PII S0031-9384(98)00028-6

# Involvement of the Anterodorsal Thalami Nuclei on the Hypophysoadrenal Response to Chronic Stress in Rats

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SUÁREZ, M., M. A. MAGLIANESI AND N. I. PERASSI. *Involvement of the anterodorsal thalami nuclei on the hypophysoadrenal response to chronic stress in rats.* PHYSIOL BEHAV **64**(1) 111–116, 1998.—Anterodorsal thalami nuclei (ADTN) exert an inhibitory influence on the hypophysoadrenal system (HAS) under basal and acute stress conditions; however, after chronic stress, the effect is different. The response to chronic immobilization stress (IMO) (forced immobilization for 15 min/day for 12 days) and variable chronic stress (V) (24-day exposure to different stressors per day) of plasma ACTH and corticosterone (C) in rats with anterodorsal thalami nuclei lesions was studied. In sham-lesioned rats, chronic immobilization stress and variable chronic stress induced a significant increase in plasma ACTH and C and a reduction of adrenal C content. After exposure of lesioned rats to chronic immobilization stress, there was a decrease of plasma ACTH compared to that in unstressed lesioned rats. In contrast, there was significant increase in ACTH levels after variable chronic stress, this increase being smaller than the variable increase elicited in sham-lesioned rats. In all stressed lesioned animals, plasma C remained unchanged. However, adrenal C content decreased significantly compared to that in unstressed lesioned rats. These findings demonstrate that anterodorsal thalami nuclei lesions attenuated the hypophysoadrenal system response to chronic stress. These data are in contrast to those obtained in previous studies under basal and acute stress conditions. The reason for this discrepancy is at present unknown, and its elucidation will require further studies.   © 1998 Elsevier Science Inc.

Chronic stress     Thalamo     ACTH     Corticosterone

THE influence of extrahypothalami limbic structures on the response of the hypothalami-hypophyso-adrenal axis to stress has been the subject of several studies, which have demonstrated that the hippocampus (8,12), amygdala (9,26), septum (4,6), and other structures modulate the hypophysoadrenal response to several types of stressors.

In previous works, we demonstrated that in rats the anterodorsal thalami nuclei (ADTN) exert an inhibitory influence on the hypophysoadrenal system (HAS) under basal and acute stress conditions. Lesions in the ADTN evoked an increase in plasma ACTH and corticosterone (C) concentrations (21,25) and potentiated the response of plasma C after acute stress (23). However, when the animals were exposed to chronic stress, the effect of ADTN lesions on the HAS was different. After exposure of lesioned rats to chronic inmobilization stress (IMO), a reduction of plasma ACTH compared to unstressed rats was evident (21,24). Possible explanations of this paradoxical result are an adaptation process, exhaustion of the releasable pool of hormones, or ACTH suppressing the feedback effect of circulating glucocorticoids. The present work aims to elucidate whether this is an adaptation process or an exhaustion of the releasable pool of hormones. With

this purpose, plasma ACTH and adrenocortical activity in response to variable chronic and unpredictable stress were evaluated in ADTN-lesioned rats.

## METHOD

Female Wistar rats were used in this study. At the time of operation the animals were about 8 weeks old and weighed 170–200 g. All animals were subjected to the same conditions; they were housed in a temperature-controlled room ($22 \pm 2°C$) under artificial illumination (12:12 h light–dark; lights on at 0700 hours), with water and food available ad libitum. The rats were handled daily by the same investigator for 30 days until they were decapitated. Details of the handling procedure are reported elsewhere (16). Decapitation was performed on diestrus. Diestrus was determined by examination of vaginal smears.

### Surgery

The animals were anesthetized with 2,2,2-tribromoethanol (200 mg/kg) and then placed in a stereotaxic instrument. Their skulls were surgically exposed and holes were drilled bilaterally, permit-

