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Lesions of the anteroventral third ventricle region exaggerate neuroendocrine and thermogenic but not behavioral responses to a novel environment

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Whyte, Douglas G., and Alan Kim Johnson. Lesions of the anteroventral third ventricle region exaggerate neuroendocrine and thermogenic but not behavioral responses to a novel environment. Am J Physiol Regul Integr Comp Physiol 292: R137–R142, 2007. First published August 10, 2006; doi:10.1152/ajpregu.00465.2006.—Mild psychological stressors provoke an acute rise in core temperature (Tc), stimulate the hypothalmo-pituitary-adrenocortical (HPA) axis, and induce various stress-related behaviors. In the present study, we examined the effect of ablation of the anteroventral third ventricle region (AV3V) on both physiological and behavioral responses to a novel environment. Tc was monitored in male Sprague-Dawley rats, with either sham or AV3V lesions, during a 5-h exposure to a novel environment. Trunk blood was collected, in a second group of rats, for the assessment of plasma levels of ACTH and corticosterone. Novelty-induced grooming and rearing behaviors were assessed in a third group of animals. Tc was elevated in all animals after 30 min in the novel environment, but the rise was exaggerated in rats with AV3V lesions (~0.5°C). AV3V-lesion rats maintained a higher core temperature for 2 h before it returned to the same level as the control group. Plasma levels of ACTH and corticosterone were also exaggerated in the AV3V lesion group after 30 min in a novel environment. In contrast to the physiological responses, the behavioral measures of grooming and rearing revealed no differences between the groups. The results from the current study suggest that neurons within the AV3V region exert an inhibitory influence on the HPA axis and fever developed in response to stressful psychological stimuli. They also confirm that the physiological and hormonal components of the stress response are independent of certain behavioral measures of stress.

Sprague-Dawley rats; grooming; adrenocorticotropic hormone; corticosterone; temperature; psychological stress

MATERIALS AND METHODS

Animals. Sixty-seven male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 325–375 g at the time of lesion, were used in this study. Rats were housed individually in hanging, wire-bottom cages (Hoeltge, Cincinnati, OH) at 23°C in a 12:12-h light-dark cycle (lights on at 0600). Rat chow (NIH-31, Harlan Teklad) and tap water were available ad libitum except during the experiment. All experimental procedures were approved by the University of Iowa Animal Care and Use Committee.

AV3V-lesion surgery. Rats were anesthetized with Equithesin (0.33 ml/100 g body wt), secured in a stereotaxic apparatus, and the skull

The anteroventral third ventricular (AV3V) region, an anatomic area that encompasses the organum vasculosum of the lamina terminalis, the ventral portion of the median preoptic nucleus (MnPO), the anteroventral periventricular nucleus, and the periventricular hypothalamic nucleus (4), is thought to play a pivotal role in the modulation of LPS-induced fever. Fos-like immunoreactivity is elevated in the AV3V region after intravenous LPS administration (8) and injection of IL-1β into this region produces fever (29). Conversely, LPS-induced fever is attenuated by ablation (3, 5, 14) or injection of cyclooxygenase inhibitors into the AV3V region (30), although the former result has recently been challenged (26). Whether the AV3V region is also involved in psychological or stress-induced fever is less apparent. Levels of Fos activity are elevated in the AV3V region after restraint stress (34); however, Hunter (14) found that AV3V lesions had no impact on stress-induced fever. More recently, Romanovsky et al. (26) found that electrolytic lesions of this region resulted in a prolonged hyperthermia that lasted upward of 3 wk. In contrast to both of these lesion studies, we have previously reported that AV3V-lesion animals produce an exaggerated stress-induced fever in response to gentle handling (35, 37).

