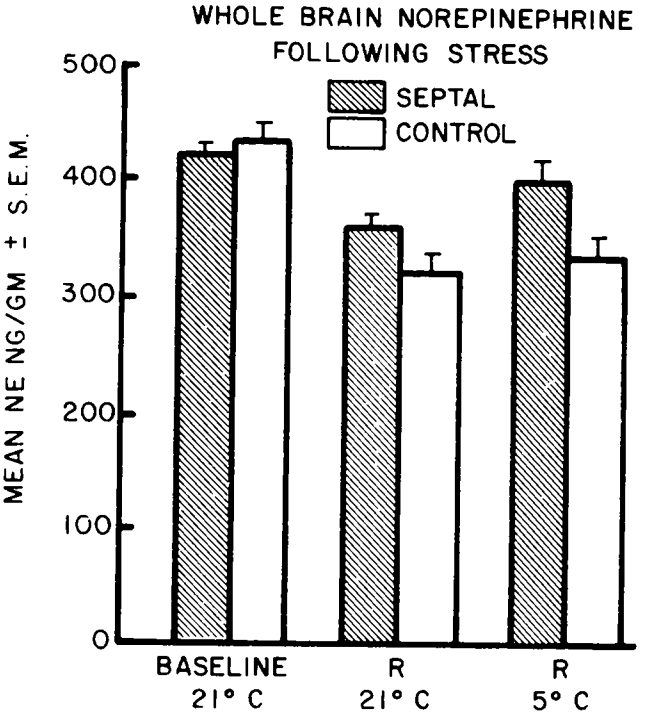

FIG. 4. Whole brain norepinephrine following stress.

Activity scores from one unrestrained control rat were not included in data analysis since they were more than 5 times greater than the next highest activity counts recorded in any group, and did not correspond to visual observations of the rat's behavior.

Rats with septal lesions were significantly more active than controls, $F(1,19)=6.62$, $p<0.025$, as can be seen in Figure 3. Activity counts of restrained rats with septal lesions were much greater than those for control or unrestrained rats with lesions during the first hour of testing, $t(10)=2.55$, $p<0.025$, but rapidly decreased over the three hour test. In contrast, the relatively low level of activity counts recorded initially for restrained control rats was comparable to that obtained for unrestrained septal and control groups, and decreased only slightly during the three hours of testing (surgery × restraint × time: $F(2,38)=4.2$, $p<0.025$).

EXPERIMENT 4: WHOLE BRAIN NOREPINEPHRINE FOLLOWING RESTRAINT

Norepinephrine has been reported to decrease following stress [32,34], and has been implicated in emotional states [20], as well as changes in motor activation [32]. Thus, we measured the effects of stress and septal lesions on whole brain levels of NE.

METHOD AND PROCEDURE

To determine the effect of stress on brain NE, 18 septal and 18 control rats were food deprived for 20 hr and then randomly assigned in a design identical to that used in Experiment 1 (no stress 21°C, restraint stress for 3 hr at either 21°C or 5°C). Rats were quickly decapitated, as in the first experiment, and brains were immediately removed and frozen with dry ice for later NE determination using a modification of several procedures [15].

RESULTS AND DISCUSSION

In keeping with the previous reports ([13] but see [4]), we found that in the non-stress condition there was no difference in whole brain NE between groups (whole brain NE ng/g ± SEM; septals=425 ± 11; controls=431 ± 19). These levels of whole brain NE in non-lesioned rats are in general agreement with the findings of other investigators [4].

As can be seen in Fig. 4, restraint stress resulted in a significant decrease in NE levels relative to unstressed controls. Under both temperature conditions, restraint resulted in a decrease in NE relative to the baseline condition. While rats with septal lesions had higher levels of NE than controls following restraint at both temperatures, this difference reached statistical significance only following restraint at 5°C (Dunnett test, $p<0.05$). Thus, as indexed by whole brain NE levels, it would appear that rats with septal lesions were less stressed than controls at this temperature.

GENERAL DISCUSSION AND SUMMARY

These four experiments attempted to determine if there were differences in stress responses between rats with septal lesions and operated controls. Rats with septal lesions had intact but attenuated stress responses as measured by less severe gastric ulceration and greater resistance to whole brain NE depletion (i.e., they had more NE) than similarly stressed controls.

The differential susceptibility to ulceration in the two groups may be related to different amounts and patterns of motor activity. Weiss and co-workers [33] suggested that the expression of an aggressive response reduced gastric lesions. In our restraint apparatus, rats with septal lesions increased motor activity such as struggling and gnawing, and it is tempting to speculate that such behavior reduced the severity of ulcers. However, increases in general activity, such as locomotion, also may be positively correlated with ulceration [24]. Further, other explanations are possible. For example, the high motor activity of rats with septal lesions may have acted to increase or preserve body temperature, thus protecting against ulceration [2].

We also found differences in brain NE as a function of stress. Septal lesions did not alter resting levels of NE. Following exposure to stressors both groups showed significantly lower levels of NE; however, control rats had significantly less NE than septals. The decrease in NE following stress is probably due to NE release exceeding synthesis [17,32]; we suggest that depletion of NE was less in the septal than control groups because displacement activity decreased the stressfulness of the situation. Rats with septal lesions are notorious for perseverating on specific stimuli [9]; in this case, such perseveration may have helped protect against ulcers.

However, not all of the indices of emotionality which we used indicated an attenuated response to stress in rats with septal lesions compared to control rats. We observed significantly higher levels of corticosterone in rats with septal lesions following restraint at 21°C. This is consistent with the work of other investigators [21] who found that rats with septal lesions released greater amounts of corticosterone than controls when exposed to apparently less stressful stimuli (handling, or a novel open field) than ours. While our corticosterone data fit nicely with the classic septal-hyperemotionality literature, less severe gastric lesions and less NE depletion do not. Why such a dissociation exists is somewhat unclear, but is not unique to this experiment [18].

In our studies, septal lesions may have induced hyperemotionality following stress (as measured by corticosterone) and hyperreactivity (as measured by motor responses to restraint). These responses may act as part of a coping mechanism which reduced ulcer severity. Stress induced release of NE may have increased activity or aggression as suggested in other studies [16,26]. This increased activity or aggressive behavior may have provided "relevant feedback", thus reducing NE utilization and ulceration. The hypothesis that the septum functions as an integral part of a comparator mechanism [9] would imply such differential weighting of responses to information derived from external and internal environmental stimulation of the organism.

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