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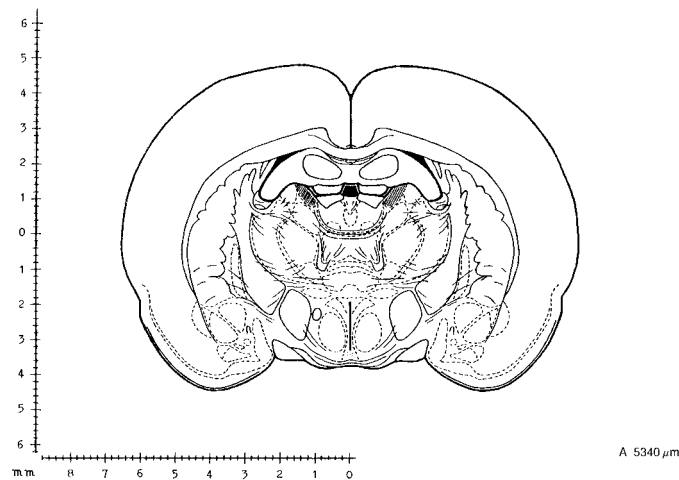
FIG. 1. Scheme of damage following lesioning of the anterodorsal thalami nuclei (plane A, 5340 $\mu$m, from the atlas of König and Klippel (15)).

ting an electrode to be stereotaxically guided into the ADTN. The stereotaxic coordinates derived from the König and Klippel atlas (15) for the ADTN were 5.3 mm anterior to the lambda, 1.2 mm lateral to the sagital suture, and 1.0 mm above the horizontal zero plane and 4.8–5.0 mm below the surface of the skull. Bilateral electrolytic lesions were made by passing 1 mA of cathodic current through the insulated tips (0.25-mm diameter) of a stainless steel electrode for 30 s. The same procedure was performed on sham-lesioned animals, but no current was passed through the electrodes. A schematic representation of the locations of the lesion points is presented in Fig. 1.

The sham-lesioned and lesioned rats were randomly assigned to three groups: a) control; b) chronic immobilization stress; and c) variable chronic stress. The control group was not subjected to any stressful procedure.

### IMO

Each morning, sham-lesioned and lesioned rats were subjected to forced immobilization. The animals were removed from their cages and restrained by placement into a 6-cm-diameter metal cylinder for 15 min/day for 12 days. Taking into account that plasma hormonal response to a stressor such as immobilization is, in general, maximal by 15 min (13), a stress duration of this length was chosen. Rats were decapitated between 0900 and 1200 hours on the day of final session of immobilization.

### Variable Chronic Stress (V)

After surgery, a 24-day variable-stressor paradigm (the modified Katz stress model (14)) was used. Individual stressors are listed in Table 1.

The type of stressor and day on which it was applied were chosen by using a random number table, except for that on Day 24. In this case, noise was used as the stressor on the day preceding the decapitation to avoid the unpredictability associated with this chronic stress model (10). The following stressors were used: a) 4 h of noise produced by an alarm bell (85 dB); b) ether anesthesia until loss of consciousness; c) two intraperitoneal (i.p.) injections of isotonic saline; d) 24 h of food deprivation; e) 1 h of restraint as described under IMO. All rats were killed on Day 25 (between 0900 and 1200 hours) by decapitation within 25 s after being taken from their home cage.

### Assays of Hormones

Immediately after decapitation, trunk blood was collected and centrifuged. Individual plasma samples were frozen and stored for subsequent determination of ACTH and C concentrations. The adrenal glands were dissected and weighed to approximately 0.1 mg, homogeneized in a saline–alcohol solution, and frozen until C