Therefore, the purpose of the current study was to directly examine stress-induced fever in the AV3V-lesion rat model. Given that stress-induced fever is just one component of a complex array of physiological and behavioral outputs elicited in response to a perceived threat, we also determined whether there were changes in the activation of the hypothalamo-pituitary-adrenocortical (HPA) axis as well as stress-related behaviors.
leveled between bregma and lambda. An electrode (24-gauge nichrome wire insulated except at the tip) was lowered on the midline, 0.2 mm posterior to bregma, 7.5 mm below dura, and anodal current (2.5 mA) passed for 15–20 s. In sham-lesion rats, the electrode was lowered 6.5 mm and no current passed. To determine the effectiveness of the lesions, overnight water intake was measured in the 24 h immediately postsurgery. Because lesions of the lamina terminals produce adipsia (4), we substituted a 10% sucrose solution in place of water and gradually weaned the rats back to water over a 2- to 3-wk period. Fluid intake was determined daily, and any animal that had not consumed 10 ml of sucrose solution was supplemented with 10 ml saline (37°C) subcutaneously. Rats did not participate in any experiment or further surgery until at least 3 wk postsurgery.

Radiotelemeter surgery. To estimate Tc in protocol 1, rats were anesthetized with Equithesin for a second surgery, and a radiotelemeter (Barrows, Magalia, CA) was implanted into the peritoneal cavity to measure peritoneal temperature (TPER). Rats were allowed at least 1 wk to recover from the surgery.

Protocol 1: change in peritoneal temperature (ΔTPER) in response to a novel environment. To minimize the effects of circadian variations on Tc and behavior, all experiments were initiated between 0745 and 0810. Care was taken in all of the protocols to ensure that rats remained undisturbed for the 14 h before the experiment. Baseline TPER was monitored for 30 min before the rats were removed from the vivarium. Animals were then transported to the experimental room (22–23°C), where they were immediately placed in individual, clean, hanging wire-bottom cages. Rats were tested in groups of 5 or 6. Rats remained undisturbed in the cages for the next 5 h, and Tc was determined every 5 min.

Protocol 2: hypothalamic-pituitary axis activation. AV3V- and sham-lesion animals were randomly assigned to one of two groups, either novel environment or home cage control. Rats in the novel environment group were transferred to a clean, hanging wire-bottom cage and left undisturbed in a sound-isolated room for 30 min, whereas home cage control animals remained in the vivarium. At the completion of the 30 min, rats were rapidly decapitated in an adjacent room and trunk blood collected into heparinized (for corticosterone determination) or EDTA (for ACTH determination) containing blood collection tubes (BD Vacutainer, Franklin Lakes, NJ) chilled on ice. Whole blood was centrifuged at 1,000 g for 15 min in a refrigerated centrifuge (Sorvall RT6000B Refrigerated Centrifuge, Du Pont, Wilmington, DE) within 15 min of being collected. Plasma was divided into 250-μl aliquots and stored at −80°C until assayed. All blood collection took place between 0815 and 0840. Rats were decapitated within 35 s of their cage being removed from the bank. To estimate Tc, colonic temperature was taken immediately after decapitation by inserting a thermistor probe (Yellow Springs Instrument, Yellow Springs, OH) 6–7 cm into the colon. Protocol 3: novelty-induced grooming. Animals were handled in the vivarium for 5 min on a daily basis for at least 7 days before the experiment. All experiments were performed between 0730 and 1030. On the day of the experiment, rats were transported to an isolated room that was illuminated with fluorescent light, placed in a clear, circular Plexiglas tub (diameter 30 cm × height 30 cm) and left for 1 h. Behavior was recorded during this period using a video camera (Sony, CCD-TRV37) and scored for grooming and rearing behaviors at a later date. Rats were all tested individually.

Plasma assays. Plasma ACTH levels were determined using a commercially available ELISA kit (MD Biosciences, St. Paul, MN). A calibration curve was constructed using synthetic rat ACTH (A-7075, Sigma-Aldrich, St. Louis, MO), dissolved in 1% acetic acid solution (0.1 mg/ml), then diluted in BSA/equine serum solution (MD Biosciences, St. Paul, MN) to 0, 7.3, 21, 66, 183, and 523 pg/ml. These concentrations were the same as those used for calibration standards of human ACTH in the kit. Plasma samples collected in EDTA-coated vials were diluted 1:2 using BSA/equine serum solution. Plasma corticosterone levels were determined using a commercially available enzyme immunoassay (EIA) kit (026-AC-14F1, American Laboratory Products, Windham, NH). Plasma samples collected in heparinized tubes were diluted 1:10 using the provided diluent.

The ACTH ELISA kit had a sensitivity of 0.46 pg/ml and intra-assay and interassay coefficients of variation of 3.1% and 5.8%, respectively. The corticosterone EIA kit had a sensitivity of 0.23 ng/ml and intra-assay and interassay coefficients of variation of 3.2% and 5.4%, respectively. All unknown samples and standards were tested in duplicate, and the values were averaged.

Behavioral measures. Each rat was assigned a random 6-digit number on the videotape, which differed from its cage identification number. In this manner, the scorer could remain blind to the lesion status of the animal in question, while scoring the videos. Results from the behavioral analyses were only matched up with the cage identification after all of the rats had been scored. Rats were scored for grooming and rearing behavior for 1 h in the novelty protocol, beginning immediately after the rat was placed in the Plexiglas tub. Grooming scores were determined using a well-validated scoring technique that has been fully described elsewhere (10). Briefly, every 15th s, rats are given a point if they are involved in a maintenance behavior, defined as licking, washing, vibrating, and scratching. Using this system the maximum possible score for a single session was 240 points. Scores are displayed as a percentage of the maximum possible score.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was performed using SigmaStat for Windows version 2.03. Data were analyzed using a two-way ANOVA with or without repeated measures and a post hoc Tukey’s test when appropriate. Water intake and maximal grooming scores were tested for significance using a Student’s t-test. Differences were accepted as significant at P < 0.05.

RESULTS

Lesions. The animals with AV3V ablations, which typically destroy the organum vasculosum of the lamina terminalis, ventral MnPO, rostral periventricular tissue, and the medial portion of the medial preoptic nucleus (1, 35, 37), showed an immediate cessation of water intake in the 24 h immediately after surgery (water intake, AV3V 8.8 ± 2.19 ml to sham 26.5 ± 3.05 ml, t66 = 4.69, P < 0.001).

Protocol 1: ΔTPER in response to a novel environment. There was no difference in the Tc of sham- and AV3V-lesion rats, while they remained in their home cages in the vivarium (−30 to 0 min, Fig. 1). Tc rose rapidly in both groups once they were transferred to an experimental cage. The Tc of sham-lesion rats reached a plateau within 30 min, after an elevation of ~0.7°C, and remained at this level for the duration of the experiment. While the elevation in Tc of AV3V-lesion rats also plateaued within a similar time span, Tc increased to a much greater extent (~1.2°C, group × time: F56,558 = 2.96, P < 0.001). AV3V-lesion animals maintained their Tc at this elevated level for the next 2 h before it returned to the level of the sham-lesion rats.

Protocol 2: HPA axis activation. On the basis of the results of the previous experiment, plasma levels of ACTH and corticosterone were determined after 30 min of exposure to the novel environment. As seen in protocol 1, there was no difference in the colonic temperature of animals that remained in their home cage; however, AV3V-lesion rats again generated a larger stress-induced fever (Fig. 2A). ACTH levels were initially similar between the two groups (Fig. 2B) and in-
creased in response to the novel environment. Although the novelty-induced increase in plasma ACTH tended to be greater in AV3V-lesion rats in comparison with sham-lesion rats, this did not reach significance \( P > 0.05 \). Basal plasma levels of corticosterone were also similar between the two groups and were elevated after exposure to the novel environment (Fig. 2C). Corticosterone levels increased to a larger extent in the AV3V-lesion group in comparison with the sham-lesion group.

Novelty-induced grooming. Sham- and AV3V-lesion rats groomed to the same extent when placed in a novel environment \( (P = 0.90; \text{Fig. 3A}) \). The temporal pattern of novelty-induced grooming was also similar between the two groups \( (\text{group} \times \text{time interaction: } F_{11,209} = 0.66, P = 0.78; \text{Fig. 3B}) \). Rearing behavior was comparable between the groups \( (\text{group} \times \text{time interaction: } F_{11,253} = 1.40, P = 0.18) \) and showed a decline over time (Fig. 4).