TABLE 1

INDIVIDUAL STRESSORS

| Day | Stressor | Hour |
|-----|----------|------|
| 1 | Immobilization | 1000 to 1100 hours |
| 2 | Noise | 1100 to 1500 hours |
| 3 | Noise | 0900 to 1300 hours |
| 4 | Immobilization | 1230 to 1330 hours |
| 5 | Ether anesthesia | 1600 hours |
| 6 | Fasting | For a 24-h period (0800 to 0800 hours) |
| 7 | Rest day | |
| 8 | Two saline injections | 1100 and 1600 hours |
| 9 | Immobilization | 1145 to 1245 hours |
| 10 | Ether anesthesia | 1030 hours |
| 11 | Two saline injections | 1000 and 1630 hours |
| 12 | Noise | 1000 to 1400 hours |
| 13 | Fasting | 0800 to 0800 hours |
| 14 | Rest day | |
| 15 | Ether anesthesia | 1100 hours |
| 16 | Noise | 1300 to 1700 hours |
| 17 | Ether anesthesia | 1230 hours |
| 18 | Immobilization | 1300 to 1400 hours |
| 19 | Ether anesthesia | 1145 hours |
| 20 | Fasting | 0800 to 0800 hours |
| 21 | Rest day | |
| 22 | Noise | 1100 to 1300 hours |
| 23 | Two saline injections | 1000 and 1630 hours |
| 24 | Noise | 0900 to 1300 hours |
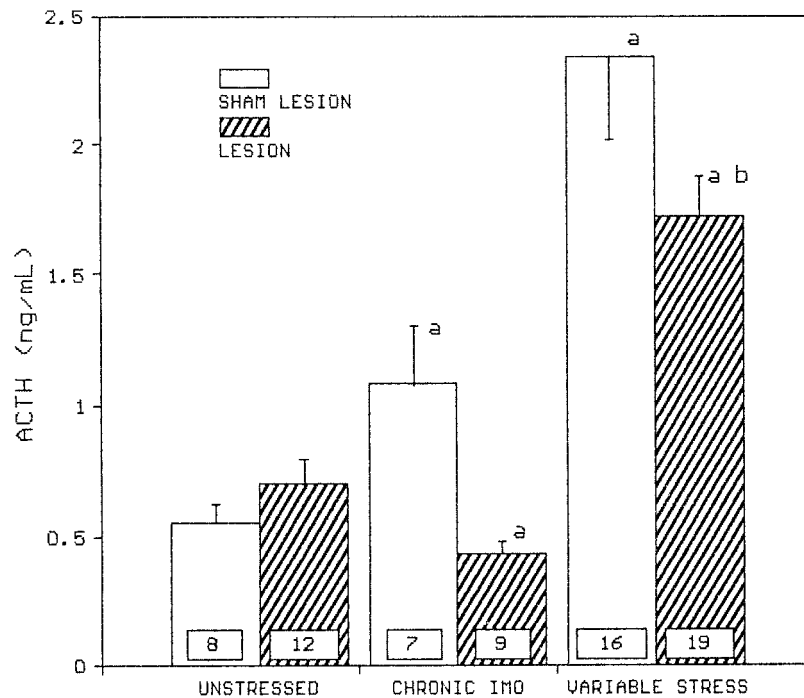
FIG. 2. Plasma ACTH response to ADTN lesions in unstressed and chronically stressed rats. Means ± SE are presented. The number of animals per group is included inside each bar. (a) Significant differences ($p < 0.05$) versus sham-lesioned unstressed group; (b) significant differences ($p < 0.05$) versus sham-lesioned stressed group.

was determined. Plasma ACTH was analyzed using a commercial radioimmunoassay kit (Incstar Corp.). The sample, first antibody, and traces were combined and incubated for 16–24 h at 2–8°C. A preprecipitated second antibody complex was added to separate the bound tracer from the free tracer. The assay was then centrifuged and decanted after a 15-min incubation at 20–25°C. Concentrations of plasma and adrenal C were determined fluorometrically according to the Rerup and Hedner method (18).

*Histology*

The brains of lesioned animals were fixed in 10% formaldehyde, subsequently sliced in a coronal plane on a cryostat-mounted microtome, and examined histologically. Data from animals with nonsatisfactory lesions were not included in the statistical evaluation.

*Statistical Method*

Statistical significance of the data was determined by ANOVA and individual group means were compared by the Student–Newman–Keul's test. Significance was set at $p < 0.05$.

RESULTS

Figure 1 illustrates the location of lesion points.

As shown in Fig. 2, the plasma ACTH concentration in unstressed rats did increase as the result of bilateral lesion of the ADTN. Figure 2 also indicates that after chronic IMO and V stress, sham-lesioned animals showed a significant increase in plasma ACTH compared to sham-lesioned unstressed rats. In lesioned rats, the levels of ACTH were differentially affected by chronic IMO or V stress. After exposure of ADTN-lesioned rats to IMO

stress, plasma ACTH dropped compared to that in unstressed lesioned or sham-lesioned rats. In contrast, there was a significant increase in ACTH concentration after V stress ($p < 0.05$) compared to that in unstressed lesioned rats (Fig. 2).

Chronic IMO and V stress induced an increase in plasma C in sham-lesioned rats, whereas in lesioned animals, plasma C remained practically unchanged compared to that in unstressed lesioned animals (Fig. 3). In all stressed groups, adrenal C content was below that found in unstressed rats. IMO and V stress-induced reduction levels occurring in lesioned rats were statistically significant, although in sham-lesioned animals, the stress-induced decrease was not significant (Fig. 4).