DISCUSSION

The current study clearly demonstrates that the degree of stress-induced fever and HPA activity are dramatically enhanced in AV3V-lesion rats. While the elevation in TC and HPA activity occur in response to the psychological stress imposed on the rat by the novel environment, they do not necessarily reflect the degree of stress experienced by the animal \( (31) \). This is illustrated by the fact, that in spite of the exaggerated TC and HPA activation, behavioral measures of stress (i.e., grooming and rearing activity) were no different between the groups. Thus it is unlikely that the data reported here are the consequence of the AV3V-lesion rats perceiving the surrounding environment as more threatening than their

Fig. 1. Peritoneal temperature (TPer) of sham- \( (n = 6) \) and AV3V-lesion rats \( (n = 5) \) during exposure to a novel environment for 5 h. Rats were moved from the vivarium to the experimental room and placed in a clean, hanging wire-bottom cage at time 0. Data are means ± SE. *Significantly different from sham-lesion animals, \( P < 0.05 \).

Fig. 2. Colonic temperature \( (A) \) and plasma levels of ACTH \( (B) \) and corticosterone \( (C) \) in home cage control (sham \( n = 10, \) AV3V \( n = 7 \)) and novelty stressed (sham \( n = 10, \) AV3V \( n = 8 \)) rats. Novelty-stressed animals were exposed to a new cage for 30 min. Data are expressed as means ± SE. *Significantly different from sham-lesion animals, \( P < 0.05 \). †Significantly different from home cage, \( P < 0.05 \).

Fig. 3. Grooming behavior in sham- \( (n = 11, \) solid bar) and AV3V-lesion rats \( (n = 10, \) open bar) during a 1-h exposure to a novel environment. Data are expressed as means ± SE and are presented as both the percent of the maximal possible grooming score \( (A) \) and the temporal pattern of grooming \( (B) \) in response to a novel environment.

Fig. 4. Rearing behavior was comparable between the groups \( (\text{group} \times \text{time interaction: } F_{11,253} = 1.40, P = 0.18) \) and showed a decline over time (Fig. 4).
As means of studies to measure TC. In the current study (explanation relates to the different techniques used by the two and thermogenic pathways in some manner. An alternative slightly larger lesions restored the balance between thermolytic warm-sensitive neurons within the MnPO responsible for the previously reported hyperthermia (26). Romanovsky et al. (26) temperature rhythm (27).

Basal thermoregulation was unaffected by the AV3V lesion. This is in contrast to a recent report in which similar lesions were found to produce a prolonged (>3 wk) hyperthermia (~2°C) (26). Although we did not measure circadian temperature rhythms in these animals, Tc did not differ between sham- and AV3V-lesion rats in the 30 min before exposure to the novel environment, nor was there any difference in the colonic temperature of the rats that remained in their home cage throughout protocol 2. Although not definitive, these data certainly suggest that AV3V-lesion rats are capable of maintaining a relatively normal Tc within the carefully controlled environment of the laboratory. However, this conclusion must be regarded with caution, as lesions of the neighboring medial preoptic nucleus increase the amplitude of the circadian temperature rhythm (27).

It remains unclear as to why our rats did not develop the previously reported hyperthermia (26). Romanovsky et al. (26) suggested that destruction of the AV3V region may eliminate warm-sensitive neurons within the MnPO responsible for the inhibition of thermogenesis. It is therefore possible that our slightly larger lesions restored the balance between thermolytic and thermogenic pathways in some manner. An alternative explanation relates to the different techniques used by the two studies to measure Tc. In the current study (protocol 1), we used a radiotelemetry system, as opposed to a colonic probe, to monitor Tc, allowing the animal to remain undisturbed until the cage switch occurred. Given that AV3V-lesion rats develop an exaggerated stress-induced fever and do not appear to habituate to a given stressor, even after extensive handling (35, 37), this may be a critical factor for the study of thermoregulation in this model.