DISCUSSION

The present results are consistent with our previous findings which showed that bilateral lesion of the ADTN in rats provoked an increase in plasma ACTH and C concentration in rats under basal conditions (21). In rats, the ADTN are connected to the cingulate cortex, especially to the retrosplenial area, and via this latter area and entorhinal area, the ADTN connect to the hippocampus, septal, amygdaloid, and mammillary nuclei (27). It is known that these areas are directly connected to the hypothalamus. Electrical stimulation of the hippocampus, septal, and amygdaloid nuclei elicits a rise in plasma C levels (19), which can be abolished by lesioning their fiber connections to the hypothalamus (7); lesioning the cingulate cortex or mammillary nuclei produced an increase in plasma C (22).

The adrenocortical response to stress in intact rats is well documented and it results in a marked elevation of C as a consequence of the activation of the hypothalamo-hypophyso-adrenal axis. In this work, chronic exposure to IMO and V stress of
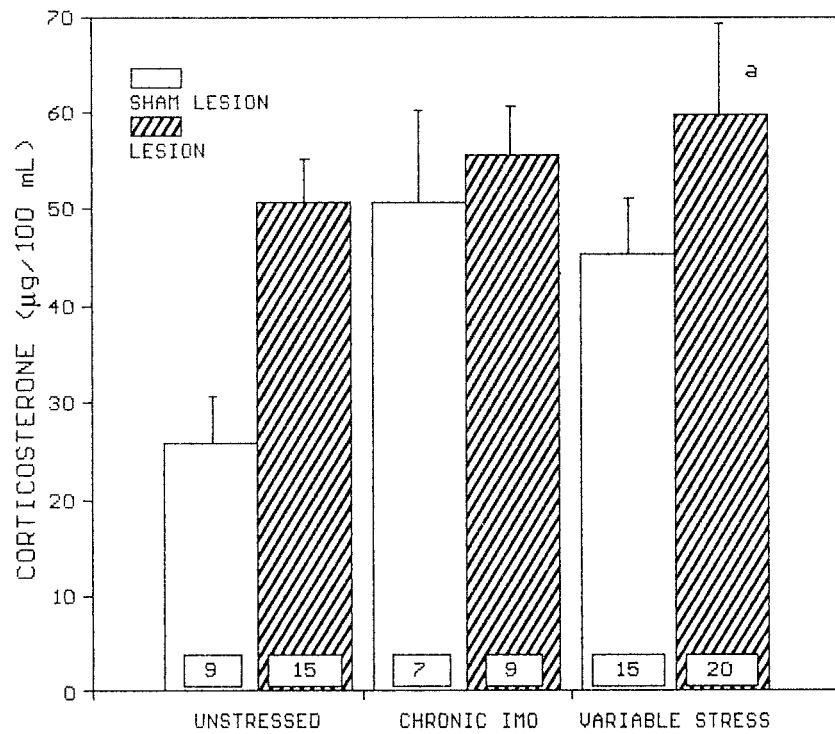
FIG. 3. Plasma corticosterone response to ADTN lesions in unstressed and chronically stressed rats. Means ± SE are presented. The number of animals per group is included inside each bar. (a) Significant differences ($p < 0.05$) versus sham-lesioned unstressed group.
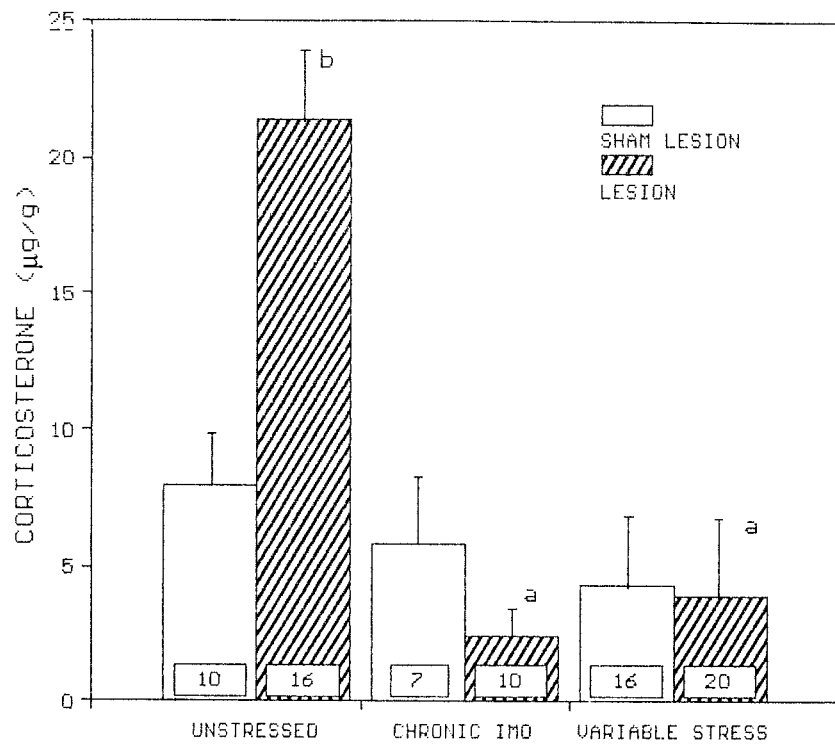