Sham-lesion rats maintained their Tc at an elevated level throughout the entire protocol (5 h). In contrast, Tc started to decline in the AV3V-lesion rats after 75 min (Fig. 1), although it did not return to baseline but rather plateaued at the same level as that of the stressed, sham-lesion animals. We interpret this to mean that AV3V-lesion rats initially exceeded the intended degree of fever (i.e., targeted temperature). Indeed, we hypothesize that the exaggerated stress-induced fever observed in AV3V-lesion rats is the consequence of a combination of an attenuated ability to lose heat, coupled with the loss of inhibitory pathways regulating heat production.

Fever is generated by minimizing heat loss while simultaneously increasing heat gain. As such, regulation of blood flow to the cutaneous vasculature is critical for the development and maintenance of a fever. Constriction of the tail vasculature initially prevents heat loss allowing the development of fever and is the primary mechanism used to balance heat production to maintain a stable Tc (11). The AV3V region, and in particular the MnPO, contains a high percentage of temperature-sensitive neurons (32), and electrical stimulation of this area promotes hindquarter dilation (19). Recent work by Yoshida et al. (38) has shown that many MnPO neurons activated by heat stress synapse in the rostral periaqueductal gray, an area crucial for tail dilation (39). Ablation of the AV3V region results in an inability to appropriately redistribute blood flow or salivate in response to high environmental temperatures and consequently produces a reduced thermal tolerance (36, 37). This loss of the ability to fine-tune cutaneous vascular tone could by itself result in the observed overheating and delayed rectification; however, evidence also exists to suggest that neurons within the MnPO also inhibit nonshivering thermogenesis (24), thereby providing a secondary drive that could push Tc higher.

HPA axis activation was also increased in the lesion group after exposure to a novel environment. AV3V-lesion rats have previously been found to produce an exaggerated HPA response to physiological stressors such as IL-1β (16) and furosemide treatment (2), although this is the first time it has been demonstrated in response to a psychological stressor. It is important to note that there was no difference in ACTH or corticosterone levels in the AV3V- and sham-lesion animals that remained in their home cage. This indicates that the elevated level of HPA axis activity was in direct response to the novel environment, not a chronic elevation induced by the lesion. These data contrast with those of Bealer and Schneider (2) who reported elevated basal levels of corticosterone in AV3V-lesion rats. In their study, animals were moved from the animal facility to the experimental room and allowed 2 h to acclimate. On the basis of the present data, we believe their rats were already stressed due to the novel environment, and this was reflected by the elevated basal levels in the experimental group.

Glucocorticoids suppress HPA axis activity (13, 17) and the febrile response (20, 22, 23) to both physical and psychological stressors. Adrenalectomized rats exhibit greater fevers after either LPS administration or exposure to an open field (22), and central administration of a glucocorticoid receptor antagonist produces a similar result (20). Given that the Tc of AV3V-lesion rats were elevated in spite of exaggerated levels of corticosterone suggests that an important negative feedback pathway was disrupted by the lesion.

Direct injections of corticosterone into the anterior hypothalamic attenuate LPS- but not stress-induced fever (23). Instead corticosterone is thought to limit the stress response to a novel environment by acting on the hippocampus, as ablation of the hippocampus or transection of the fornix enhances stress-induced fever (23), whereas hippocampal stimulation inhibits corticosterone release (7). However, the limbic system does not communicate directly with the paraventricular nucleus but rather relays information through various nuclei, including...
those within the AV3V region (13, 18). This synaptic relay allows limbic information to be integrated with homeostatic information such as fluid balance and thermoregulatory status prior to the activation of any effector mechanisms. Neurons projecting from the AV3V region to the paraventricular nucleus are predominantly GABAergic, indicating that they provide an inhibitory input to the HPA axis (12). Electrolytic ablation of the AV3V region would therefore eliminate these important inhibitory inputs, as well as any fibers of passage and consequently result in the exaggerated HPA and febrile response that we observed.

In summary, the present data are the first to demonstrate that AV3V-lesion rats exhibit an exaggerated degree of fever and HPA activation in response to a mild psychological stressor. These data indicate that neurons within the AV3V region have an important modulatory role in the physiological, but not behavioral, responses to psychological stressors. They also support the hypothesis that physical and psychological stressors are processed by distinct neural pathways.

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