FIG. 4. Adrenal corticosterone response to ADTN lesions in unstressed and chronically stressed rats. Means ± SE are presented. The number of animals per group is included inside each bar. (a) Significant differences ($p < 0.05$) versus sham-lesioned unstressed group; (b) significant differences ($p < 0.05$) versus lesioned unstressed group.

sham-lesioned rats enhanced hypophysoadrenal activity, as reflected by the increased plasma ACTH and C. The plasma ACTH rise after IMO stress was lower than that after V stress (70.9% and 325.4%, respectively). In lesioned animals, plasma ACTH goes below basal values after IMO stress, whereas after variable stress, this parameter is higher than that in unstressed lesioned animals. These results and those obtained from sham-lesioned rats, in which the response of hormone to IMO stress was lower than that in V stressed animals, suggest that the exposure of the animal to the same type of stressor over time could provoke an adaptation process. (2).

Our results differ from those of Sapolsky et al. (20) on rats with lesioned hippocampus. In these animals, Sapolsky et al. found a higher response of HAS to chronic stress.

On the other hand, greater plasma ACTH concentration after V stress in lesioned rats indicates that the plasma ACTH decrease after chronic IMO is not due to exhaustion of secretory capacity of corticotrophs.

The V stress-induced increase in lesioned rats was lower than the V stress-elicited increase in sham-lesioned rats. The data expressed as a percentage of change of unstressed plasma ACTH values showed an increase of 145.7% in lesioned rats and 325.4% in sham-lesioned rats. Such attenuation of the pituitary response in lesioned chronically stressed animals may involve a number of mechanisms, including increased glucocorticoid feedback, a decrease in hypothalamic reaction of corticotropin releasing hormone (CRH), exhaustion of the secretory capacity of the corticotrophs, and a decrease in hypophyso receptors for ACTH regulators. Considering the higher levels of corticoids after ADTN lesions and assuming that the first stress exposure resulted in a large increase in plasma ACTH and, therefore, plasma C, it is possible that the inhibition reflects a glucocorticoid feedback.

On the other hand, considering the high levels of ACTH in lesioned animals, we can infer that CRH release is also increased, and it is known that corticotrophs become refractory to CRH after prolonged exposure to this peptide (5). This desensitization involves the loss of CRH receptors. Aguilera et al. (1) reported that during prolonged IMO stress, the concentration of pituitary CRH receptors decreases.

In unstressed conditions, lesion of ADTN evoked a significant increase in plasma and adrenal C concentrations. After IMO stress, ACTH levels were lower and plasma C remained elevated. The dissociation between plasma ACTH and plasma C concentration could be a result of direct sympathetic activation of the adrenal cortex or another mechanism. There is evidence suggesting that a number of non-ACTH-dependent mechanisms may be involved in control of adrenocortical secretion, including direct autonomous innervation (11), local paracrine mechanisms involving chemical messengers produced in the adrenal medulla, and other peptides that directly stimulate the adrenal cortex (3,17). The similarity between plasma C values of unstressed and poststressed animals could be due to the fact that the maximum C response is reached, considering the low adrenal C content.

It is well accepted that the HAS is activated in different ways in response to different stressful stimuli; therefore, we can assume that under stress conditions the thalamic nuclei may be inhibited from somewhere within the limbic system, thus evoking a desinhibition of the hypothalamic targets and enhancing the production and release of ACTH. In conclusion, the present results suggest that the lower response of HAS to IMO stress could be a result of an adaptation process rather than the exhaustion of a releasable pool of hormones. However, the lower activity of the HAS in rats with lesioned ADTN during chronic stress is more difficult to explain and its elucidation requires further studies.

## ACKNOWLEDGEMENTS

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